SPRINGER NATURE
Reference

Michele I. Morris
Camille Nelson Kotton
Cameron R. Wolfe
Editors

Emerging Transplant Infections

Clinical Challenges and Implications

Springer

Emerging Transplant Infections

Michele I. Morris • Camille Nelson Kotton • Cameron R. Wolfe
Editors

Emerging Transplant Infections

Clinical Challenges and Implications

With 132 Figures and 198 Tables

Springer

Editors
Michele I. Morris
Immunocompromised Host Service
Division of Infectious Diseases
University of Miami Miller School of Medicine
Miami, FL, USA

Camille Nelson Kotton
Transplant and Immunocompromised Host Infectious Diseases, Infectious Diseases Division, Massachusetts General Hospital
Harvard Medical School
Boston, MA, USA

Cameron R. Wolfe
Division of Infectious Diseases
Duke University School of Medicine
Durham, NC, USA

ISBN 978-3-030-25868-9 ISBN 978-3-030-25869-6 (eBook)
ISBN 978-3-030-25870-2 (print and electronic bundle)
https://doi.org/10.1007/978-3-030-25869-6

© Springer Nature Switzerland AG 2021
All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

The field of Transplant Infectious Diseases has emerged over recent decades to meet the challenges of caring for immunocompromised solid organ and stem cell transplant recipients. Innovations in surgical and cellular therapies and changes in transplant immunosuppression have impacted the variety of bacterial, viral, fungal, and parasitic organisms that may cause disease in this population. Transplant programs worldwide now recognize the need for expertise in the management of infectious complications and consider infectious disease specialists an essential part of the transplant team. Clinical trials in post-transplant prophylaxis and treatment have optimized the understanding of common infections such as cytomegalovirus, facilitating the establishment of clinical guidelines. Such guidelines are constantly subject to modification as ongoing changes in antimicrobial susceptibility have altered historically validated approaches to infection management. New guidance has also been required to address unique presentations of infections previously rarely seen in transplant recipients. Current transplant protocols must reflect the impact of global human migration and expanded access to transplantation in patients with previous exposure to rare tropical diseases. Trials of new immunotherapeutic agents are essential as we improve management of transplant rejection or disease relapse, but may facilitate the emergence of latent or previously unrecognized microorganisms. Novel molecular diagnostics have led to the identification of new pathogens which may be observed first in this vulnerable patient population. For the transplant teams seeking to optimize management of these infections, a comprehensive textbook which can be regularly updated is most welcome.

This premier edition of the *Emerging Transplant Infections* textbook was prepared by an international team of experts to provide an authoritative and current resource for transplant specialists seeking up-to-date guidance in the diagnosis and treatment of infectious complications of transplant. Information can be accessed electronically via SpringerLink or through current and future editions of the printed textbook. Chapters will be added and updated to reflect the constantly changing nature of the field. This edition contains 69 chapters divided into seven parts. The introductory part provides an overview of the field and encompasses general topics including transplant immunosuppression, infection control and prevention, and pre-transplant screening. This is followed by four pathogen-based parts devoted to bacterial, viral, fungal, and parasitic diseases of major importance in transplant

candidates and recipients. Authors were asked to share information on disease epidemiology, diagnosis, and management with an emphasis on the clinical impact on both solid organ and stem cell transplantation. A part focused on emerging clinical challenges follows which addresses cardiac device infections and other new areas of focus in the transplant world. The final part provides invaluable information using a syndrome-based approach that will enhance the ability of all readers to identify emerging infectious diseases. Chapters include dermatologic and ophthalmologic presentations as well as a review of emerging diagnostics and a section devoted to pediatric diseases.

It has been over three years since three Transplant Infectious Disease physicians joined together to develop a clinical reference that would assist our colleagues in navigating the challenging and rapidly changing field of emerging infections in transplantation. This project would not have been possible without the outstanding work of an international team of authors who were willing to dedicate their time and expertise in support of this effort. Many thanks to all of the contributors for their invaluable assistance. Thanks also to the editorial staff at Springer Nature, including Nadina Persaud who conceived of the project in late 2017, Alexa Steele who guided us in development, and Sonal Nagpal who provided us consistent production support throughout the project. We hope this collection is not only a useful tool in clinical care but also reflects the passion and commitment of all of us to share our knowledge with the goal of improving outcomes for the transplant patients who entrust us with their care.

June 2021

Michele I. Morris
Camille Nelson Kotton
Cameron R. Wolfe

Contents

Volume 1

Part I General Introduction 1

1 Impact of Infection on Transplant Outcomes: Transplant Infectious Disease Expertise as an Essential Component of Transplant Programs 3
Michele I. Morris

2 Introduction to Immunosuppression, Tolerance, and Infection Risk 11
Cameron R. Wolfe

3 Changing Definition of Immunosuppression: Targeted Therapies and Resulting Emerging Infections and Their Prevention 25
Nicolas J. Mueller

4 Stem Cell Transplantation: Infection Risk by Transplant Type and Emerging Trends in Infection Management 43
Gowri Satyanarayana, Katie S. Gatwood, and Whitney J. Nesbitt

5 Evolving Epidemiology of Transplant Infections: Impact of Population Shifts and Climate Change 83
Tirdad T. Zangeneh and Mohanad Al-Obaidi

6 Hospital Epidemiology and Infection Control in the Transplant Center 99
Gopi Patel and Sarah Hochman

7 Antimicrobial Stewardship for Transplant Candidates and Recipients 131
Catherine Liu and Erica J. Stohs

8 Antimicrobial Prophylaxis in the Age of Emerging Transplant Infections 155
Jonathan Hand

9 **Pretransplant Screening for Infection Exposure: Approach to the Patients with Epidemiologic and Geographic Risk Factors** . . . 171
Deepa Nanayakkara and Joanna Schaenman

10 **Transplant Tourism and the Traveling Transplant Recipient: Infection Mitigation and Treatment Strategies** 201
C. Arianne Buchan

11 **Preventative Health in Solid Organ Transplant: Safe Living Post-transplant** 239
Barbra M. Blair

12 **Vaccination Strategies in Solid Organ and Stem Cell Transplant** 253
Shilpa R. Jain and Deepali Kumar

Part II Bacterial Infections Introduction 285

13 **Multidrug-Resistant Organisms: Pre-transplant Evaluation and Management** 287
Anoma Nellore and Rachael A. Lee

14 **Multidrug-Resistant Organisms: Posttransplant Management for Extended-Spectrum Beta-Lactamase Producers and Carbapenem-Resistant Enterobacterales** 313
Jessica Howard-Anderson and Stephanie M. Pouch

15 ***Pseudomonas aeruginosa* Infections in Transplant: Epidemiology and Emerging Treatment Options** 343
John I. Hogan, Brandon K. Hill, and Costi D. Sifri

16 **Management of Acinetobacter Infections in the Immunosuppressed Host** 371
Emily Baneman and Meenakshi M. Rana

17 ***Burkholderia* in Transplant: Important to Speciate and Important to Treat** 391
Gregory Snell, Olivia Smibert, and Elizabeth Tullis

18 ***Mycobacterium tuberculosis* in Transplantation** 409
Aruna K. Subramanian

19 **Rapidly Growing Mycobacterial Infections in Transplant: Evolving Epidemiology and Treatment Options** 425
Nicholas A. Turner and Arthur W. Baker

20 **Slow-Growing Nontuberculous Mycobacteria in Transplant** 459
Esther Benamu and Joanna Nelson

21 ***Nocardia* Infections in Transplantation: Diagnosis, Management, and Impact on Outcomes** 487
Reuben J. Arasaratnam and Alejandro Restrepo

22 ***Mycoplasma* and *Ureaplasma* Infections in Transplantation: A Big Impact Despite the Lack of a Cell Wall** 505
Peter W. Schreiber and Nicolas J. Mueller

23 **Clostridioides Difficile: Where Should the Management Strategies in Transplant Vary?** 519
Jennifer C. Pisano and Kathleen M. Mullane

Volume 2

Part III Viral Infections Introduction **545**

24 **Cytomegalovirus in Solid Organ Transplant Recipients: Prevention, Diagnosis, and Treatment** 547
Camille Nelson Kotton

25 **Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment** 573
Jose F. Camargo

26 **Herpes Simplex and Varicella Zoster in Transplant Candidates and Recipients** 617
Richard A. Zuckerman

27 **Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorders** 655
Masaki Yamada, Andrew Nowalk, and Michael Green

28 **HHV-6, HHV-7, and HHV-8: Forgotten Viruses in Transplantation** 683
Ghady Haidar

29 **Influenza Infections in Solid Organ and Stem Cell Transplant Recipients** 709
Yoichiro Natori

30 **Respiratory Syncytial Virus in Transplant: Stopping the Short-Term Spread, Solving the Longer-Term Problems** 725
A. J. Wright and A. Mah

31 **Respiratory Viral Pathogens in Solid Organ and Hematopoietic Stem Cell Transplant Recipients** 743
Steven A. Pergam and Michael G. Ison

32 Transplant Adenovirus Infection: Clinical Syndromes, Diagnosis, and Management 781
Maricar Malinis and Marwan M. Azar

33 BK Polyomavirus After Solid Organ and Hematopoietic Cell Transplantation: One Virus – Three Diseases 807
Fabrice E. Graf and Hans H. Hirsch

34 Challenges of Human Papillomavirus Infection in Solid Organ and Hematopoietic Stem Cell Transplant Recipients 833
Cristina Brickman and Peter Chin-Hong

35 Solid Organ Transplantation in HIV Positive Patients 859
Elmi Muller and Peter Stock

36 Hepatitis A Infections in Transplant 877
Rebekah J. Lane

37 Hepatitis E Infections in Transplants 909
Olivier Marion and Nassim Kamar

38 Hepatitis B and Hepatitis D Infections in the Transplant Setting 927
Brian Kim and Norah A. Terrault

39 Emerging Management of Hepatitis C in Transplant 951
Lorena Puchades and Marina Berenguer

40 West Nile Virus and Transplantation 971
Marilyn E. Levi and Marian G. Michaels

41 Emerging Tropical Viral Infections: Dengue, Chikungunya, and Zika 987
Alexandre Machado de Sant' Anna Carvalho and Clarisse Martins Machado

42 Yellow Fever: Prevention in Transplant Candidates and Emerging Treatment Data for Liver Transplant 1011
Alice Tung Wan Song and Luiz Augusto Carneiro D'Albuquerque

43 Viral Encephalitides Including Rabies and Lymphocytic Choriomeningitis Virus: Impact in Transplant Donors and Recipients 1027
Aalok Khole and Nicole M. Theodoropoulos

Part IV Fungal Infections Introduction **1043**

44 *Candida* Infections in Solid Organ Transplantation 1045
Nicolas Barros and Michael K. Mansour

45 *Aspergillus* in Solid Organ and Stem Cell Transplant Patients: Emerging Options for Diagnosis and Management 1077
Armelle Pérez-Cortés Villalobos and Shahid Husain

46 Management of Mucorales Infections in Transplant Patients 1109
Sharon C.-A. Chen and Monica A. Slavin

47 Non-*Aspergillus* Fungal Infections in Transplant Patients 1139
Sanjay G. Revankar

48 Management of Cryptococcosis in Transplant Candidates and Recipients 1155
John W. Baddley and Graeme N. Forrest

49 *Pneumocystis jiroveci* in Transplant: Recognizing Risk, Understanding Prevention, and Implementing Treatment 1183
Shmuel Shoham and M. Veronica Dioverti Prono

50 Coccidioidomycosis in Transplantation 1207
Brandon T. Nokes and Janis E. Blair

51 Emerging Epidemics in Transplant: Histoplasmosis, Blastomycosis, and Paracoccidioidomycosis 1225
Jeremy Nel, Anne Lachiewicz, and David van Duin

Volume 3

Part V Parasitic Infections Introduction 1251

52 *Toxoplasma gondii* in Solid Organ and Stem Cell Transplant: Prevention and Treatment 1253
Hélène Guegan and Florence Robert-Gangneux

53 Strongyloides: Emerging Challenges and Prevention 1281
Ricardo M. La Hoz and Alfredo G. Puing

54 Chagas Disease: Coming to a Transplanted Patient Near You 1293
Lígia Camera Pierrotti and Karim Yaqub Ibrahim

55 Leishmaniasis in Transplant Candidates and Recipients: Diagnosis and Management 1341
Wanessa T. Clemente and Paulo H. O. Mourão

56 Malaria in Transplant Candidates and Recipients: Diagnosis and Management 1373
Pilar Martín-Dávila, Francesca F. Norman, and Rogelio López-Vélez

57 Pathogenic Intestinal Parasites in Transplant Recipients 1397
Joelle I. Rosser and Brian G. Blackburn

58 ***Acanthamoeba*, *Naegleria*, and *Balamuthia* in Transplant Patients: An Emerging Threat** 1451
Yoram A. Puius, Margaret L. Aldrich, and Jennifer R. Cope

Part VI Emerging Clinical Challenges **1473**

59 Ventricular Assist Devices, ECMO, and Cardiac Support Devices: Challenges in the Bridge to Transplant 1475
Hao Tran and Saima Aslam

60 Emerging Transplant Infections: Cystic Fibrosis 1495
Omar H. Mohamedaly and Carrie A. Johnson

61 Vascular Composite Allografts (VCA): New Transplants, New Infections 1513
Julie M. Steinbrink, Timothy L. Pruett, and Linda C. Cendales

Part VII Considering Emerging Infections in Differential Diagnosis **1533**

62 Emerging Diagnostics for Transplant Infectious Diseases 1535
Marwan M. Azar, David C. Gaston, and Maricar Malinis

63 Rashes to Recognize in the Immunocompromised Transplant Patient: Focus on the Solid Organ Transplant Recipient 1577
Melodi Javid Whitley, Adela R. Cardones, and Sarah A. Myers

64 Ophthalmic Infections in Transplant 1595
Jacob E. Lemieux, Lucia Sobrin, and Miriam B. Barshak

65 Pulmonary Syndromes in Transplantation 1615
Sonya Trinh and Alfred Luk

66 Diarrhea in Transplant: Diagnosis and Management 1639
Diana F. Florescu and Andrea Zimmer

67 Altered Mental Status in the Solid Organ Transplant Recipient . . . 1661
Dilek Ince

68 Transplant Infectious Disease Evaluation of Cytopenias 1673
Rachel Miller and Annette Schlueter

69 Common and Emerging Infections in Pediatric Transplant Recipients 1709
Jennifer E. Schuster, Hilary Miller-Handley, and Lara Danziger-Isakov

Index 1727

About the Editors

Michele I. Morris, M.D., FIDSA, FAST, is Professor of Clinical Medicine and Director of the Immunocompromised Host Service in the Division of Infectious Diseases at the University of Miami Miller School of Medicine. She has spent the last 17 years of her career focusing her clinical practice and research efforts on transplant infectious diseases, with special attention to the area of emerging infections in transplant. Dr. Morris is also Medical Director of Infection Control and Antimicrobial Stewardship at Sylvester Cancer Center, and Fellowship Director of the Transplant Infectious Diseases fellowship at the University of Miami/Jackson Memorial Hospital. She has published over 90 peer-reviewed articles and 8 book chapters. Dr. Morris has delivered numerous presentations at national and international meetings, including invited lectures on five continents. She is a past president of the Transplantation Society's Transplant Infectious Disease Section. She is an active member of the American Society of Transplantation's Infectious Diseases Community of Practice (ID-COP), and a previous executive board member.

Acknowledgments:
I am privileged to be a member of the international community of transplant infectious disease specialists whose outstanding teamwork is demonstrated in this textbook. An enormous thanks to Camille Kotton and Cameron Wolfe – your dedication and incredible collaboration made this project possible. Thanks also to my daughters Elana and Kayla, who somehow taught me more about life and love (and popular culture) than I could ever teach them. And to my husband, Joel Fishman, who has been there since the beginning,

thank you for sharing your life with me. Your unwavering love and support has shaped my growth as a physician and a person.

Finally, from all of us in the field of Transplant Infectious Diseases, thank you to our amazing patients for your resilience and optimism and to transplant donors everywhere for making it all possible.

Camille Nelson Kotton, M.D., FIDSA, FAST, is Clinical Director of the Transplant Infectious Disease and Immunocompromised Host Program at the Massachusetts General Hospital and Associate Professor of Medicine, Harvard Medical School, Boston, Massachusetts. She was chair of the Infectious Disease Community of Practice of the American Society of Transplantation (2012–2018). From 2007 to 2013, she was the president of the Transplant Infectious Disease Section of The Transplantation Society. Highlights of her time as president include the development of international guidelines on CMV management after solid organ transplant, published in *Transplantation* (2010, 2013, 2018). She is the first transplant infectious disease specialist to be a councilor of The Transplantation Society (2020). Her clinical interests include cytomegalovirus, donor-derived infections, zoonoses, and travel and tropical medicine in the transplant setting.

She would very much like to thank her family for their wonderful support over the years, including her sons Benjamin and David, and her husband Darrell, both for extensive homework projects like this one, and for strength during the COVID-19 pandemic.

Dr. Cameron R. Wolfe is an Associate Professor of Medicine in the Division of Infectious Disease at Duke University, specializing in transplant infectious diseases emerging pathogens, HIV, and viral hepatitis. He grew up and trained in Australia, and has research interests in the management of pandemic disease in the general community and especially respiratory viruses in the immunocompromised. He lectures at the University of North Carolina, School of Public Health, as well as the Duke University Medical School. More than anything, Cameron is proud of the grace shown by his two sons, Callum and Lachlan, and his wife, Sarah, as this project has come to fruition!

Contributors

Margaret L. Aldrich Division of Pediatric Infectious Diseases, Department of Pediatrics, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY, USA

Mohanad Al-Obaidi Infectious Diseases, Banner – University Medical Center Tucson (BUMC-T), Tucson, AZ, USA

Reuben J. Arasaratnam Division of Infectious Diseases and Geographic Medicine, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Saima Aslam Division of Infectious Diseases and Global Public Health, University of California San Diego, La Jolla, CA, USA

Marwan M. Azar Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT, USA

John W. Baddley University of Maryland, Institute of Human Virology, Baltimore, MD, USA

Arthur W. Baker Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, USA

Duke Center for Antimicrobial Stewardship and Infection Prevention, Durham, NC, USA

Emily Baneman Icahn School of Medicine at Mount Sinai, New York, NY, USA

Nicolas Barros Division of Infectious Diseases, Indiana University School of Medicine, Indianapolis, IN, USA

Miriam B. Barshak Division of Infectious Disease, Massachusetts General Hospital, Boston, MA, USA

Esther Benamu School of Medicine, Division of Infectious Diseases, University of Colorado, Aurora, CO, USA

Marina Berenguer Department of Gastroenterology, Hepatology Unit and Instituto de Investigación La Fe, Hospital Universitari i Politècnic La Fe, Valencia, Spain

Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Valencia, Spain

School of Medicine, University of Valencia, Valencia, Spain

Brian G. Blackburn Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA

Janis E. Blair Division of Infectious Diseases, Mayo Clinic Arizona, Phoenix, AZ, USA

Barbra M. Blair Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston, MA, USA

Harvard Medical School, Boston, MA, USA

Cristina Brickman University of California San Francisco, San Francisco, CA, USA

C. Arianne Buchan Divisoin of Infectious Diseases, Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada

The University of Ottawa, Ottawa, ON, Canada

The Ottawa Hospital Research Institute, Ottawa, ON, Canada

Jose F. Camargo Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, FL, USA

Adela R. Cardones Department of Dermatology, Duke University Medical Center, Durham, NC, USA

Luiz Augusto Carneiro D'Albuquerque Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Liver Surgery and Transplantation Laboratory LIM37, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Alexandre Machado de Sant' Anna Carvalho Department of Internal Medicine, Saint Joseph Mercy Ann Arbor, Ann Arbor, MI, USA

Linda C. Cendales Department of Surgery, Duke University Medical Center, Durham, NC, USA

Sharon C.-A. Chen Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, New South Wales Health Pathology, Westmead Hospital, Sydney, NSW, Australia

Marie Bashir Institute for Emerging Infections and Biosecurity, The University of Sydney, Sydney, NSW, Australia

The National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Peter Chin-Hong University of California San Francisco, San Francisco, CA, USA

Wanessa T. Clemente Faculty of Medicine, Departamento de Propedêutica Complementar, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil

Digestive Transplant Service, Liver Transplant Group, Instituto Alfa de Gastroenterologia do Hospital das Clínicas, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil

Jennifer R. Cope Waterborne Disease Prevention Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Lara Danziger-Isakov Division of Pediatric Infectious Diseases, Department of Infectious Disease, Cincinnati Children's Hospital and University of Cincinnati, Cincinnati, OH, USA

M. Veronica Dioverti Prono Infectious Diseases, Johns Hopkins University, Baltimore, MD, USA

Diana F. Florescu Division of Infectious Diseases, Transplant Infectious Diseases Program, Transplant Surgery Division, University of Nebraska Medical Center, Omaha, NE, USA

Graeme N. Forrest VA Portland Healthcare System, Portland, OR, USA

David C. Gaston Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT, USA

Katie S. Gatwood Department of Pharmaceutical Services, Vanderbilt University Medical Center, Nashville, TN, USA

Fabrice E. Graf Transplantation and Clinical Virology, Department Biomedicine, University of Basel, Basel, Switzerland

Michael Green Department of Pediatrics, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Department of Surgery, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Hélène Guegan Univ Rennes, CHU Rennes, Inserm, EHESP, Irset UMR_S 1085, Rennes, France

Centre Hospitalier Universitaire de Rennes, Rennes, France

Ghady Haidar Department of Medicine, Division of Infectious Diseases, University of Pittsburgh and UPMC, Pittsburgh, PA, USA

Jonathan Hand Division of Infectious Diseases, Ochsner Health, University of Queensland School of Medicine, Ochsner Clinical School, New Orleans, LA, USA

Brandon K. Hill Department of Pharmacy Clinical Services, UVA Health, Charlottesville, VA, USA

Hans H. Hirsch Transplantation and Clinical Virology, Department Biomedicine, University of Basel, Basel, Switzerland

Clinical Virology, Laboratory Medicine, University Hospital Basel, Basel, Switzerland

Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

Sarah Hochman Department of Medicine, Division of Infectious Disease and Immunology, New York University School of Medicine, New York, NY, USA

John I. Hogan Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA, USA

Jessica Howard-Anderson Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

Shahid Husain Multi-Organ Transplant Unit, Division of Infectious Diseases, University Health Network, Toronto, ON, Canada

Karim Yaqub Ibrahim Infectious Disease Clinical Division, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil

Dilek Ince Department of Internal Medicine, Division of Infectious Diseases, University of Iowa Hospitals, Iowa City, IA, USA

Michael G. Ison Division of Infectious Diseases and Organ Transplantation, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Shilpa R. Jain Transplant Infectious Diseases, University Health Network, Toronto, ON, Canada

Carrie A. Johnson Pulmonary, Allergy, and Critical Care Medicine, Duke University, Durham, NC, USA

Nassim Kamar Department of Nephrology, Dialysis and Organs Transplantation, CHU Rangueil, INSERM U1043, IFR –BMT, Paul Sabatier University, Toulouse, France

Aalok Khole Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, USA

Brian Kim Department of Medicine, Division of Gastrointestinal and Liver Diseases, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA

Camille Nelson Kotton Transplant and Immunocompromised Host Infectious Diseases, Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Deepali Kumar Transplant Infectious Diseases, University Health Network, Toronto, ON, Canada

Ricardo M. La Hoz Division of Infectious Diseases and Geographic Medicine, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

Anne Lachiewicz Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Rebekah J. Lane Departments of General Medicine and Infectious Disease, Auckland City Hospital, Auckland, New Zealand

Rachael A. Lee Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA

Jacob E. Lemieux Division of Infectious Disease, Massachusetts General Hospital, Boston, MA, USA

Marilyn E. Levi Health Systems Bureau, Division of Transplantation, Human Resources Services Administration, Rockville, MD, USA

Catherine Liu Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Division of Allergy and Infectious Diseases, University of Washington, Seattle, WA, USA

Rogelio López-Vélez National Referral Unit for Tropical Diseases, Infectious Diseases Department, Ramón y Cajal University Hospital, Madrid, Spain

Alfred Luk Department of Medicine, Adult Infectious Diseases Section, Tulane University School of Medicine, New Orleans, LA, USA

Clarisse Martins Machado Virology Laboratory (LIM 52 - HCFMUSP), Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil

HSCT Program – Amaral Carvalho Foundation, Jahu, Brazil

Israel Institute of Education and Research, Hospital Albert Einstein, São Paulo, Brazil

A. Mah Division of Infectious Disease, University of British Columbia, Vancouver, BC, Canada

Maricar Malinis Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA

Michael K. Mansour Transplant Infectious Diseases and Immunocompromised Host Service, Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA

Department of Medicine, Harvard Medical School, Boston, MA, USA

Olivier Marion Department of Nephrology, Dialysis and Organs Transplantation, CHU Rangueil, INSERM U1043, IFR –BMT, Paul Sabatier University, Toulouse, France

Pilar Martín-Dávila Transplant Infection Unit, Infectious Diseases Department, Ramón y Cajal University Hospital, Madrid, Spain

Marian G. Michaels Pediatric Infectious Diseases, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Rachel Miller Division of Infectious Diseases, Department of Internal Medicine, Duke University, Durham, NC, USA

Hilary Miller-Handley Division of Pediatric Infectious Diseases, Department of Infectious Disease, Cincinnati Children's Hospital and University of Cincinnati, Cincinnati, OH, USA

Omar H. Mohamedaly Pulmonary, Allergy and Critical Care Medicine, Duke University, Durham, NC, USA

Michele I. Morris Immunocompromised Host Service, Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, FL, USA

Paulo H. O. Mourão Infection Control and Hospital Epidemiology, Laboratory Medicine, Hospital das Clínicas, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil

Nicolas J. Mueller Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, Zürich, Switzerland

Kathleen M. Mullane Department of Medicine, Section of Infectious Diseases and Global Health, University of Chicago, Chicago, IL, USA

Elmi Muller Department of Surgery, University of Cape Town, Cape Town, South Africa

Sarah A. Myers Department of Dermatology, Duke University Medical Center, Durham, NC, USA

Deepa Nanayakkara Division of Infectious Diseases, City of Hope, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Yoichiro Natori Division of Infectious Disease, Department of Medicine, University of Miami Miller School of Medicine and Miami Transplant Institute, Miami, FL, USA

Jeremy Nel University of the Witwatersrand, Johannesburg, South Africa

Anoma Nellore Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA

Joanna Nelson Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Whitney J. Nesbitt Department of Pharmaceutical Services, Vanderbilt University Medical Center, Nashville, TN, USA

Brandon T. Nokes Division of Pulmonary, Critical Care, and Sleep Medicine, University of California, San Diego, CA, USA

Francesca F. Norman National Referral Unit for Tropical Diseases, Infectious Diseases Department, Ramón y Cajal University Hospital, Madrid, Spain

Andrew Nowalk Department of Pediatrics, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Gopi Patel Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Armelle Pérez-Cortés Villalobos Multi-Organ Transplant Unit, Division of Infectious Diseases, University Health Network, Toronto, ON, Canada

Steven A. Pergam Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center and University of Washington, Seattle, WA, USA

Lígia Camera Pierrotti Infectious Disease Clinical Division, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil

Jennifer C. Pisano Department of Medicine, Section of Infectious Diseases and Global Health, University of Chicago, Chicago, IL, USA

Stephanie M. Pouch Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

Timothy L. Pruett Department of Surgery, University of Minnesota, Minneapolis, MN, USA

Lorena Puchades Department of Gastroenterology, Hepatology Unit and Instituto de Investigación La Fe, Hospital Universitari i Politècnic La Fe, Valencia, Spain

Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Valencia, Spain

Alfredo G. Puing Division of Infectious Diseases, Department of Medicine, City of Hope National Medical Center, Duarte, CA, USA

Yoram A. Puius Division of Infectious Diseases, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY, USA

Meenakshi M. Rana Icahn School of Medicine at Mount Sinai, New York, NY, USA

Alejandro Restrepo Division of Infectious Diseases, Department of Internal Medicine, Baylor College of Medicine, Houston, TX, USA

Sanjay G. Revankar Division of Infectious Diseases, Wayne State University School of Medicine, Detroit, MI, USA

Florence Robert-Gangneux Univ Rennes, CHU Rennes, Inserm, EHESP, Irset UMR_S 1085, Rennes, France

Centre Hospitalier Universitaire de Rennes, Rennes, France

Study Group for Clinical Parasitology (ESGCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Basel, Switzerland

Joelle I. Rosser Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA

Gowri Satyanarayana Division of Infectious Diseases, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

Joanna Schaenman Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Annette Schlueter Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA, USA

Peter W. Schreiber Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland

Jennifer E. Schuster Division of Infectious Diseases, Children's Mercy Hospital, Kansas City, MO, USA

Shmuel Shoham Johns Hopkins University School of Medicine, Baltimore, MD, USA

Costi D. Sifri Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA, USA

Monica A. Slavin The National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Olivia Smibert Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Gregory Snell Lung Transplant Service, Alfred Hospital and Monash University, Melbourne, VIC, Australia

Lucia Sobrin Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA, USA

Alice Tung Wan Song Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Liver Surgery and Transplantation Laboratory LIM37, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Julie M. Steinbrink Division of Infectious Diseases, Duke University Medical Center, Durham, NC, USA

Peter Stock Department of Surgery, University of California, San Francisco, San Francisco, CA, USA

Erica J. Stohs Division of Infectious Diseases, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA

Aruna K. Subramanian Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA

Norah A. Terrault Department of Medicine, Division of Gastrointestinal and Liver Diseases, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA

Nicole M. Theodoropoulos Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, USA

Hao Tran Division of Cardiovascular Medicine, University of California San Diego, La Jolla, CA, USA

Sonya Trinh Department of Infectious Diseases, Ochsner Health, New Orleans, LA, USA

Elizabeth Tullis St Michael's Hospital, Department of Medicine, University of Toronto, Toronto, ON, Canada

Nicholas A. Turner Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, USA

Duke Center for Antimicrobial Stewardship and Infection Prevention, Durham, NC, USA

David van Duin Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Melodi Javid Whitley Department of Dermatology, Duke University Medical Center, Durham, NC, USA

Cameron R. Wolfe Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, USA

A. J. Wright Division of Infectious Disease, University of British Columbia, Vancouver, BC, Canada

Masaki Yamada Division for Advanced Medicine for Virus Infections, National Center Child Health and Development, Tokyo, Japan

Department of Pediatrics, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Tirdad T. Zangeneh Infectious Diseases, University of Arizona College of Medicine, Tucson, AZ, USA

Andrea Zimmer Division of Infectious Diseases, University of Nebraska Medical Center, Omaha, NE, USA

Richard A. Zuckerman Infectious Disease and International Health, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

Part I

General Introduction

1 Impact of Infection on Transplant Outcomes: Transplant Infectious Disease Expertise as an Essential Component of Transplant Programs

Michele I. Morris

Contents

Introduction 3
The Essential Role of Infectious Disease Specialists in the Care of Transplant Patients 4
Conclusion 8
References 8

Introduction

As an introduction to this textbook focused on emerging transplant infections, it is appropriate to begin with the emergence of the burgeoning field of transplant infectious diseases. Outcomes for both solid organ and hematopoietic stem cell transplantation have improved with the development of safer and more effective immunosuppression. Techniques in organ procurement and preservation as well as methods for hematopoietic stem cell harvest and infusion have evolved, contributing to significant expansion in the availability and safety of transplant. As transplant procedures have become more routine and better standardized, improved access to transplantation services internationally has expanded the number of potential recipients and the variety of infectious complications. Most recently, recognition of the relative importance of infection prevention and treatment in overall transplant success has reinforced the essential role of transplant infectious diseases subspecialists in the management of solid organ and hematopoietic stem cell transplant candidates and recipients.

M. I. Morris (✉)
Immunocompromised Host Service, Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, FL, USA
e-mail: mmorris2@med.miami.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_1

The Essential Role of Infectious Disease Specialists in the Care of Transplant Patients

Infectious complications are now a more frequent cause of hospitalization after solid organ transplant than allograft rejection, largely due to the use of more efficacious and potent immunosuppressive therapies [1]. In a review of 1414 hospital admissions of 531 solid organ transplant recipients at a tertiary transplant center, infectious disease complications were identified in 603 (43%) of the admissions impacting 306 (58%) of the patients studied with an associated 10% inhospital mortality [2]. The major cause of mortality in hematopoietic stem cell transplant recipients is relapse of the primary disease, but non-relapse mortality (NRM) is frequently attributed to infection. The Center for International Blood and Marrow Transplant Research (CIBMTR) reported mortality data for US stem cell transplant recipients undergoing transplant in 2015–2016. Overall mortality within the first 100 days of allogeneic transplant was attributed to infection in 19% of HLA-matched sibling donor recipients and 21% of unmatched donor recipients [3]. A study of over 15,000 adult and pediatric stem cell transplant recipients who survived at least 2 years post-allogeneic transplant found infection to be a primary or contributory cause of mortality in 30% [4].

In both solid organ and hematopoietic stem cell recipients, the net state of immunosuppression significantly influences the risk of infectious complications. In addition to immunosuppressive medications, the patient's immune status is altered by other therapeutic interventions including surgical procedures and postoperative complications, the presence of indwelling catheters and drains, antineoplastic chemotherapy, and antimicrobial treatment. The overall level of immune dysfunction is also impacted by concomitant diseases such as diabetes, cirrhosis, renal dysfunction, genetic immunodeficiencies, and autoimmune conditions, as well as the presence of infections including cytomegalovirus, HIV, and hepatitis B and C. Immune modulation inherent to the transplant process may cause cytopenias which predispose to infection. Finally, the presence of allograft rejection and/or graft versus host disease alters the immune response of the transplant recipient well in advance of the effects of immunomodulatory therapies used to control these conditions. In the face of this multilayered attack on the immune defenses, a variety of opportunistic pathogens often emerge that require the assistance of experts in infectious disease diagnosis and treatment.

The importance of general infectious disease (ID) expertise in the successful management of common bacterial infections is well recognized. Multiple published studies from throughout the world have reported a significantly lower mortality rate in patients with *Staphylococcus aureus* bacteremia who received ID consultative care [5–11]. All of these studies included a significant immunocompromised population, with a variety of solid organ transplant and hematologic malignancy patients as well as others receiving cancer chemotherapy. Reported improvements in outcome were so profound that many hospitals have implemented mandatory infectious disease consultation for patients with *Staphylococcus aureus* bacteremia [12]. Improved survival has also been seen with early infectious disease (ID) consultation

in patients with candidemia [13, 14]. The benefits of ID involvement have been observed in the treatment of sepsis as well. In a study of emergency department management of severe sepsis, early involvement of infectious disease consultants was associated with increased identification of an etiologic diagnosis for sepsis (9% vs. 42%, $p < 0.001$) and more appropriate empiric antibiotic therapy (30% vs. 79%, $p < 0.001$) [15]. A review of Medicare claims submitted on behalf of over 200,000 hospitalized beneficiaries with a diagnosis of infection evaluated the impact of ID consultation on patient mortality, utilization of resources, and overall costs of care. This study found that the patient cohort treated with ID consultation demonstrated lower mortality (odds ratio 0.87) and fewer readmissions to the hospital (odds ratio 0.96) with no increase in Medicare charges and a 3.7% decrease in ICU length of stay. ID involvement within the first 2 hospital days was associated with lower 30-day mortality, 3.8% shorter overall and 5.1% shorter ICU hospitalizations, decreased readmission, and lower Medicare costs [16]. A French study of 4188 patient hospitalizations using the French national database revealed that patients receiving care in the ID ward had significantly lower mortality, shorter length of stay, and lower hospitalization costs [17].

The influence of infectious disease specialists on patient outcomes extends beyond the bedside due to the crucial role ID experts play in infection prevention. Medical center antimicrobial stewardship and infection control programs are of particular benefit to immunocompromised transplant candidates and recipients who often experience prolonged or recurrent hospitalization and are most vulnerable to infection. Numerous studies demonstrate the value of ID intervention in controlling antimicrobial resistance by lowering inappropriate antimicrobial use and implementing appropriate infection control interventions [18]. Active participation of transplant infectious disease specialists as leaders in infection prevention activities may improve transplant physician engagement and significantly benefit transplant outcomes [19].

Solid organ transplant patients with bacteremic sepsis are noted to have lower associated mortality than non-transplant patients, a finding that was attributed to immunomodulation of the inflammatory response due to immunosuppressive therapy [20]. This interpretation of the study findings was disputed in a letter that cited the importance of ID consultants as an integral part of the multidisciplinary transplant team, which may have improved access to early subspecialty care and could potentially impact sepsis outcomes in this population [21]. A Canadian study of transplant center admissions showed that ID consultation provided to solid organ transplant recipients was associated with increased hospital stay and costs but decreased 28-day mortality (hazard ratio 0.33) and readmission rates (16.9% vs. 23.9%). In the same study, early ID referral (within 2 days of admission) was found to reduce median length of stay and hospitalization costs [2].

A review of the specific benefits provided by ID participation in patient care attributed the improvement to specific diagnostic and therapeutic areas of expertise. These include the achievement of a more rapid diagnosis through the performance of a detailed history and physical examination and targeted use of laboratory testing, followed by timely initiation of appropriate antimicrobial therapy. Cognitive specialties such as ID have frequently been utilized through curbside consultation

without face-to-face direct patient interaction. Although informal telephone consultations may facilitate access to ID expertise in medical centers with limited staffing, the benefits afforded by ID involvement in patient care may be reduced. In one Finnish study of patients with *Staphylococcus aureus* bacteremia, clinical management incorporating bedside ID specialty consultation was associated with shorter fever duration ($p = 0.003$) and decreased mortality at 7, 28, and 90 days ($p = 0.001$, 0.001, <0.001, respectively) when compared with care supported through telephone ID consultation [22]. In addition to providing expedited diagnosis and treatment, ongoing infectious disease management by ID specialists may ensure adherence to the treatment plan and facilitate monitoring for drug-drug interactions and treatment-related toxicities. ID faculty may also provide support for the delivery of outpatient parenteral antimicrobial therapy to transplant recipients, which can shorten hospital stays and reduce costs [23].

It remains to be seen whether remote consultation through the use of electronic medical records will be effective in extending access to transplant ID expertise in areas that lack such ID specialty support. A recent study of electronic ID consultation in the US Veterans Administration system found that electronic consultations actually generated an increase in overall consult volume, with more rapid access to ID specialists supporting the time-sensitive clinical decisions often required in infection management [24]. As healthcare systems increasingly seek to manage patient populations in an effort to improve patient care and lower costs, outcomes data is needed to define the appropriate role for electronic consultation [25]. A systematic review of the use of provider to provider electronic consultation including literature from five countries concluded that further research is required to define the correct methods for implementation and safety and efficacy monitoring when clinical management is delivered remotely [26]. The allocation of remote access to the electronic medical records of transplant recipients has the potential to extend transplant center specialty care to patients who live far from major centers while insuring more accurate communication and comprehensive data transfer.

Expansion of the reach of transplant ID care is an emerging topic of interest, as the benefit of ID specialty care is not confined to major transplant centers. Despite the limited availability of trained ID specialists in many countries, there is growing worldwide recognition of the value of such expertise, including a detailed justification for the expansion of ID services in Germany medical centers [27]. In the United States, the national certification for transplant center approval and reapproval includes a requirement for access to ID specialists as an essential member of the clinical team [28]. Subspecialty training in transplant ID is now available at over a dozen centers in North America [29] with a recommended curriculum that includes training in the care of infections in solid organ and hematopoietic stem cell transplant recipients and frequently incorporates instruction in the management of infectious complications of cancer chemotherapy and mechanical circulatory support devices [30].

ID specialists have been recognized for their contributions to cancer care, including patients with hematologic malignancies and hematopoietic stem cell transplant recipients. Infectious complications cause half of all deaths in patients with acute myeloid leukemia (AML) and are a significant consideration for the timing of

consolidative chemotherapy and hematopoietic stem cell transplant in this population. Ninety five percent of AML patients experience at least one episode of neutropenic fever [31]. Inexperienced management may delay future chemotherapy leading to fatal disease progression. Close collaboration between hematologists and ID specialists familiar with leukemia care is now a recognized standard for a successful leukemia service [32]. A single-center retrospective review of outpatient ID consultative practice in a comprehensive cancer center found that ID specialists identified alternative diagnoses in 53% of consult patients, with 46% found to have a different infectious disease. Nearly one-third of patients (29%) were noted to have a noninfectious etiology for their clinical presentation. ID involvement was determined to significantly contribute to patient care in 62% of the 598 patient charts evaluated [33]. A review of ID specialty impact that included both inpatient and outpatient oncology practice data noted benefits including up to 34% increase in adherence to clinical guidelines, up to 52% increase in prescription of appropriate antimicrobial therapy duration, and up to 17% decrease in mortality [34]. The value of ID consultants in supporting transition of care after hospital discharge was also highlighted, along with their role in the prevention of recurrent and future infections.

The reemergence of vaccine preventable viruses such as measles in previously low-risk populations has underscored the importance of pre-transplant screening and vaccination of transplant candidates. Vaccination status should be reviewed and updated whenever possible, particularly in solid organ transplant candidates likely to respond prior to initiation of immunosuppression. In a study of 393 patients awaiting kidney or kidney pancreas transplant, those receiving ID care were significantly more likely to receive appropriate pre-transplant immunization [35]. Pre-transplant consultation also provides an opportunity to engage patients and their families in future infection prevention and safe living techniques more generally, including targeted counseling for the avoidance of identified microbial threats [36]. Consultation can also include travel medicine advice directed specifically to immunocompromised patients.

Recognition of the value that transplant ID specialists provide to a program is crucial, particularly since many infection prevention initiatives such as protocol development and antimicrobial stewardship cannot be financed through direct patient billing. A study of institutional support of ID specialists in North America found great variation between transplant centers, with a trend toward increased support in larger programs. In centers performing over 250 transplants annually, 80% of programs provided some financial support not obtained through individual patient care charges. Transplant ID programs were in place at 36 of the 42 transplant centers that participated in the study. The inclusion of ID subspecialists as part of the interdisciplinary transplant team was recognized in this study as the standard of care [37]. In an era in which post-transplant infections are a major factor impacting reportable metrics such as allograft and patient survival after transplantation, ID expertise is instrumental to the success of any transplant program. In this rapidly changing area of medical practice, ID specialists possess unique skills required to update transplant teams on the infection risks associated with novel immunosuppressants and the emerging pathogens that may harm patients receiving such therapies.

Among the challenges of transplant ID practice is the paucity of randomized clinical trials of infection management in the immunocompromised transplant population. Much of clinical care is based on single-center observational studies, individual case reports, and expert opinion. This is especially true for newer surgical and immunosuppressive techniques and also emerging biothreats. Guidance documents such as the latest edition of the solid organ transplant infectious diseases guidelines endorsed by the American Society of Transplantation's ID Community of Practice and published in 2019 in a special issue of Clinical Transplantation are invaluable as information sources [38]. Transplant ID specialists are frequently tasked to develop center specific protocols for infection screening and management focused on local exposure risk and practice. Protocol development and standardization of practice can lower patient morbidity and mortality, but infectious disease practice is constantly changing [18]. The ongoing emergence of new and increasingly resistant infectious pathogens such as *Candida auris* and the worldwide spread of the SARS-CoV-2 virus and other treatment resistant viruses are good examples. Expansion of older threats like influenza and measles disproportionately impacts immunocompromised populations. Climate change and global warming accelerate vector expansion, thereby enhancing disease spread. Changing immigration patterns and worldwide travel have erased the borders that previously protected transplant recipients from exposure to exotic geographically restricted organisms. Recognition of the unique and sometimes atypical presentations of such rare infections in an immunosuppressed transplant patient is an essential part of modern transplant practice. All of these factors mandate the development of a textbook devoted to *Emerging Transplant Infections*.

Conclusion

This collection will highlight the cutting-edge information required to provide transplant care today. Each chapter is focused on a unique problem or pathogen-facing transplant professionals. The international team of experts have been tasked with creating a concise overview of the latest knowledge in the field of transplant infectious diseases. The outstanding work of these well-recognized professionals will be a contribution to this emerging subspecialty, but no textbook can ever replace the value of bedside consultation with transplant ID specialists. As clinical practice evolves and new microbiologic threats emerge, this living textbook can provide a roadmap to guide ID specialists in the care of their most challenging and vulnerable patients.

References

1. Dharnidharka VR, Stablein DM, Harmon WE. Post-transplant infections now exceed acute rejection as cause for hospitalization: a report of the NAPRTCS. Am J Transplant. 2004;4(3):384–9.

2. Hamandi B, Husain S, Humar A, Papadimitropoulos EA. Impact of infectious disease consultation on the clinical and economic outcomes of solid organ transplant recipients admitted for infectious complications. Clin Infect Dis. 2014;59(8):1074–82.
3. D'Souza A, Fretham C. Current uses and outcomes of hematopoietic stem cell transplantation (HCT): CIBMTR summary slides; 2018. https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/pages/index.aspx. Accessed 14 Aug 2020.
4. Norkin M, Shaw BE, Brazauskas R, Tecca HR, Leather HL, Gea-Banacloche J, et al. Characteristics of late fatal infections after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2019;25(2):362–8.
5. Bai AD, Showler A, Burry L, Steinberg M, Ricciuto DR, Fernandes T, et al. Impact of infectious disease consultation on quality of care, mortality, and length of stay in *Staphylococcus aureus* bacteremia: results from a large multicenter cohort study. Clin Infect Dis. 2015;60(10):1451–61.
6. Honda H, Krauss MJ, Jones JC, Olsen MA, Warren DK. The value of infectious diseases consultation in *Staphylococcus aureus* bacteremia. Am J Med. 2010;123(7):631–7.
7. Kaech C, Elzi L, Sendi P, Frei R, Laifer G, Bassetti S, et al. Course and outcome of *Staphylococcus aureus* bacteraemia: a retrospective analysis of 308 episodes in a Swiss tertiary-care centre. Clin Microbiol Infect. 2006;12(4):345–52.
8. Lahey T, Shah R, Gittzus J, Schwartzman J, Kirkland K. Infectious diseases consultation lowers mortality from *Staphylococcus aureus* bacteremia. Medicine. 2009;88(5):263–7.
9. Nagao M, Iinuma Y, Saito T, Matsumura Y, Shirano M, Matsushima A, et al. Close cooperation between infectious disease physicians and attending physicians can result in better management and outcome for patients with *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect. 2010;16(12):1783–8.
10. Rieg S, Peyerl-Hoffmann G, de With K, Theilacker C, Wagner D, Hubner J, et al. Mortality of *S. aureus* bacteremia and infectious diseases specialist consultation – a study of 521 patients in Germany. J Infect. 2009;59(4):232–9.
11. Robinson JO, Pozzi-Langhi S, Phillips M, Pearson JC, Christiansen KJ, Coombs GW, et al. Formal infectious diseases consultation is associated with decreased mortality in *Staphylococcus aureus* bacteraemia. Eur J Clin Microbiol Infect Dis. 2012;31(9):2421–8.
12. Tissot F, Calandra T, Prod'hom G, Taffe P, Zanetti G, Greub G, et al. Mandatory infectious diseases consultation for MRSA bacteremia is associated with reduced mortality. J Infect. 2014;69(3):226–34.
13. Farmakiotis D, Kyvernitakis A, Tarrand JJ, Kontoyiannis DP. Early initiation of appropriate treatment is associated with increased survival in cancer patients with *Candida glabrata* fungaemia: a potential benefit from infectious disease consultation. Clin Microbiol Infect. 2015;21(1):79–86.
14. Ishikane M, Hayakawa K, Kutsuna S, Takeshita N, Ohmagari N. The impact of infectious disease consultation in candidemia in a tertiary care hospital in Japan over 12 years. PLoS One. 2019;14(4):e0215996.
15. Viale P, Tedeschi S, Scudeller L, Attard L, Badia L, Bartoletti M, et al. Infectious diseases team for the early management of severe sepsis and septic shock in the emergency department. Clin Infect Dis. 2017;65(8):1253–9.
16. Schmitt S, McQuillen DP, Nahass R, Martinelli L, Rubin M, Schwebke K, et al. Infectious diseases specialty intervention is associated with decreased mortality and lower healthcare costs. Clin Infect Dis. 2014;58(1):22–8.
17. Brouqui P, Jouve E, Romain F, Sambuc R, Raoult D. Are infectious disease doctors better at caring for infectious diseases than other specialists? Clin Infect Dis. 2014;58(10):1486–7.
18. McQuillen DP, MacIntyre AT. The value that infectious diseases physicians bring to the healthcare system. J Infect Dis. 2017;216(Suppl 5):S588–S93.
19. Seo SK, Lo K, Abbo LM. Current state of antimicrobial stewardship at solid organ and hematopoietic cell transplant centers in the United States. Infect Control Hosp Epidemiol. 2016;37(10):1195–200.

20. Kalil AC, Syed A, Rupp ME, Chambers H, Vargas L, Maskin A, et al. Is bacteremic sepsis associated with higher mortality in transplant recipients than in nontransplant patients? A matched case-control propensity-adjusted study. Clin Infect Dis. 2015;60(2):216–22.
21. Bahr NC, Beaudoin A, Drekonja D. Rapid access to comprehensive care may explain better outcomes in persons with sepsis with solid organ transplant versus those without solid organ transplant. Clin Infect Dis. 2015;60(12):1869–70.
22. Forsblom E, Ruotsalainen E, Ollgren J, Jarvinen A. Telephone consultation cannot replace bedside infectious disease consultation in the management of *Staphylococcus aureus* bacteremia. Clin Infect Dis. 2013;56(4):527–35.
23. Harrison J, Hossain MA, Morsy M, Ghazanfar A. Outpatient parenteral antibiotic therapy in a renal transplant population: a single-center experience. Saudi J Kidney Dis Transpl. 2015;26(6):1121–9.
24. Strymish J, Gupte G, Afable MK, Gupta K, Kim EJ, Vimalananda V, et al. Electronic consultations (e-consults): advancing infectious disease care in a large veterans affairs healthcare system. Clin Infect Dis. 2017;64(8):1123–5.
25. McQuillen DP, Schmitt SK. Declare the past, diagnose the present, foretell the future: electronic consultations in infectious disease practice. Clin Infect Dis. 2017;64(8):1126–8.
26. Vimalananda VG, Gupte G, Seraj SM, Orlander J, Berlowitz D, Fincke BG, et al. Electronic consultations (e-consults) to improve access to specialty care: a systematic review and narrative synthesis. J Telemed Telecare. 2015;21(6):323–30.
27. Rieg S, Kupper MF. Impact of an infectious diseases consultation service on the quality of care and the survival of patients with infectious diseases. Z Evid Fortbild Qual Gesundhwes. 2015;109(7):500–10.
28. Centers for Medicare & Medicaid Services (CMS), HHS. Requirements for approval and re-approval of transplant centers to perform organ transplants. Fed Regist. 2007;42:CFR parts 405, 482, 488, and 498(15205).
29. IDCOP. Fellowship training opportunities in transplant infectious diseases. American Society of Transplantations (AST) website. 2019. https://www.myast.org/communities-practice/infectious-disease/fellowship-training-opportunities-transplant-infectious
30. Avery R, Clauss H, Danziger-Isakov L, Davis J, Doucette K, van Duin D, et al. Recommended curriculum for subspecialty training in transplant infectious disease on behalf of the American Society of Transplantation infectious diseases Community of Practice Educational Initiatives Working Group. Transpl Infect Dis. 2010;12(3):190–4.
31. Cannas G, Pautas C, Raffoux E, Quesnel B, de Botton S, de Revel T, et al. Infectious complications in adult acute myeloid leukemia: analysis of the Acute Leukemia French Association-9802 prospective multicenter clinical trial. Leuk Lymphoma. 2012;53(6):1068–76.
32. Mulanovich V, Kontoyiannis DP. Acute myeloid leukemia and the infectious diseases consultant. Leuk Lymphoma. 2018;59(6):1284–91.
33. Pongas G, Hamilos G, Rolston KV, Kontoyiannis DP. Formal adult infectious disease specialist consultations in the outpatient setting at a comprehensive cancer center (1998–2008): diverse and impactful. Support Care Cancer. 2012;20(2):261–5.
34. Granwehr BP, Kontoyiannis DP. The impact of infectious diseases consultation on oncology practice. Curr Opin Oncol. 2013;25(4):353–9.
35. Kasper AK, Pallotta AM, Kovacs CS, Spinner ML. Infectious diseases consult improves vaccination adherence in kidney transplant candidates. Vaccine. 2018;36(34):5112–5.
36. Ariza-Heredia EJ, Kontoyiannis DP. Our recommendations for avoiding exposure to fungi outside the hospital for patients with haematological cancers. Mycoses. 2014;57(6):336–41.
37. Schaenman JM, Kumar D, Kotton CN, Danziger-Isakov L, Morris MI. Transplant center support for infectious diseases. Transpl Infect Dis. 2017;19(5). https://doi.org/10.1111/tid.12746.
38. Special issue – Transplant infectious diseases clinical transplantation: Wiley online library. 2019. https://onlinelibrary.wiley.com/journal/13990012

Introduction to Immunosuppression, Tolerance, and Infection Risk

2

Cameron R. Wolfe

Contents

Introduction 11
T-Cell, B-Cells, and the Innate Immune System Involvement in Rejection 13
Other Elements of the Immune System Involved in Transplantation 16
Therapeutics Impacting Immunosuppression 17
Calcineurin Inhibitors 17
Antiproliferative Agents 19
mTOR Inhibitors 19
Corticosteroids 19
Co-stimulation Blockade 20
Induction Therapies 20
Anti-CD25 Monoclonal Antibodies 20
Anti-CD52 Monoclonal Antibodies 21
Polyclonal Antibodies 21
Conclusion 21
Key Points 22
References 22

Introduction

The human immune system is arguably one of the most complex, multifaceted systems in the human body, comprising not only fixed, evolutionarily older components, known as the innate immune system, but also adaptable arms capable of learning and remembering pathogens. These are commonly referred to as the adaptive immune system. For both hematopoietic stem cell transplantation (HSCT) and solid organ transplantation (SOT), as our understanding has grown of the roles the immune system plays in graft rejection, tolerance, and infection, so too have our

C. R. Wolfe (✉)
Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, USA
e-mail: Cameron.Wolfe@duke.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_2

techniques been refined to try to strike a balance with therapeutic immunosuppression. We now recognize that rejection can be broadly divided into acute versus chronic rejection, and cellular versus antibody mediated rejection, although there is both overlap and interaction between each. Understanding the evolution of our immune system, the different pathways by which stimulation and proliferation occur, and the different timelines at play, can help us ensure our patients get the most out of their transplant.

Almost 70 years ago, when deceased donor kidney transplant was first attempted, organ rejection was rapid, given limited understanding of rejection, and no therapeutic avenues to impede the alloimmune response [1, 2]. In fact, initially, the only transplants that were successful for any length of time were between genetically identical donor and recipient pairs, such as the transplant between the Herrick twin brothers in 1954 in Boston [3]. Not long after that T cells were recognized as the major effector cells engaging the graft directly, leading to rejection. T-cell knockout mouse models were able to demonstrate that fully mismatched solid organ allografts underwent little or no rejection. When T cells were reconstituted, rejected ensued.

There are three key elements to any immunological decision faced by the human immune system. Each of these is areas for potential control in SOT, to prevent organs being rejected, and to ultimately allow tolerance. Firstly, cellular "context," that is, how efficiently adhesion molecules bind immune cells to foreign material, and how much co-stimulation exists allowing the response to be cemented. Secondly, "specificity," that is, an antigen's affinity for immune recognition after which activation begins. Finally, the "magnitude," that is, how much cytokine enhancement is present and therefore the degree by which immune activation is amplified.

Regarding specificity, immune recognition of foreign antigen is incredibly broad, especially via T-cell receptors. It evolves early in our life and yet remarkably allows for self-recognition. This usually occurs in the thymus, where immature thymocytes from the bone marrow undergo random reassortment of their TCR genes, and then checking for self-recognition. Cells that do not interact with the MHC complex and thereby are ineffective for the purpose of surveillance are apoptosed. Subsequently, cells with high-affinity for self-recognition are also destroyed, thereby minimizing risk of auto-immunity and leaving only cells that are active, and primed to recognize anything else.

In regards to the "context" of an immune system interaction, binding of any foreign material – specifically donor tissue in the case of organ transplant – to the recipient's immune system should not be viewed as a static process. Binding can be enhanced or restricted by inflammation. It can also be pharmacologically blocked by co-stimulation blocking drugs, thereby altering the magnitude of any immune response, even when recognition of foreign material is underway.

Time and recipient age also impacts immune system responsiveness, and therefore likelihood of rejection. Over time, "immunosenescense" begins – this is characterized by reduced thymus activity and increased reliance on homeostatic proliferation, that is, the response to things seen before, rather than the ability to respond robustly to new foreign entities. This leaves the immune system both prone to greater infection, but also potentially more tolerant of the foreign antigen

challenge posed by transplantation [4]. In another way, the naïve (hard to activate) immune system of a patient's youth moves to the memory (easy to activate and strong effector) state of our adulthood, to the exhausted (easy to activate but largely impotent) immune state of our elder years. The impact on rejection and tolerance is therefore very different at different stages of our life.

Finally, the immune system and its responsiveness is impacted by many other variables, for example, loss of regenerative capacity as we age, infections and vaccines priming our memory, trauma to a solid organ graft itself, allowing inflammation and accelerated immunologic recognition to name a few.

T-Cell, B-Cells, and the Innate Immune System Involvement in Rejection

Alloimmunity is the process by which our adaptive immune system recognizes self, and also targets non-self-antigens. It is, therefore, the focus of the majority of antirejection efforts posttransplant. T-cell function plays the most significant role in alloimmunity, and therefore rejection, although B-cells and other adaptive antigen presenting cells (APC) also play a significant role in the identification and presentation of foreign material to the immune system. While there are two major alloantigens that we respond to, ABO blood group antigens and human leucocyte (HLA) antigens, this chapter will focus on the HLA interactions, as they appear the most significant for transplant [5].

Foreign antigen is usually recognized shortly after a solid organ transplant is reperfused. At that point, alloantigen is gathered and processed, typically by macrophages acting as an APC, but occasionally by other cells including dendritic cells and vascular endothelial cells [6, 7]. Presentation to the adaptive immune system occurs usually through 3 pathways: first, the CD3 complex on T-lymphocyte surfaces are engaged through T-cell receptor-major histocompatibility-allopeptide complex engagement (see Fig. 1). The second pathway occurs via cellular co-stimulation. Specifically, CD80/86 on an APC binds antigen to CD28 on a T-cell. Both pathway 1 and 2 result in positive signaling through the calcineurin-calcium pathway, in addition to upregulated NF-κβ and protein kinase signaling. These, in turn, lead to more interleukin-2 and other cytokines produced by the now activated T-cell. Negative feedback loops do exist to prevent overstimulation and ongoing ultimately damaging proliferation. For example, there are increased surface inhibitory molecules expressed on cytotoxic t-lymphocyte (specifically, "antigen 4," or CTLA-4) on an activated T-cell, which have a higher affinity to CD80 and 86, thereby reducing costimulation through CD28, and eventually bringing proliferation under control [8]. With the advent of many new "checkpoint inhibitors" that seek to take the harness off the immune system in cancer, we may see new avenues of immunomodulation enter the transplant space, although at this time, their role is unclear. A final pathway exists, whereby secreted IL-2 binds to the IL-2R on the surface of T-lymphocytes. This activates the mammalian target of rapamycin (mTOR), which in turn regulates cellular proliferation and amplification.

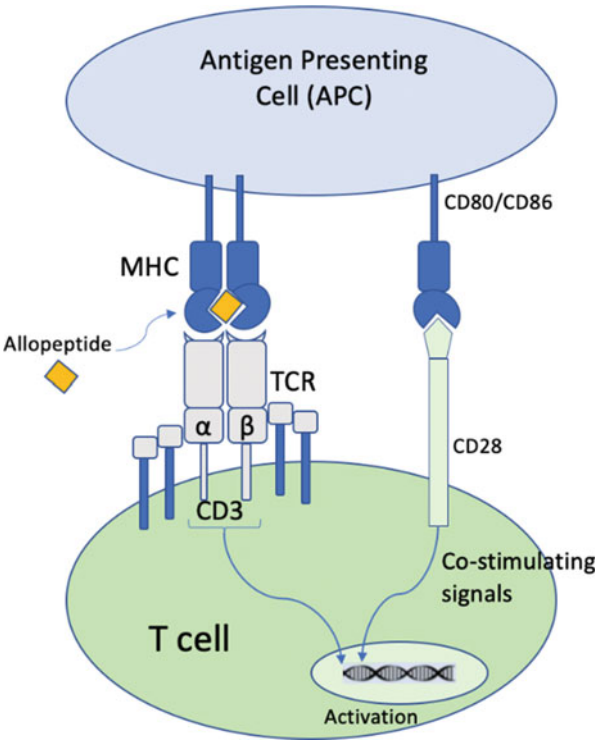


Fig. 1 T-cell receptor-major histocompatibility-allopeptide complex

Allorecognition also occurs via both donor and recipient APCs. Donor APCs within a graft can migrate out to present donor antigen to the recipient's immune system. This is sometimes referred to as "direct allo-recognition." Whereas when the recipient's antigen presenting cells penetrate the donor tissue, an MHC-II response occurs, with more intense reactivity resulting.

T-cell mediated rejection is most likely in the first year after transplant and diminishes thereafter, as graft tolerance develops. Risk factors for cellular rejection include presensitization (where the presence of donor-specific antibodies (DSAs) already exist, or where a recipient's propensity to react against a wide variety of human population tissue is already high (measured by "panel reactive antibody" or PRA). Additional factors include African-American ethnicity, prolonged cold ischemia time (see below), delayed graft function, and recipient age 18 years of age or younger. Finally, those with a previous episode of rejection, patients receiving their second transplant, and those who are noncompliant with medication are all at greater risk [9].

B cells also typically become upregulated in rejection. When a B-cell encounters antigen bound to complement, particularly in the presence of activated T-lymphocytes, the antigen can be internalized by the B-cell Receptor on the cell surface, fragmented, and returned to the surface via CD40 receptors. With the help of

T-lymphocytes (specifically the CD40-Ligand on their surface), B cells become activated, proliferate, and differentiate under the influence of IL-4, 5 and 6 [5]. B cell proliferation leads to direct differentiation into the relatively short-lived plasma cell, whose principal function is the amplified production of antibody. Ultimately therefore the end point of B-cell involvement in the immune response to transplant is to increase antibody-mediated rejection risk.

When acute antibody mediated rejection is occurring, antibodies typically bind to endothelium within the graft, thereby triggering further immune system activation through complement-dependent and independent mechanisms. Complement activation in turn recruits NK cells, neutrophils, and macrophages into graft tissue, leads to enhanced platelet aggregation, and ultimately generates capillaritis, thrombotic microangiopathy, and tissue destruction with a reduction in function of the graft. Although the clinical appearance of antibody-mediated rejection may appear different in different tissues, the immunologic pathway is the same. In kidney transplant, for example, glomerulitis is the most visible endpoint. In a lung transplant, acute capillaritis is also seen, although diffuse alveolar damage or organizing pneumonitis can also be present, mimicking infection [10]. Although there are acute and chronic forms of antibody mediated rejection, they present differently across the spectrum of SOT tissue types and are beyond the scope of this chapter.

Ultimately, more so than B-cells, T-lymphocytes are both sufficient and necessary for rejection of allogenic tissues, both solid organ and HSCT. Patients that are at high risk for T cell-mediated rejection (TCMR) are also at high risk for antibody-mediated rejection (ABMR).

Consequently, most antirejection focus is appropriately directed towards their control (refer to later in this chapter, and also to ▶ Chap. 3, "Changing Definition of Immunosuppression: Targeted Therapies and Resulting Emerging Infections and Their Prevention," by Nicolas J. Mueller for further details). However, the innate immune system does play a role, in fact one that can present earlier than the T cell response. In reality, the innate and adaptive immune systems are involved in a complex multifaceted interaction that is beyond the focus of this chapter.

One area where the innate immune system is particularly important for SOT is the injury and rejection associated with cold-time ischemia and reperfusion damage. Procurement injury and ischemia-reperfusion injury occur to varying degrees in all grafts, more for those with longer "cold-times" out of the body or with inadequate tissue perfusion during a donor's terminal illness. As a result of these antigen-independent insults and the consequent cellular damage in the grafted organ, a number of chemicals signaling "danger" to the primitive elements of the immune system are released. These include heparan sulfate, hyaluronan, heat-shock proteins, and the HMGB1 (high mobility group box 1) protein from within the nucleus. Such danger signals activate toll-like receptors (TLRs) and the pro-inflammatory RAGE (Receptor for Advanced Glycation End-products) that are found on donor dendritic cells (which act as APCs) thereby enhancing the subsequent adaptive immune response previously described. Expanding the discussion of Toll-like receptors (TLRs), these are in fact seen in multiple innate cells including macrophages,

epithelial cells and neutrophils, as well as dendritic cells. They recognize a variety of pathogen-associated molecular proteins, many common in transplant (viral hepatitis, cytomegalovirus (CMV), respiratory syncytial virus, herpes simplex virus, etc.) but can also help regulate rejection [11]. As more has been learned about the role of TLR's in graft regulation, especially when combined with clinical data about longer term sequelae, it became clear that the response to injury sustained during the transplant process was directly tied to subsequent allograft rejection and therefore should be minimized if at all possible [12, 13].

Other Elements of the Immune System Involved in Transplantation

We have a more nascent understanding of a number of the elements of the immune system, as they pertain to rejection and tissue tolerance. NK cells are one of the cellular arms of the innate immune system involved in rejection of transplanted tissue. Although technically lymphocytes, they lack the adaptive elements associated with the thymically nurtured T cell receptor. After a transplant, NK-cells are rapidly activated and contain KIR (killer cell immunoglobulin-like receptors) on their surface which regulate the killing activity of the cell; with more binding to self-aligned receptors on the graft tissue, KIR cannot activate the killing function of the NK cell. With less binding, that is, through a missing-self strategy, activation of cell occurs leading to tissue destruction [14]. NK cells typically act indirectly on their target cells, inducing apoptosis via granzyme and perforin release. Furthermore, NK secrete proinflammatory cytokines – specifically IFN-γ and TNF- α to increase expression of APC MHC molecules and costimulation receptors. This has the effect of promoting APC maturation and thereby amplification of T cell alloreactivity.

However, the precise role of NK cells in solid organ rejection is not as clear as their T cell cousins [15]. Firstly, there is some data to support the ability of NK cells to tilt the balance of overall Th1 (proinflammatory) and Th2 (regulatory) response, thereby actually promoting tolerance under certain circumstances. They can do this by focusing their toxic cytokine response on APCs themselves, thereby decreasing drive to alloreactivity [16]. Interestingly, standard immunosuppression appears not to have as much impact on NK cell activity as it does on T cells; we do not have enough understanding of the impact of novel therapeutics on NK function and number to consider directed therapy at this time.

Technically, the human microbiome is also part of innate immunity, providing host defense through the breadth and magnitude of microbial flora. It also helps modulate the balance between inflammation and immune homeostasis in both the nontransplanted and allografted patient [17]. Frequently during HSCT and SOT, the microbiome is damaged, either through the direct impact of antimicrobials, or as a result of surgical techniques and immunosuppression. Current research efforts designed to improve understanding of the role of the microbiome in regulating rejection and tolerance are ongoing.

Therapeutics Impacting Immunosuppression

Perhaps the easiest way to think about therapeutic immunosuppression as it pertains to SOT is to break up interventions into those occurring at the time of transplant, typically known as "induction therapies," and those occurring under more chronic situations, known as "maintenance therapy." In the sphere of HSCT, typically maintenance therapy is instituted with an aim of reducing the transplanted bone marrow's recognition of the host. Many new therapeutics and biologic agents are being developed that act on very focused sections of the immune system. Some of these are discussed in ► Chap. 3, "Changing Definition of Immunosuppression: Targeted Therapies and Resulting Emerging Infections and Their Prevention."

Induction agents are generally used to overwhelm the immune system for a short period of time; they are usually administered immediately prior to and just after transplantation. Their goal is to reduce acute rejection, typically in "high-risk" patients, as described earlier.

Cellular rejection therapy most commonly also mimics induction agents, with high-dose steroids followed by antibody therapy being the mainstay of most programs.

Common induction agents include corticosteroids (methylprednisone), polyclonal antibodies (antithymocyte globulin, antilymphocyte globulin), and monoclonal antibodies (alemtuzumab, basixilimab).

Maintenance agents typically begin shortly after transplant, are typically tapered steadily over the first 6–12 months after SOT, and try to strike a long term balance between rejection risk, infection risk, and side effect profile. Precise dosing is often required to achieve that balance, and therapeutic drug monitoring is often employed, often with the help of transplant pharmacists (Fig. 2). Common induction agents also include corticosteroids (prednisone), but additionally calcineurin inhibitors (tacrolimus, cyclosporine), antiproliferative agents (mycophenolate mofetil, azathioprine), mTOR inhibitors (sirolimus, everolimus) and co-stimulation blockers (belatacept).

Calcineurin Inhibitors

Long the backbone of most antirejection regimens across the world, calcineurin inhibitors work to block intracellular amplification of "nuclear factor of amplified T-cells" (NFAT), which subsequently blocks production of IL2, a major cytokine that stimulates T-cell activation and proliferation. Tacrolimus and cyclosporine are the two drugs in this class, both available in oral and IV formulations, with sublingual tacrolimus an option, and extended and short release versions of tacrolimus also available. Both are metabolized by both liver and kidney and excreted primarily in bile [18].

Common issues that ID physicians run into with calcineurin inhibitors include drug interactions, with azole antifungals, protease inhibitors, direct acting hepatitis C antivirals, and a number of antibiotics all having substantial impact on calcineurin metabolism. Common side effects of long term use include increased infection risk,

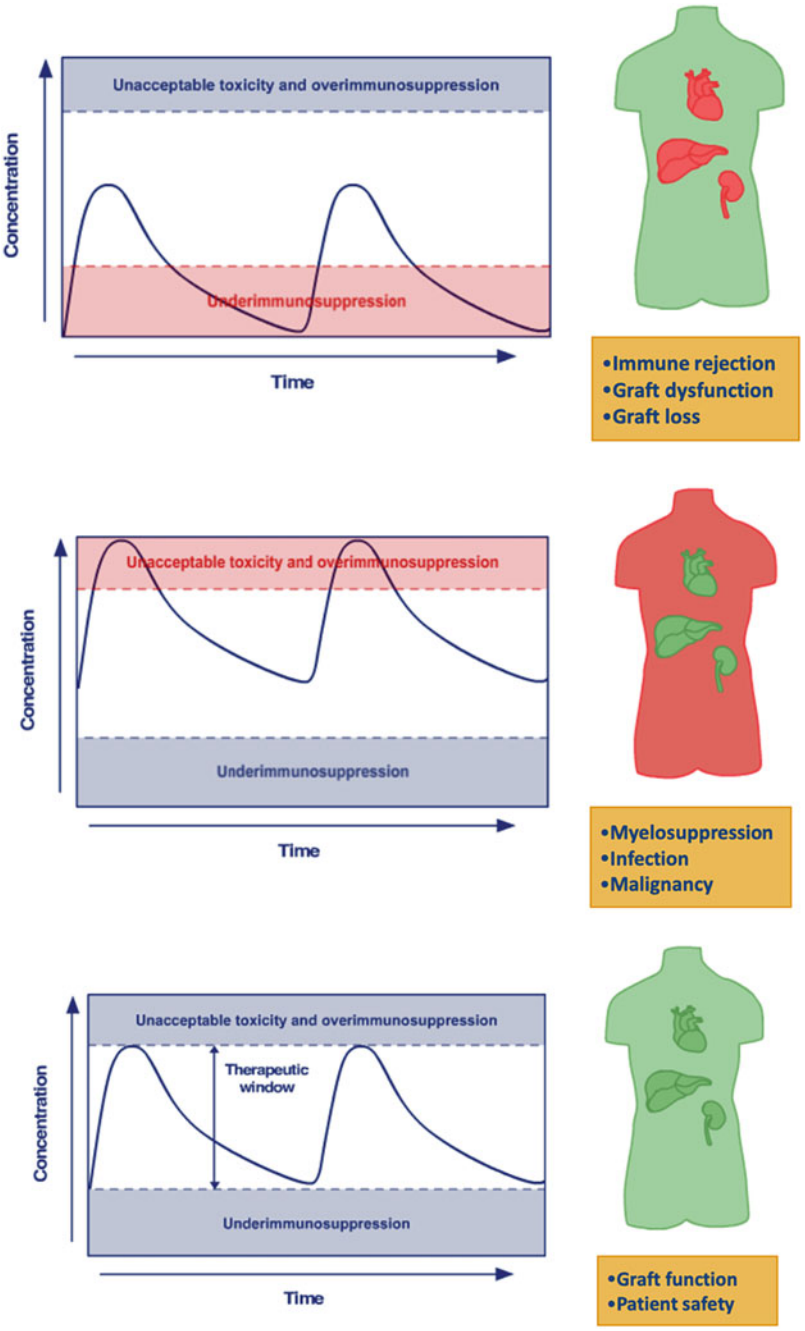


Fig. 2 Therapeutic drug monitoring for immunosuppression

especially in the viral, fungal, and mycobacterial realms, chronic renal dysfunction, hypertension, gum hyperplasia, tremors, and electrolyte disturbances.

Antiproliferative Agents

The two most common antiproliferative agents, azathioprine and mycophenolate mofetil, both act on intracellular components of lymphocyte division by decreasing the metabolism of purines and they may inhibit DNA and RNA synthesis. Azathioprine is used less commonly now, given long term risks with skin cancer development and the sense that mycophenolate seemed more efficacious in the long term. While both drugs lead commonly to dose-dependent cytopenias, mycophenolate is often associated with oral ulceration and chronic gastrointestinal side effects, especially diarrhea. The combination of oral ulceration, cytopenias, and diarrhea should make people consider mycophenolate toxicity, although often exclusion of infection is also required, especially CMV. Drug levels are available, specifically mycophenolic acid (MPA). Again, like the calcineurin inhibitors, the long term goal is to reduce antiproliferative dose to a minimum over the first year, thereby balancing risks and benefits [19, 20].

mTOR Inhibitors

Sirolimus and Everolimus are the two most widely available mammalian targets of rapamycin (mTOR) inhibitors, and work by blocking the signaling molecule that propagates the cell cycle and causes proliferation of T cells. Sirolimus was originally developed as an antifungal, although subsequently its immunosuppressive effects were noted. They are typically given in replacement of a calcineurin inhibitor, although can be given in addition to, or to supplement an antiproliferative agent in certain situations. Cytopenia and hyperlipidemia are commonly seen side effects, as well as impaired wound healing, something that has limited their wider use. Less common side effects include interstitial pneumonitis, which can be difficult to clinically separate from infectious pneumonia. Like the calcineurin inhibitors, they are extensively metabolized by the cyp450 system, and therapeutic drug monitoring is important [21].

Corticosteroids

Corticosteroids come in a variety of strengths and can be administered in a variety of ways, although they all fundamentally act to inhibit cytokine production and expression, thereby working as an anti-inflammatory. They suppress humoral responses also. At the cellular level, steroids passively diffuse through the outer membrane,

binding to intracellular receptors to form a complex that can move into the nucleus and interact with glucocorticoid-responsive elements (GREs). The interactions with GREs negate various proinflammatory genes, such as interleukin (IL)-1-alpha and IL-1-beta, as well as promoting the synthesis of numerous anti-inflammatory cytokines [22].

Prednisone is the most commonly used corticosteroid in the maintenance phase. Like most immunosuppressants, doses are tapered quickly over the first year of a transplant, often down to a chronic level of 5 mg/day, or discontinued altogether if a satisfactory level of tolerance is achieved. Side effects can be acute and long term. Acute issues include hyperglycemia, delirium, and insomnia. Longer term impacts include thinning of the skin, acne, Cushingoid body habitus changes, growth suppression, bone metabolic complications, glaucoma and cataracts, hyperlipidemia, and accelerated atherosclerosis. There is a dose-dependent infection risk and also suppression of endogenous adrenal function that results in a need to wean chronic steroids carefully.

Co-stimulation Blockade

Belatacept is a comparatively new form of immunosuppression, which binds to the CD80/86 on antigen presenting cells blocking T cell activation promoting anergy and apoptosis. Unlike other immunosuppressants, it is given as an IV infusion, typically monthly, although it may be used more frequently as an adjunct to induction therapy. Infection issues are uncommon but possible, with unique issues being seen in the EBV-naïve population who are at a markedly increased risk of EBV-associated posttransplant lymphoproliferative disorder (PTLD) should they acquire EBV through the transplanted graft or afterwards [23]. Furthermore, post-marketing surveillance has resulted in confirmed cases of aspergillosis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy (PML) in $<1\%$ of patients treated with belatacept therapy [24].

Induction Therapies

A number of induction drugs can be used for transplant. Each of these also has a role to play in treatment of acute and sometimes chronic rejection. High dose corticosteroids are commonly used and have the same acute side effects as noted above. Additional agents include:

Anti-CD25 Monoclonal Antibodies

Basiliximab is the most commonly used of the monoclonal antibodies targeting CD25. It is a nondepleting chimeric/humanized monoclonal antibody, targeted against IL-2 receptors on activated T cells. As a result of its action, it blocks IL-2

mediated responses, thereby direction limiting T cell proliferation. The drug is typically administered intraoperatively [25].

Anti-CD52 Monoclonal Antibodies

Alemtuzumab is used frequently as an induction agent because of its profound and prolonged impact on lymphocyte function. As a humanized monoclonal, it results in antibody dependent lysis of T cells, B cells, NK cells, monocytes, and macrophages. CD4 cell counts have become a standard way of measuring the impact of alemtuzumab and often reflect substantial immunosuppression. Infection is the most significant side effect, particularly those impacted by impaired T-cell function (fungal infections, latent viruses, and mycobacteria). Given the impact can often last 6–12 months, most centers would combine the use of alemtuzumab induction with prophylactic antiviral and/or antifungal agents [26].

Polyclonal Antibodies

Two types of polyclonal antibody therapies exist: antithymocyte globulin, derived from rabbits, and antilymphocyte globulin, derived from horses. Both are produced by immunizing the animals with human cells and harvesting subsequent antibodies. Consequently, antibodies are produced against multiple targets within the human cell. This leads to the broad action of the drug, but also the multiple side effects. Some of the more immediate effects are infusional, and the products should be administered slowly, typically over 4–6 h, with premedication. Longer term side effects again stem from lymphocyte mediated infection risk [27].

Conclusion

Through continual advances in our understanding of the human immune system, we have been able to drive the fields of solid organ and bone marrow transplant forward. We now have far better ways to control and prevent rejection of solid organ transplants. Our recognition of the various ways our innate and adaptive immune systems interact to seek out and control infection and rejection has helped us control them. Although organ allograft rejection, and subsequent loss of graft function, remains one of the most challenging problems facing solid organ allotransplantation, countless developments in therapeutic immunosuppression have occurred in the years since the first solid organ transplants took place. They have enabled us to minimize rejection risk and improve the life expectancy and graft survival for many of our patients, albeit with the cost of greater infection risk. Ultimately, the field continues to work toward ways of measuring and achieving organ tolerance, whereby both rejection risk and the threat of infection are minimized.

Key Points

- Absent control, solid organ transplant rejection remains one of the most common causes of transplant graft failure. It can be acute or chronic, and predominantly cellular or antibody mediated.
- The human immune system regulates rejection and tolerance through interactions with both innate and adaptive immune mechanisms that are not static, rather they evolve and diminish again over time as we age.
- Balancing therapeutic immunosuppression with infection and malignancy risk is crucial to long-term graft survival. Often more significant immunosuppression is required at the time of transplant, or shortly thereafter, for bone marrow engraftment to prevent rejection and encourage tolerance.
- Triggers of graft rejection can be associated with different forms of rejection and also need to be controlled in different ways with cellular and humoral rejection.
- Thanks to multiple different therapeutic agents now available, immunosuppression can be tailored more precisely for everyone, and altered over time to continually adjust to rejection in ways that are more precise than were historically available to transplant clinicians.

References

1. Linden P. History of solid organ transplantation and organ donation. Crit Care Clin. 2009;25(1):165–84.
2. De Vito Dabbs A, Dauber JH, Hoffman LA. Rejection after organ transplantation: a historical review. Am J Crit Care. 2000;9(6):419–29.
3. Transplant Pioneers Recall Medical Milestone. NPR. December 20, 2004. Retrieved 20 Dec 2019.
4. Chen J, Li J, Wu Q. Maintenance of naïve CD8 T cells in nonagenarians by leptin, IGFBP3 and T3. Mech Ageing Dev. 2010;131(1):29–37.
5. Lim M, Kohli J, Blood R. Immunosuppression for kidney transplantation: where are we now and where are we going? Transplant Rev. 2017;31(1):10–7.
6. Cruse J, Lewis R, Wang H. Immunology guidebook. Amsterdam: Academic; 2004. p. 267–76.
7. DeWolf S, Sykes M. Alloimmune T cells in transplantation. J Clin Invest. 2017;127(7): 2473–81.
8. De Bruyn P, Van Gestel D, Ost P, et al. Immune checkpoint blockade for organ transplant patients with advanced cancer: how far can we go? Curr Opin Oncol. 2019;31(2):54–64.
9. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2015 annual data report: kidney. Am J Transplant. 2017;17(Suppl 1):21–116.
10. Witt CA, Gaut JP, Yusen RD, Byers DE, Iuppa JA, Bennett Bain K, Alexander Patterson G, Mohanakumar T, Trulock EP, Hachem RR. Acute antibody-mediated rejection after lung transplantation. J Heart Lung Transplant. 2013;32(10):1034–40. Epub 2013 Aug 13.
11. Zhang SY, Jouanguy E, Sancho-Shimizu V, et al. Human toll-like receptor-dependent induction of interferons in protective immunity to viruses. Immunol Rev. 2007;220(1):225–36.
12. Zhao H, Alam A, Soo AP, George AJT, Ma D. Ischemia-reperfusion injury reduces long term renal graft survival: mechanism and beyond. EBioMedicine. 2018;28:31–42.
13. Lu C, Penfield J, Kielar M, Vazquez M, Jeyarajah R. Hypothesis: is renal allograft rejection initiated by the response to injury sustained during the transplant process? Kidney Int. 1999;55(6):2157–68.

14. Villard J. The role of natural killer cells in human solid organ and tissue transplantation. J Innate Immun. 2011;3(4):395–402.
15. Benichou G, Yamada Y, Aoyama A, Madsen J. Natural killer cells in rejection and tolerance of solid organ allografts. Curr Opin Organ Transplant. 2011;16(1):47–53.
16. Yu G, Xu X, Vu MD, Kilpatrick ED, Li XC. NK cells promote transplant tolerance by killing donor antigen-presenting cells. J Exp Med. 2006;203(8):1851–8.
17. Surana N, Kasper K. Deciphering the tete-a-tete between the microbiota and the immune system. J Clin Invest. 2014;124(10):4197–203.
18. Wiederrecht G, Lam E, Hung S, Martin M, Sigal N. The mechanism of action of FK-506 and cyclosporin A. Ann N Y Acad Sci. 1993;696:9–19.
19. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology. 2000;47(2–3):85–118.
20. Sandborn WJ. A review of immune modifier therapy for inflammatory bowel disease: azathioprine, 6-mercaptopurine, cyclosporine, and methotrexate. Am J Gastroenterol. 1996;91(3):423–33.
21. Kelly PA, Gruber SA, Behbod F, Kahan BD. Sirolimus, a new, potent immunosuppressive agent. Pharmacotherapy. 1997;17(6):1148–56.
22. Almawi WY, Beyhum HN, Rahme AA, Rieder MJ. Regulation of cytokine and cytokine receptor expression by glucocorticoids. J Leukoc Biol. 1996;60(5):563.
23. Sam T, Gabardi S, Tichy EM. Risk evaluation and mitigation strategies: a focus on belatacept. Prog Transplant. 2013;23(1):64–70.
24. Nulojix. (webpage) Risk evaluation and mitigation strategy for increased risk of PTLD, predominantly involving the CNS, and PML with NULOJIX. Available from http://www.nulojix.com/hcp/rems.aspx?cm_mc_uid=03785373987414557419388&cm_mc_sid_51110000=1459792841
25. Liu CL, Fan ST, Lo CM, et al. Interleukin-2 receptor antibody (basiliximab) for immunosuppressive induction therapy after liver transplantation: a protocol with early elimination of steroids and reduction of tacrolimus dosage. Liver Transpl. 2004;10(6):728–33.
26. Hanaway MJ, Woodle ES, Mulgaonkar S, et al. Alemtuzumab induction in renal transplantation. N Engl J Med. 2011;364(20):1909–19.
27. Bhorade SM, Stern E. Immunosuppression for lung transplantation. Proc Am Thorac Soc. 2009;6(1):47–53.

3 Changing Definition of Immunosuppression: Targeted Therapies and Resulting Emerging Infections and Their Prevention

Nicolas J. Mueller

Contents

Introduction 26
Belatacept 26
Key Findings and Suggestions 27
Basiliximab 27
Key Findings and Suggestions 28
Rituximab and Obinutuzumab 28
Key Findings and Suggestions 29
Alemtuzumab 29
Key Findings and Suggestions 30
Eculizumab 30
Key Findings and Suggestions 31
Ruxolitinib 32
Key Findings and Suggestions 32
Ibrutinib 33
Key Findings and Suggestions 33
Bortezomib 34
Key Findings and Suggestions 34
Brentuximab 34
Key Findings and Suggestions 35
Conclusions 35
References 35

N. J. Mueller (✉)
Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, Zürich, Switzerland
e-mail: Nicolas.Mueller@usz.ch

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_13

Introduction

Biologics are therapeutic biological products manufactured in and/or isolated from natural resources. In a broader sense cells, blood, and components of blood, such as immunoglobulins, are sometimes included in the definition, whereas some prefer a more narrow definition emphasizing the active genetic manipulation involved in the manufacturing process. The introduction of these therapies has had a major impact in many fields of medicine. Indeed, in transplantation, the use of polyclonal antibodies against T cells (i.e., antithymocyte globulin) was one of the first clinical uses of a biologic product. A number of newer targeted therapies have more recently found their way into the field of transplantation. Biologic agents can be grouped by the target, the intended use, or the mechanisms of action. This chapter will concentrate on newer agents used in solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT), even if used for non-approved indications. The emphasis will be on the potential for infection, whereas indications (often overlapping) and mode of action will not be covered in detail. The note of caveat expressed in an earlier review still holds true: It remains very challenging attributing a specific infection to a specific immunosuppressive agent, given the complex interplay of many factors contributing to the "net state" of immunosuppression [1] as well as the common use of multiple drugs at the same time in the transplant setting. The rapid introduction of many biologics into clinical practice with few studies and less than optimal monitoring of infectious events has further complicated a solid assessment of risks. Also, rates of infections are never absolute rates but compared between interventions, highlighting the importance of the comparator arms in the respective trials.

Contrary to other patient groups treated with biologics, such as patients with neurologic or rheumatologic diseases, recipients of a solid organ or hematopoietic stem cells are usually screened for both active and latent infections, and appropriate prevention strategies are routinely implemented [2]. Standard prevention measures (updated vaccination status, screening for infections pretransplant, antiviral, and anti-*Pneumocystis* prophylaxis as per local guidelines, hepatitis B prevention if indicated, treatment of latent tuberculosis if required) are only mentioned in the specific chapters if warranted by an unusual pattern but are otherwise assumed. The recommendations are derived from expert statements, published trials, and personal opinion, and they are intended to help guide prevention measures but must be adapted to the individual patient.

Belatacept

The selective co-stimulatory blocking agent belatacept is used in induction and maintenance immunosuppression therapy after kidney transplantation. Both de novo and conversion protocols have been studied [3]. In liver transplant patients, an early study demonstrated decreased patient and allograft survival and higher rates of rejection, leading to the recommendation not to use belatacept in these patients [4].

A number of later smaller studies did however not replicate these findings [5, 6]. In thoracic transplantation, data are very limited, and belatacept is rarely used [3].

Infectious risks. Initial findings indicated that patients on belatacept experienced a small but reproducible increase in the rate of posttransplant lymphoproliferative disorders (PTLD). This increase was predominantly observed in Epstein Barr virus (EBV)-seronegative patients experiencing a primary infection [7]. This led to an FDA boxed warning not to use it in EBV-seronegative recipients.

In two large long-term randomized studies comparing belatacept with cyclosporine for maintenance immunosuppression (BENEFIT and BENEFIT-EXT), rates of serious infections did not differ between the two belatacept arms and the cyclosporine comparator [8, 9]. A Cochrane systematic review did not detect a PTLD signal, neither overall nor in subgroups such as EBV-negative recipients or patients receiving higher belatacept doses [10]. With the exception of EBV-associated PTLD in the initial studies, the remaining infections were those expected in an immunosuppressed population and did not differ, despite the concern that belatacept may specifically hamper de novo immune responses such as in a CMV high-risk constellation [11, 12].

Key Findings and Suggestions

- The main concern is the increased rate of EBV-associated PTLD, limiting its use to patients who are EBV-seropositive.
- Overall, infection rates were similar between belatacept and standard immunosuppression (usually a CNI-based regimen).
- Standard posttransplant infection prevention measures apply.

Basiliximab

Basiliximab is an anti-CD25 directed nondepleting antibody (interleukin-2 receptor antagonist) mainly used as part of an induction regimen in SOT. Occasionally, it serves as replacement immunosuppression for relevant calcineurin inhibitor toxicity and has been successfully used as treatment in refractory graft-versus-host disease after HSCT in small case series.

Infectious risks. The initial landmark trial comparing induction therapy with antithymocyte globulin versus basiliximab in recipients of a kidney graft showed a lower incidence of infection with basiliximab; however a recent meta-analysis including this trial did not report a difference in infection rates [13, 14]. In a large study on induction therapy from Brazil, a lower rate of CMV infection and disease was observed in the group receiving basiliximab, reduced dose tacrolimus and everolimus as compared to antithymocyte globulin [15]. A pediatric study using the ISHLT database did show a small but not significant increase in infections in the antithymocyte cohort as compared to basiliximab [16]. No excess infectious risk was

observed in a recent meta-analysis of basiliximab in liver transplant recipients [17]. A hypersensitivity reaction is very rare but can be mistaken for an infectious event.

Key Findings and Suggestions

- Overall, infection rates were similar or lower between basiliximab and comparator induction immunosuppression (usually an antithymocyte globulin regimen).
- Rarely, a hypersensitivity reaction can mimic an infectious event.
- Standard posttransplant infection prevention measures apply.

Rituximab and Obinutuzumab

Both anti-CD20-directed antibodies are mainly used in hematological malignancies, leading to a rapid elimination of most human B cells. Obinutuzumab is a member of a new generation of anti-CD20 humanized monoclonal antibodies; *in vitro* studies demonstrated an enhanced potency. Experience with rituximab in SOT is limited. It is part of desensitization protocols for highly sensitized patients or in ABO-incompatible kidney transplantation and used for some glomerular disease and antibody-mediated rejection [18]. Across the different organs, it is a cornerstone in the treatment of posttransplant lymphoproliferative disorder (PTLD). Obinutuzumab has occasionally been used in the context of allogeneic HSCT performed for chronic lymphocytic leukemia [19].

Infectious risk. Rituximab has rarely been used in controlled trials in both SOT and HSCT, rendering an estimation of the infectious risk difficult. Some papers describe a moderate increase of infectious complications with rituximab [20, 21]. In studies in patients without antiviral (anti cytomegalovirus) prophylaxis, rates of cytomegalovirus infections were high in the rituximab group [22]. Kamar et al. reported a higher infectious-related mortality in SOT recipients receiving rituximab for various indications; the only independent predictor was the concomitant use of antithymocyte globulin [23]. In pediatric kidney transplantation, use of rituximab was associated with a higher risk of infection, in particular if used in conjunction with additional immunosuppressive interventions, such as plasmapheresis [24]. Not Rituximab all studies did see a difference: In a case-control comparing highly sensitized patients with and without rituximab, no difference in severe infections was noted [25]. A systematic review of randomized trials of rituximab as induction therapy in non-sensitized patients did not reveal an additional infectious risk [26]. The data on overall infections highlight a number of inherent challenges: few of these trials were controlled, and the concomitant medication including immunosuppression differed considerably, as did the enrolled population (ABO incompatible and highly sensitized patients). Therefore, the risk attributed to rituximab varied from none to high.

Some specific concerns need mentioning. Fatal cases of reactivation of hepatitis B (mainly in the non-transplant population) lead to an FDA-issued black box warning

in 2013. When used in SOT patients, antiviral prophylaxis should be considered in patients at higher risk (HBsAg-positive or HBsAg-negative/anti-HBc-positive seroconstellation) [27, 28]. Of two recent reports in kidney transplantation, one reported an increased reactivation of hepatitis B under a standard, but not on a low-dose regimen [29, 30]. Occasional case reports describe the association with progressive multifocal leukoencephalopathy due to JC virus; there is concern that this may be a more significant risk with the newer anti-CD20 agents [31].

Whether the increased potency of obinutuzumab will result in more serious infectious complications remains to be seen; a similar pattern of infectious complications can be expected as seen for rituximab. Two cases of disseminated enteroviral infections were published in 2015 [32]. An FDA-issued black box warning includes hepatitis B reactivation and danger of progressive multifocal leukoencephalopathy.

An interesting concept was described in a pilot study by Schachtner et al., where rituximab was given to EBV-seronegative recipients of a living EBV-positive donor to prevent EBV primary infection. Three out of five remained EBV-negative, and none showed EBV viremia, favorably comparing to a Rituximab cohort of deceased kidney donation from an EBV-positive donor to an EBV-negative recipient [33].

Key Findings and Suggestions

- Overall, infection rates may be increased, especially with the use of higher doses and when given with additional immunosuppressive agents.
- A preventive strategy for cytomegalovirus should be initiated, if not already in place.
- In all patients with a diagnosis of either active or latent hepatitis B, prophylaxis should be evaluated, depending on seroconstellation/activity (as measured by HBV DNA) [27].
- Standard posttransplant infection prevention measures apply.

Alemtuzumab

The hallmark of alemtuzumab is its long-lasting depletion of lymphocytes and its subsets, e.g., CD4 helper T cells, which can last as long as 1 year after transplantation [34]. The main indication is induction therapy.

Infectious risk. One would expect that this profound and lasting effect on lymphocytes should translate in a higher rate of infections, but in most studies, a clear signal is missing. Contrary to the personal experience, a similar rate of infections between the alemtuzumab arm and the comparator was reported in the majority of studies in SOT [35, 36]. Whether this is due to the study setting with careful evaluation and strict prevention measures in place is unknown, as data from larger registries are missing. Looking more at specific pathogen groups, fungal and bacterial infections did not differ, whereas in some settings, specific viral infections were slightly increased (BK viremia), whereas, interestingly, some even reduced

(cytomegalovirus), nicely summarized in [37]. Similar rates between alemtuzumab and comparators were reported after induction therapy in HSCT, also in a pediatric population [38–40]. The few studies that have analyzed the repeated use, e.g., for rejection treatment, did however find an increased rate of infection (4.5% vs. 21% in the alemtuzumab group [41]).

Key Findings and Suggestions

- Overall, infection rates were similar when used as induction therapy, but data is limited to patients in a study setting, with antimicrobial preventive measures in place.
- SOT and HSCT populations do not differ in risk of infection.
- Infections rates were higher if given repeatedly, e.g., as part of a rejection treatment. In this setting, a prevention strategy for cytomegalovirus and a *Pneumocystis* prophylaxis should be initiated, if not in place.
- Standard posttransplant infection prevention measures apply.

Eculizumab

The mechanism of action of eculizumab, blocking the cleavage and activity of complement factor 5, explains the increased risk of infection for encapsulated bacteria and, in particular, with *Neisseria* spp. Its main use is in patients with paroxysmal nocturnal hemoglobinuria [42, 43] and atypical hemolytic uremic syndrome-associated thrombotic microangiopathy [44]. For these two FDA-approved indications, eculizumab is used prior and after transplantation [45]. HSCT-associated thrombotic microangiopathy has been successfully treated with eculizumab [46, 47]. Small studies have tested eculizumab in desensitization protocols or as treatment of antibody-mediated rejection, with mixed results [48–51]; nonetheless its use has been increasing after solid organ transplant.

Infectious risk. The risk is directly related to the inhibition of the terminal cleavage of complement and specifically infections with *Neisseria* spp. Interestingly, invasive infections with other capsulated organisms like *Streptococcus pneumoniae* are rare, likely due to alternative mechanisms such as opsonization [52]. For *Neisseria gonorrhoeae*, disseminated courses have been associated with use of eculizumab [53, 54], the largest being a recent case series [55]. Unusual *Neisseria* species have been reported as well [56]. Several severe cases of meningococcal disease were reported, also in vaccinated individuals [57–59]. It remains unknown if infections other than due to *Neisseria* spp. are increased, but so far, no robust signal was observed [52]. In an open-label, randomized study in pediatric kidney recipients on the role of eculizumab in ischemia-reperfusion injury, four participants in the eculizumab arm did lose their graft to a flu-like infection (none were vaccinated against influenza), but the infection was not characterized further, and significance is unclear [60].

Prevention strategies are therefore primarily directed against gonococcal and meningococcal disease. For gonococcal disease prevention, active screening of patients and partners who are sexually active and at risk for acquisition of new infections is recommended. The cornerstone of prevention of meningococcal disease is vaccination in combination with antibacterial prophylaxis. Conjugate quadrivalent meningococcal vaccines should be used against serotypes ACWY, in conjunction with a serotype B vaccine, where available. For the ACWY vaccines, most will recommend two initial doses separated by at least 4 weeks, the initial dose 2 weeks before first dose of eculizumab, and a booster every 5 years [61]. A systemic antibacterial prophylaxis with meningococcal coverage such as penicillin, amoxicillin, azithromycin, or ciprofloxacin is initiated at the same time. The main unresolved question in prevention is whether longer concomitant antibacterial prophylaxis is warranted for the duration of eculizumab therapy in properly vaccinated individuals. Most statements advocate for prolonged chemoprophylaxis for the duration of eculizumab therapy in immunocompromised patients [62]. Monitoring antibody titers against meningococci is not widely available.

Key Findings and Suggestions

- Due to its mechanisms, eculizumab predisposes to invasive infection with *Neisseria* spp.
- Gonococcal disease prevention consists of screening of at-risk sexually active patients and their partners, by PCR.
- Vaccination with a conjugated quadrivalent serotype ACWY vaccine together with a recombinant serotype B vaccine is strongly recommended, ideally at least 2 weeks prior to first dose of eculizumab. A second dose after 4 weeks (or later) and a booster every 5 years are recommended for the quadrivalent vaccine, whereas for the serotype B vaccine, best practice is unknown, although its immunogenicity is less durable.
- Additional systemic antibacterial prophylaxis is recommended for at least 4 weeks after last vaccine dose. Most expert statements favor continuous chemoprophylaxis for the duration of eculizumab therapy in immunocompromised individuals due to reports of breakthrough infections. Penicillin V is preferred over ciprofloxacin for ecological reasons.
- Vaccine failures have been reported. It might be prudent to include meningococcal vaccination as required immunization upon listing, especially in highly sensitized patients, which may result in better vaccine responses prior to the onset of immunosuppression.
- Standard posttransplant infection prevention measures apply, in particular a documented vaccination against pneumococci.

Ruxolitinib

Ruxolitinib is a Janus kinase inhibitor approved for the treatment in myelofibrosis and polycythemia vera [63–65]. More recently, it has been studied in steroid-refractory graft-versus-host disease (GvHD) including bronchiolitis obliterans syndrome in adult and pediatric recipients of an allogeneic HSCT [66–68]. Randomized trials are planned, but the literature on infectious risk in this very vulnerable population is very limited [69]. It is not being used after SOT.

Infectious risk. Most of the knowledge comes from its use in myelofibrosis and polycythemia vera [70]. These initial studies did report a higher rate of infection in the ruxolitinib groups, but in later trials, only a mild to moderate effect was seen [71]. In the transplant setting, prior use of a Janus kinase inhibitor did not adversely affect the outcomes of allogeneic HSCT, including the rate of infections [72]. In patients treated for refractory GvHD, the type of infections reported included viral, fungal, and bacterial infections, affecting over 50% of patients, but it was not possible to discern the role of ruxolitinib [66]. Cytomegalovirus reactivation was observed in 25–30% of all patients [73]. Antimicrobial prophylaxis was specified in one pediatric series and consisted of antifungal prophylaxis, cotrimoxazole against *Pneumocystis jirovecii*, and antiviral prophylaxis if GvHD was acute [68]. Lussana et al. have summarized the infectious risk in randomized trials in myelofibrosis and polycythemia vera [74]. The only infection significantly associated with ruxolitinib was herpes zoster. In a long-term follow-up study of patients taking ruxolitinib for myelofibrosis, infection risk correlated with disease severity and a history of a previous infection: infections were observed in 28% of all analyzed patients (23%, bacterial; 5%, viral; only three patients with a fungal infection) [75]. Rare (opportunistic) infections were seen early on, including reactivation hepatitis B, toxoplasmosis, and cryptococcosis, prompting the question of appropriate antimicrobial prophylaxis [76–80]. Biologic drugs do not always enhance the risk for infections and can be used to help treat infections: ruxolitinib was successfully used as treatment in a case of chronic mucocutaneous candidiasis caused by STAT1 mutation, an effect believed to be mediated by the induction of interleukin 17 [81], and was used to treat a patient with relapsed refractory chronic active Epstein–Barr virus infection [82].

Key Findings and Suggestions

- The share of ruxolitinib on the burden of infectious complications in patients with GvHD does not seem higher than current standard of care, although comparison is challenging given the already intense immunosuppression of patients with treatment-refractory GvHD.
- Often, these patients are on antiviral, antifungal, and *Pneumocystis* prophylaxis. It is prudent to continue or put patients on broad antimicrobial prophylaxis if ruxolitinib is used as a therapeutic option for GvHD.

- Herpes zoster was the only infection significantly increased in the ruxolitinib patients when given for myeloproliferative neoplasms, such that antiviral prophylaxis would be recommended. Use of a zoster vaccine should be evaluated before start of ruxolitinib.
- Standard posttransplant infection prevention measures apply.

Ibrutinib

Ibrutinib is approved for a number of B cell-derived hematological malignancies, such as chronic lymphocytic leukemia, Waldenstrom's macroglobulinemia, mantle-cell lymphoma, and marginal zone lymphoma [83, 84]. If judged from the literature on primary immunodeficiency where the X-linked agammaglobulinemia mimicking the predicted effect of ibrutinib results in severe, life-threatening bacterial infections, one would expect a similar increased risk with ibrutinib use [85]. However, the therapeutic effects on the hematological malignancies seem to offset these risks, and the data on the infectious risks has shown only a moderate increase. Bacterial infections (pneumonia, urinary tract infection) predominate [84, 86]. The main data comes from studies in patients with chronic lymphocytic leukemia, but smaller studies for the other indications yielded similar results [87]. A steady but small number of cases of invasive fungal infections have been reported, prompting a call to action to improve the knowledge and data collection on the use of this and similar compounds [88]. Indeed, the authors state the same challenges: often, the risk is not defined through one factor alone, but is a complex interplay between various risk factors. Much less is known about the added infectious risk when ibrutinib is used in the treatment of chronic GvHD. The largest observational trial did include 42 patients, of whom 69% had an infection (any grade) and 36% (15 patients) a grade $\geq$3 infection [89]. In agreement with the previous experience, pneumonia was the lead infection.

Key Findings and Suggestions

- Ibrutinib is used in patients with chronic GvHD. The small studies so far do not allow a concise infection risk stratification.
- The main infections seen are pneumonia, skin/soft tissue, and urinary tract infections.
- Reports of invasive fungal infections are increasing, so while still low, it has led to a heightened awareness and a call to improve our knowledge.
- Most patients with chronic GvHD are on antiviral, antifungal, and anti-*Pneumocystis* prophylaxis. It is prudent to continue or put patients on a broad antimicrobial prophylaxis if ibrutinib is used as a therapeutic option for GvHD.

Bortezomib

Besides its indication for multiple myeloma and relapsed or refractory mantle cell lymphoma, bortezomib has been tested as part of a preventive combination treatment for GvHD [90–92]. Occasionally, it has been used for the treatment of humoral rejection after SOT. Owing to the key effect of this drug, depletion of T cells, a higher risk of viral infections is expected and confirmed by the literature [93]. Indeed, a higher risk of herpes zoster described in the initial studies prompted many centers to adopt a specific antiviral prevention strategy [94]. An Australian study did see a higher rate of severe influenza courses in patients treated with bortezomib [95]. Fungal infections and other opportunistic infections, such as *Pneumocystis*, were not found to be increased or related to underlying disease progression [96]. Bortezomib did not increase the infection risk when it was tested in a three-arm GvHD prevention study [92].

Key Findings and Suggestions

- Antiviral prophylaxis is strongly recommended in VZV-seropositive individuals for the duration of bortezomib use, given the augmented rates of herpes zoster (patients on acyclovir, famvir, or valacyclovir or on cytomegalovirus prophylaxis with (val)ganciclovir are protected, but not on maribavir or letermovir).
- Herpes zoster recombinant vaccine should be considered in patients with planned bortezomib use, where possible.
- Bortezomib per se did not increase the infectious risk when used in a large three-arm study as prevention for GvHD.
- Most patients with chronic GvHD are on an antiviral, antifungal, and anti-*Pneumocystis* prophylaxis. It is prudent to continue or put patients on a broad antimicrobial prophylaxis if ibrutinib is used as a therapeutic.
- Standard posttransplant infection prevention measures apply.

Brentuximab

Brentuximab vedotin is an anti-CD30 monoclonal antibody-drug conjugate approved for the treatment of relapsed/refractory Hodgkin lymphoma and anaplastic large T-cell lymphoma. In the context of transplantation, it is used for recurrent Hodgkin lymphoma after allogeneic HSCT [97]. In the initial studies including as part of a consolidation therapy after autologous HSCT, no excess risk was noted, but patients were on a prophylactic regimen for herpesviruses and *Pneumocystis* [98–100]. In the study by Gopal et al. after allogeneic HSCT, severe infections were reported in 24% of patients, including *Pneumocystis*, *Pseudomonas*, and *Aspergillus* pneumonias. Dissecting the role of brentuximab is not possible [97]. In addition, a small series and single cases of progressive multifocal

leukoencephalopathy were reported, some occurring rather early after the end of treatment, leading to a specific FDA warning [101].

Key Findings and Suggestions

- An excess infection risk attributable to brentuximab was not seen in the larger initial studies, nor when used as salvage therapy for recurrent disease after allogeneic HSCT. Most patients however were on *Pneumocystis* and herpesviral prophylaxis.
- Herpesviral and *Pneumocystis* prophylaxis should be considered when on brentuximab.
- Rare cases of PML were observed, resulting in an FDA warning to stop therapy in case of clinical suspicion and initiate a diagnostic work-up.
- Standard posttransplant infection prevention measures apply.

Conclusions

The advent of biologic therapies has rendered the estimation of the infectious risk even more challenging. Not all pathways targeted result in an enhanced infection risk, and for some molecules, the spectrum of expected infections can be quite narrow. In transplant recipients, these agents are never used alone but are part of a combination of measures resulting in an overall "net state" of immunosuppression, which is difficult to quantify. The importance of well-maintained cohorts and registries, as well as post-marketing reporting to regulatory agencies, to detect rare signals in real-life patients cannot be overestimated.

References

1. Mueller NJ. New immunosuppressive strategies and the risk of infection. Transpl Infect Dis. 2008;10(6):379–84.
2. Malinis M, Boucher HW, AST Infectious Diseases Community of Practice. Screening of donor and candidate prior to solid organ transplantation-guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13548.
3. Perez CP, Patel N, Mardis CR, Meadows HB, Taber DJ, Pilch NA. Belatacept in solid organ transplant: review of current literature across transplant types. Transplantation. 2018; 102(9):1440–52.
4. Klintmalm GB, Feng S, Lake JR, Vargas HE, Wekerle T, Agnes S, et al. Belatacept-based immunosuppression in de novo liver transplant recipients: 1-year experience from a phase II randomized study. Am J Transplant. 2014;14(8):1817–27.
5. LaMattina JC, Jason MP, Hanish SI, Ottmann SE, Klassen DK, Potosky D, et al. Safety of belatacept bridging immunosuppression in hepatitis C-positive liver transplant recipients with renal dysfunction. Transplantation. 2014;97(2):133–7.

6. Schwarz C, Rasoul-Rockenschaub S, Soliman T, Berlakovich GA, Steininger R, Muhlbacher F, et al. Belatacept treatment for two yr after liver transplantation is not associated with operational tolerance. Clin Transplant. 2015;29(1):85–9.
7. Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, et al. Costimulation blockade with belatacept in renal transplantation. N Engl J Med. 2005;353(8):770–81.
8. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, et al. Belatacept and long-term outcomes in kidney transplantation. N Engl J Med. 2016;374(4):333–43.
9. Durrbach A, Pestana JM, Florman S, Del Carmen RM, Rostaing L, Kuypers D, et al. Long-term outcomes in belatacept- versus cyclosporine-treated recipients of extended criteria donor kidneys: final results from BENEFIT-EXT, a phase III randomized study. Am J Transplant. 2016;16(11):3192–201.
10. Masson P, Henderson L, Chapman JR, Craig JC, Webster AC. Belatacept for kidney transplant recipients. Cochrane Database Syst Rev. 2014;11:CD010699.
11. Xu H, Perez SD, Cheeseman J, Mehta AK, Kirk AD. The allo- and viral-specific immunosuppressive effect of belatacept, but not tacrolimus, attenuates with progressive T cell maturation. Am J Transplant. 2014;14(2):319–32.
12. Bassil N, Rostaing L, Mengelle C, Kallab S, Esposito L, Guitard J, et al. Prospective monitoring of cytomegalovirus, Epstein-Barr virus, BK virus, and JC virus infections on belatacept therapy after a kidney transplant. Exp Clin Transplant. 2014;12(3):212–9.
13. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D, Thymoglobulin Induction Study Group. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med. 2006;355(19):1967–77.
14. wWang K, Xu X, Fan M. Induction therapy of basiliximab versus antithymocyte globulin in renal allograft: a systematic review and meta-analysis. Clin Exp Nephrol. 2018;22(3):684–93.
15. Tedesco-Silva H, Felipe C, Ferreira A, Cristelli M, Oliveira N, Sandes-Freitas T, et al. Reduced incidence of cytomegalovirus infection in kidney transplant recipients receiving everolimus and reduced tacrolimus doses. Am J Transplant. 2015;15(10):2655–64.
16. Butts RJ, Dipchand AI, Sutcliffe D, Bano M, Dimas V, Morrow R, et al. Comparison of basiliximab vs antithymocyte globulin for induction in pediatric heart transplant recipients: an analysis of the International Society for Heart and Lung Transplantation database. Pediatr Transplant. 2018;22(4):e13190.
17. Zhang GQ, Zhang CS, Sun N, Lv W, Chen BM, Zhang JL. Basiliximab application on liver recipients: a meta-analysis of randomized controlled trials. Hepatobiliary Pancreat Dis Int. 2017;16(2):139–46.
18. Sood P, Hariharan S. Anti-CD20 blocker rituximab in kidney transplantation. Transplantation. 2018;102(1):44–58.
19. Al-Sawaf O, Herling CD, Holtick U, Scheid C, Cramer P, Sasse S, et al. Venetoclax plus rituximab or obinutuzumab after allogeneic hematopoietic stem cell transplantation in chronic lymphocytic leukemia. Haematologica. 2019;104:e224.
20. Grim SA, Pham T, Thielke J, Sankary H, Oberholzer J, Benedetti E, et al. Infectious complications associated with the use of rituximab for ABO-incompatible and positive cross-match renal transplant recipients. Clin Transplant. 2007;21(5):628–32.
21. Lee J, Lee JG, Kim S, Song SH, Kim BS, Kim HO, et al. The effect of rituximab dose on infectious complications in ABO-incompatible kidney transplantation. Nephrol Dial Transplant. 2016;31(6):1013–21.
22. Nishida H, Ishida H, Tanaka T, Amano H, Omoto K, Shirakawa H, et al. Cytomegalovirus infection following renal transplantation in patients administered low-dose rituximab induction therapy. Transpl Int. 2009;22(10):961–9.
23. Kamar N, Milioto O, Puissant-Lubrano B, Esposito L, Pierre MC, Mohamed AO, et al. Incidence and predictive factors for infectious disease after rituximab therapy in kidney-transplant patients. Am J Transplant. 2010;10(1):89–98.

24. Gulleroglu K, Baskin E, Moray G, Ozdemir H, Arslan H, Haberal M. Rituximab therapy and infection risk in pediatric renal transplant patients. Exp Clin Transplant. 2016;14(2):172–5.
25. Scemla A, Loupy A, Candon S, Mamzer MF, Martinez F, Zuber J, et al. Incidence of infectious complications in highly sensitized renal transplant recipients treated by rituximab: a case-controlled study. Transplantation. 2010;90(11):1180–4.
26. Cheungpasitporn W, Thongprayoon C, Edmonds PJ, Bruminhent J, Tangdhanakanond K. The effectiveness and safety of rituximab as induction therapy in ABO-compatible non-sensitized renal transplantation: a systematic review and meta-analysis of randomized controlled trials. Ren Fail. 2015;37(9):1522–6.
27. Te H, Doucette K. Viral hepatitis: guidelines by the American Society of Transplantation Infectious Disease Community of Practice. Clin Transplant. 2019;33:e13514.
28. Martin ST, Cardwell SM, Nailor MD, Gabardi S. Hepatitis B reactivation and rituximab: a new boxed warning and considerations for solid organ transplantation. Am J Transplant. 2014; 14(4):788–96.
29. Lee J, Park JY, Kim DG, Lee JY, Kim BS, Kim MS, et al. Effects of rituximab dose on hepatitis B reactivation in patients with resolved infection undergoing immunologic incompatible kidney transplantation. Sci Rep. 2018;8(1):15629.
30. Masutani K, Omoto K, Okumi M, Okabe Y, Shimizu T, Tsuruya K, et al. Incidence of hepatitis B viral reactivation after kidney transplantation with low-dose rituximab administration. Transplantation. 2018;102(1):140–5.
31. Focosi D, Tuccori M, Maggi F. Progressive multifocal leukoencephalopathy and anti-CD20 monoclonal antibodies: what do we know after 20 years of rituximab. Rev Med Virol. 2019;29:e2077.
32. Dendle C, Gilbertson M, Korman TM, Golder V, Morand E, Opat S. Disseminated enteroviral infection associated with obinutuzumab. Emerg Infect Dis. 2015;21(9):1661–3.
33. Schachtner T, Reinke P. Pretransplant prophylactic rituximab to prevent Epstein-Barr virus (EBV) viremia in EBV-seronegative kidney transplant recipients from EBV-seropositive donors: results of a pilot study. Transpl Infect Dis. 2016;18(6):881–8.
34. Watson CJ, Bradley JA, Friend PJ, Firth J, Taylor CJ, Bradley JR, et al. Alemtuzumab (CAMPATH 1H) induction therapy in cadaveric kidney transplantation – efficacy and safety at five years. Am J Transplant. 2005;5(6):1347–53.
35. Morgan RD, O'Callaghan JM, Knight SR, Morris PJ. Alemtuzumab induction therapy in kidney transplantation: a systematic review and meta-analysis. Transplantation. 2012; 93(12):1179–88.
36. Hanaway MJ, Woodle ES, Mulgaonkar S, Peddi VR, Kaufman DB, First MR, et al. Alemtuzumab induction in renal transplantation. N Engl J Med. 2011;364(20):1909–19.
37. Helfrich M, Ison MG. Opportunistic infections complicating solid organ transplantation with alemtuzumab induction. Transpl Infect Dis. 2015;17(5):627–36.
38. Cupit-Link MC, Nageswara Rao A, Warad DM, Rodriguez V, Khan S. EBV-PTLD, adenovirus, and CMV in pediatric allogeneic transplants with alemtuzumab as part of pretransplant conditioning: a retrospective single center study. J Pediatr Hematol Oncol. 2018;40(8):e473–8.
39. Schneidewind L, Neumann T, Knoll F, Zimmermann K, Smola S, Schmidt CA, et al. Are the polyomaviruses BK and JC associated with opportunistic infections, graft-versus-host disease, or worse outcomes in adult patients receiving their first allogeneic stem cell transplantation with low-dose alemtuzumab? Acta Haematol. 2017;138(1):3–9.
40. Neumann T, Schneidewind L, Thiele T, Pink D, Schulze M, Schmidt C, et al. No indication of increased infection rates using low-dose alemtuzumab instead of anti-thymocyte globulin as graft-versus-host disease prophylaxis before allogeneic stem cell transplantation. Transpl Infect Dis. 2018;20(1). https://doi.org/10.1111/tid.12822.
41. Peleg AY, Husain S, Kwak EJ, Silveira FP, Ndirangu M, Tran J, et al. Opportunistic infections in 547 organ transplant recipients receiving alemtuzumab, a humanized monoclonal CD-52 antibody. Clin Infect Dis. 2007;44(2):204–12.

42. Brodsky RA, Young NS, Antonioli E, Risitano AM, Schrezenmeier H, Schubert J, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood. 2008;111(4):1840–7.
43. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006;355(12):1233–43.
44. Legendre CM, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368(23):2169–81.
45. Johnson CK, Leca N. Eculizumab use in kidney transplantation. Curr Opin Organ Transplant. 2015;20(6):643–51.
46. Elsallabi O, Bhatt VR, Dhakal P, Foster KW, Tendulkar KK. Hematopoietic stem cell transplant-associated thrombotic microangiopathy. Clin Appl Thromb Hemost. 2016;22(1):12–20.
47. Rudoni J, Jan A, Hosing C, Aung F, Yeh J. Eculizumab for transplant-associated thrombotic microangiopathy in adult allogeneic stem cell transplant recipients. Eur J Haematol. 2018;101(3):389–98.
48. Locke JE, Magro CM, Singer AL, Segev DL, Haas M, Hillel AT, et al. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. Am J Transplant. 2009;9(1):231–5.
49. Biglarnia AR, Nilsson B, Nilsson T, von Zur-Muhlen B, Wagner M, Berne C, et al. Prompt reversal of a severe complement activation by eculizumab in a patient undergoing intentional ABO-incompatible pancreas and kidney transplantation. Transpl Int. 2011;24(8):e61–6.
50. Bentall A, Tyan DB, Sequeira F, Everly MJ, Gandhi MJ, Cornell LD, et al. Antibody-mediated rejection despite inhibition of terminal complement. Transpl Int. 2014;27(12):1235–43.
51. Stegall MD, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. Am J Transplant. 2011;11(11):2405–13.
52. Benamu E, Montoya JG. Infections associated with the use of eculizumab: recommendations for prevention and prophylaxis. Curr Opin Infect Dis. 2016;29(4):319–29.
53. Gleesing J, Chiwane S, Rongkavilit C. Gonococcal septic shock associated with eculizumab treatment. Pediatr Infect Dis J. 2012;31(5):543.
54. Hublikar S, Maher WE, Bazan JA. Disseminated gonococcal infection and eculizumab – a "high risk" connection? Sex Transm Dis. 2014;41(12):747–8.
55. Crew PE, Abara WE, McCulley L, Waldron PE, Kirkcaldy RD, Weston EJ, et al. Disseminated gonococcal infections in patients receiving eculizumab: a case series. Clin Infect Dis. 2018;69(4)596–600, https://doi.org/10.1093/cid/ciy958.
56. Crew PE, McNamara L, Waldron PE, McCulley L, Jones SC, Bersoff-Matcha SJ. Unusual *Neisseria* species as a cause of infection in patients taking eculizumab. J Infect. 2019;78(2):113–8.
57. Reher D, Fuhrmann V, Kluge S, Nierhaus A. A rare case of septic shock due to *Neisseria meningitidis* serogroup B infection despite prior vaccination in a young adult with paroxysmal nocturnal haemoglobinuria receiving eculizumab. Vaccine. 2018;36(19):2507–9.
58. Friedl C, Hackl G, Schilcher G, Rosenkranz AR, Eller K, Eller P. Waterhouse-Friderichsen syndrome due to *Neisseria meningitidis* infection in a young adult with thrombotic microangiopathy and eculizumab treatment: case report and review of management. Ann Hematol. 2017;96(5):879–80.
59. Struijk GH, Bouts AH, Rijkers GT, Kuin EA, ten Berge IJ, Bemelman FJ. Meningococcal sepsis complicating eculizumab treatment despite prior vaccination. Am J Transplant. 2013;13(3):819–20.
60. Kaabak M, Babenko N, Shapiro R, Zokoyev A, Dymova O, Kim E. A prospective randomized, controlled trial of eculizumab to prevent ischemia-reperfusion injury in pediatric kidney transplantation. Pediatr Transplant. 2018;22(2). https://doi.org/10.1111/petr.13129.

61. Cohn AC, MacNeil JR, Clark TA, Ortega-Sanchez IR, Briere EZ, Meissner HC, et al. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2013;62(RR-2):1–28.
62. Winthrop KL, Mariette X, Silva JT, Benamu E, Calabrese LH, Dumusc A, et al. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Soluble immune effector molecules [II]: agents targeting interleukins, immunoglobulins and complement factors). Clin Microbiol Infect. 2018;24(Suppl 2):S21–40.
63. Vannucchi AM, Kiladjian JJ, Griesshammer M, Masszi T, Durrant S, Passamonti F, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med. 2015;372(5):426–35.
64. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9): 799–807.
65. Verstovsek S, Mesa RA, Gotlib J, Gupta V, DiPersio JF, Catalano JV, et al. Long-term treatment with ruxolitinib for patients with myelofibrosis: 5-year update from the randomized, double-blind, placebo-controlled, phase 3 COMFORT-I trial. J Hematol Oncol. 2017;10(1):55.
66. Modi B, Hernandez-Henderson M, Yang D, Klein J, Dadwal S, Kopp E, et al. Ruxolitinib as salvage therapy for chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2019;25(2):265–9.
67. Schoettler M, Duncan C, Lehmann L, Furutani E, Subramaniam M, Margossian S. Ruxolitinib is an effective steroid sparing agent in children with steroid refractory/dependent bronchiolitis obliterans syndrome after allogenic hematopoietic cell transplantation. Bone Marrow Transplant. 2019;54:1158.
68. Gonzalez Vicent M, Molina B, Gonzalez de Pablo J, Castillo A, Diaz MA. Ruxolitinib treatment for steroid refractory acute and chronic graft vs host disease in children: clinical and immunological results. Am J Hematol. 2019;94(3):319–26.
69. von Bubnoff N, Ihorst G, Grishina O, Rothling N, Bertz H, Duyster J, et al. Ruxolitinib in GvHD (RIG) study: a multicenter, randomized phase 2 trial to determine the response rate of Ruxolitinib and best available treatment (BAT) versus BAT in steroid-refractory acute graft-versus-host disease (aGvHD) (NCT02396628). BMC Cancer. 2018;18(1):1132.
70. Cervantes F, Vannucchi AM, Kiladjian JJ, Al-Ali HK, Sirulnik A, Stalbovskaya V, et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. Blood. 2013;122(25):4047–53.
71. Harrison CN, Vannucchi AM, Kiladjian JJ, Al-Ali HK, Gisslinger H, Knoops L, et al. Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. Leukemia. 2016;30(8):1701–7.
72. Shanavas M, Popat U, Michaelis LC, Fauble V, McLornan D, Klisovic R, et al. Outcomes of allogeneic hematopoietic cell transplantation in patients with myelofibrosis with prior exposure to Janus kinase 1/2 inhibitors. Biol Blood Marrow Transplant. 2016;22(3):432–40.
73. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. Leukemia. 2015;29(10):2062–8.
74. Lussana F, Cattaneo M, Rambaldi A, Squizzato A. Ruxolitinib-associated infections: a systematic review and meta-analysis. Am J Hematol. 2018;93(3):339–47.
75. Polverelli N, Palumbo GA, Binotto G, Abruzzese E, Benevolo G, Bergamaschi M, et al. Epidemiology, outcome, and risk factors for infectious complications in myelofibrosis patients receiving ruxolitinib: a multicenter study on 446 patients. Hematol Oncol. 2018;36:561.
76. Caocci G, Murgia F, Podda L, Solinas A, Atzeni S, La Nasa G. Reactivation of hepatitis B virus infection following ruxolitinib treatment in a patient with myelofibrosis. Leukemia. 2014;28(1):225–7.
77. Wysham NG, Sullivan DR, Allada G. An opportunistic infection associated with ruxolitinib, a novel janus kinase 1,2 inhibitor. Chest. 2013;143(5):1478–9.

78. Wathes R, Moule S, Milojkovic D. Progressive multifocal leukoencephalopathy associated with ruxolitinib. N Engl J Med. 2013;369(2):197–8.
79. Goldberg RA, Reichel E, Oshry LJ. Bilateral toxoplasmosis retinitis associated with ruxolitinib. N Engl J Med. 2013;369(7):681–3.
80. Heine A, Brossart P, Wolf D. Ruxolitinib is a potent immunosuppressive compound: is it time for anti-infective prophylaxis? Blood. 2013;122(23):3843–4.
81. Mossner R, Diering N, Bader O, Forkel S, Overbeck T, Gross U, et al. Ruxolitinib induces interleukin 17 and ameliorates chronic mucocutaneous candidiasis caused by STAT1 gain-of-function mutation. Clin Infect Dis. 2016;62(7):951–3.
82. Jin Z, Wang Y, Wang J, Zhang J, Wu L, Wang Z. Long-term survival benefit of ruxolitinib in a patient with relapsed refractory chronic active Epstein-Barr virus. Ann Hematol. 2019; 98:2003.
83. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013;369(1):32–42.
84. Tillman BF, Pauff JM, Satyanarayana G, Talbott M, Warner JL. Systematic review of infectious events with the Bruton tyrosine kinase inhibitor ibrutinib in the treatment of hematologic malignancies. Eur J Haematol. 2018;100(4):325–34.
85. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993;72(2):279–90.
86. Williams AM, Baran AM, Meacham PJ, Feldman MM, Valencia HE, Newsom-Stewart C, et al. Analysis of the risk of infection in patients with chronic lymphocytic leukemia in the era of novel therapies. Leuk Lymphoma. 2018;59(3):625–32.
87. Wang ML, Blum KA, Martin P, Goy A, Auer R, Kahl BS, et al. Long-term follow-up of MCL patients treated with single-agent ibrutinib: updated safety and efficacy results. Blood. 2015;126(6):739–45.
88. Chamilos G, Lionakis MS, Kontoyiannis DP. Call for action: invasive fungal infections associated with ibrutinib and other small molecule kinase inhibitors targeting immune signaling pathways. Clin Infect Dis. 2018;66(1):140–8.
89. Miklos D, Cutler CS, Arora M, Waller EK, Jagasia M, Pusic I, et al. Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. Blood. 2017;130(21):2243–50.
90. Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352(24):2487–98.
91. San Miguel JF, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, Kropff M, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359(9):906–17.
92. Bolanos-Meade J, Reshef R, Fraser R, Fei M, Abhyankar S, Al-Kadhimi Z, et al. Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced-intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203). Lancet Haematol. 2019;6(3): e132–43.
93. Kim SJ, Kim K, Kim BS, Lee HJ, Kim H, Lee NR, et al. Bortezomib and the increased incidence of herpes zoster in patients with multiple myeloma. Clin Lymphoma Myeloma. 2008;8(4):237–40.
94. Redelman-Sidi G, Michielin O, Cervera C, Ribi C, Aguado JM, Fernandez-Ruiz M, et al. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Immune checkpoint inhibitors, cell adhesion inhibitors, sphingosine-1-phosphate receptor modulators and proteasome inhibitors). Clin Microbiol Infect. 2018;24(Suppl 2):S95–107.

95. Teh BW, Worth LJ, Harrison SJ, Thursky KA, Slavin MA. Risks and burden of viral respiratory tract infections in patients with multiple myeloma in the era of immunomodulatory drugs and bortezomib: experience at an Australian Cancer Hospital. Support Care Cancer. 2015;23(7):1901–6.
96. Teh BW, Teng JC, Urbancic K, Grigg A, Harrison SJ, Worth LJ, et al. Invasive fungal infections in patients with multiple myeloma: a multi-center study in the era of novel myeloma therapies. Haematologica. 2015;100(1):e28–31.
97. Gopal AK, Ramchandren R, O'Connor OA, Berryman RB, Advani RH, Chen R, et al. Safety and efficacy of brentuximab vedotin for Hodgkin lymphoma recurring after allogeneic stem cell transplantation. Blood. 2012;120(3):560–8.
98. Younes A, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. J Clin Oncol. 2012;30(18):2183–9.
99. Moskowitz CH, Nademanee A, Masszi T, Agura E, Holowiecki J, Abidi MH, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;385(9980): 1853–62.
100. Pro B, Advani R, Brice P, Bartlett NL, Rosenblatt JD, Illidge T, et al. Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol. 2012;30(18):2190–6.
101. Carson KR, Newsome SD, Kim EJ, Wagner-Johnston ND, von Geldern G, Moskowitz CH, et al. Progressive multifocal leukoencephalopathy associated with brentuximab vedotin therapy: a report of 5 cases from the Southern Network on Adverse Reactions (SONAR) project. Cancer. 2014;120(16):2464–71.

Stem Cell Transplantation: Infection Risk by Transplant Type and Emerging Trends in Infection Management

4

Gowri Satyanarayana, Katie S. Gatwood, and Whitney J. Nesbitt

Contents

Introduction 44
Risk of Infection Post-HSCT 45
General Immune Reconstitution After Hematopoietic Stem Cell Transplantation 45
Aplastic Period 46
NK Cells and T-Cells 46
B-Cells and Immunoglobulins 47
Graft-Versus-Host Disease 47
Special Donor Populations 48
Umbilical Cord Blood Transplantation 48
Haploidentical Transplantation 49
Pre-HSCT Recipient Infectious Disease Evaluation 49
Prophylaxis and Management of Infection Post-HSCT 50
Bacterial Infections 50
Diagnosis 51
Management 52
Bacterial Pneumonia 53
Diagnosis 54
Management 55
Fungal Infections 57

G. Satyanarayana (✉)
Division of Infectious Diseases, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
e-mail: gowri.satyanarayana@vumc.org

K. S. Gatwood · W. J. Nesbitt
Department of Pharmaceutical Services, Vanderbilt University Medical Center, Nashville, TN, USA
e-mail: katie.s.gatwood@vumc.org; whitney.nesbitt@vumc.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_3

Yeasts 57
Disease Epidemiology 57
Diagnosis 58
Management 59
Molds 60
Disease Epidemiology 60
Clinical Impact 60
Diagnosis 61
Management 63
Viral Infections 64
Herpes Simplex Virus 64
Varicella Zoster 64
Cytomegalovirus 65
Diagnosis 65
Epstein-Barr Virus 67
Adenovirus 67
BK Virus 68
Parasitic Infections 68
Toxoplasmosis 68
Post-HSCT Vaccinations 69
Conclusions 69
Key Points 71
Cross-References 71
References 72

Introduction

Since its inception in the 1960s, HSCT has revolutionized the management of malignant and nonmalignant diseases. With the development of protocols using reduced intensity conditioning regimens and alternate donor sources, such as cord blood and haploidentical donors, an increasing number of patients are eligible for allogeneic HSCT.

Infections after HSCT can result from chemotherapy-related neutropenia in addition to immunosuppression caused by medications used for prophylaxis and treatment of graft-versus-host disease (GVHD) and GVHD itself [1]. A large prospective multicenter study of infections after HSCT reported an infection in 93% of transplant patients in their cohort, with 21% infection-attributable mortality [2]. Bacterial infections such as bacteremia and *Clostridioides difficile* infection were the most common infectious complications, followed by viral infections and fungal diseases. Another large study evaluating infections in patients undergoing non-matched related donor HSCT for treatment of acute leukemia reported bacterial or viral infections in over 50% of patients and fewer with fungal diseases [3].

This chapter will highlight the risk of infectious complications based on the type of HSCT and discuss new trends in management of specific infections in this patient population.

Risk of Infection Post-HSCT

General Immune Reconstitution After Hematopoietic Stem Cell Transplantation

A major determinant of risk of infection after HSCT is immune reconstitution of the different compartments of the immune system. Deficiencies in natural killer (NK) cells, T-cells, B-cells, or immunoglobulins can predispose patients to infection within and beyond 1 year post-HSCT (Fig. 1) and are highly dependent on multiple patient- and transplant-related factors, which will determine the kinetics and extent of recovery of each component (Table 1).

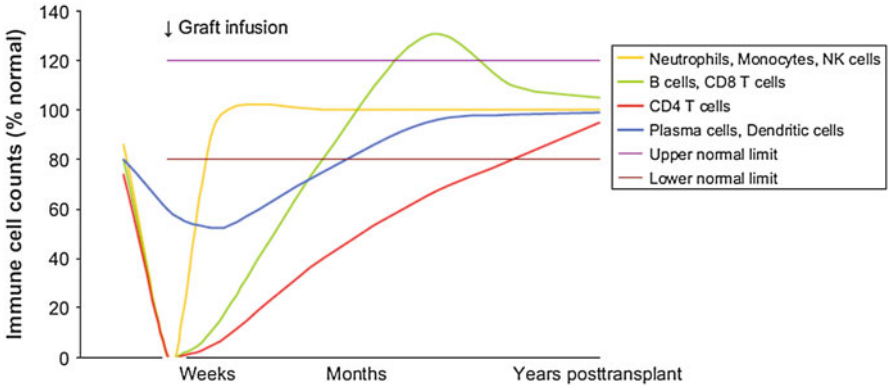


Fig. 1 Timeline of immune reconstitution post-allo HCT. (**License**: 4657680706894. PMID 19747629)

Table 1 Factors impacting post-allogeneic HCT immune reconstitution

Pretransplant factors	Transplant factors	Posttransplant factors
Age	Graft source	Thymic function
Comorbidities	Donor-recipient matching	GVHD
Underlying disease	CD34+ cell dose	Immunosuppressive agents for GVHD treatment
Prior treatment	Conditioning regimen intensity	Infection
	Ex vivo T-cell depletion	Nutritional status
	T-cell depletion (serotherapy)	Disease relapse

Aplastic Period

The initial phase after autologous and allogeneic HSCT is an aplastic phase produced by the conditioning regimen. The major determinant of infection risk during this time period is the depth and duration of neutropenia. Time to neutrophil recovery can be variable and is influenced by the source of hematopoietic cells. On average, recovery can be seen within 14 days after peripheral blood, 21 days after bone marrow, and 30 days after umbilical cord blood transplants [4]. CD34+ cell dose is directly correlated with time to engraftment and risk of infection during this period, with higher doses shortening the duration of neutropenia [4]. Infections occurring during this timeframe are commonly caused by bacterial and fungal pathogens. The extent of myelosuppression tends to be less severe with the use of reduced intensity conditioning regimens, as compared to myeloablative regimens [5]. Additionally, patients with prolonged periods of pretransplant neutropenia, such as patients with aplastic anemia or recent chemotherapy administration, are at an increased risk for infection during the posttransplant aplastic period, particularly with regard to fungal pathogens [5].

NK Cells and T-Cells

Natural killer (NK) cell recovery is dependent on the generation of donor-derived lymphoid progenitor cells [5]. NK cell numbers may be deficient up to 1 year post-allogeneic HSCT but are typically the first lymphoid cell line to fully recover [5]. T-cell reconstitution following allogeneic HSCT occurs via two distinct pathways. First, in the early posttransplant period, CD8+ memory T-cells preferentially expand, leading to an inverted CD4+/CD8+ ratio during this timeframe [4]. These are largely donor-derived T-cells although recipient cells which have survived conditioning chemotherapy may also be present. These T-cells are not especially responsive to central tolerance and will be more likely to cause GVHD [6]. Mature naïve CD4+ T-cell generation, comparatively, occurs in the thymus via donor-derived lymphoid progenitors and, therefore, is slower than CD8+ T-cell formation [4, 5]. It typically takes at least 100 days to reach a CD4+ T-cell count of 200/μL, which is one of the most reliable surrogate markers for estimating immune reconstitution in the HSCT population [4, 7]. However, many factors can impact thymic function and activity posttransplant and further delay reconstitution of the full T-cell repertoire [6]. The thymus is particularly sensitive to the high-dose chemotherapy used in HSCT conditioning regimens with damage occurring to both thymic epithelial cells and the thymic stroma [6]. Lymphodepleting agents, such as antithymocyte globulin and alemtuzumab, used to prevent GVHD and graft rejection or occasionally given pretransplant, as in the case of aplastic anemia, also lead to significant thymocyte depletion [6]. Age is another key factor impacting immune reconstitution due to age-related thymic involution resulting in decreased naïve T-cell generation [8]. This is particularly relevant as the upper age limit among patients receiving transplants has steadily increased over the last decade with improvements in transplant approaches,

such as the use of reduced intensity and nonmyeloablative conditioning regimens. Studies have shown markers of thymic-derived naïve T-cells remain low for 3–6 months after HSCT [9]. Therefore, sufficient T-cell reconstitution may not occur until 6–12 months post-allogeneic HSCT, at the earliest. Deficiencies in these cytotoxic lymphocyte cells predispose patients to viral infections and fungal diseases in particular.

B-Cells and Immunoglobulins

B-cell recovery after allogeneic HSCT can range from 1 to 5 years post-HSCT and is impacted by antigen exposure, interactions with CD4+ T-cells, and degree of GVHD [4, 7, 10]. Naïve B-cells often reconstitute before memory B-cells [4, 10]. Within 3–6 months after allogeneic HSCT, normal levels of serum IgM can be measured, followed by IgG and IgA [4]. Administration of antithymocyte globulin significantly impacts naïve and memory B-cell regeneration, delaying recovery by approximately 5 months [4]. Impaired B-cell and immunoglobulin recovery can predispose patients to infections due to encapsulated bacteria and viruses. Additionally, even once full recovery of B-cell numbers occurs, impaired responses to vaccines have been documented, and it is generally recommended to obtain titers following vaccination to ensure adequate protection [4].

Graft-Versus-Host Disease

GVHD occurs post-HSCT when donor T-cells respond to genetically defined proteins on host cells encoded by the major histocompatibility complex (MHC), minor histocompatibility antigens, or both [11, 12]. A sequence of steps including activation and proliferation of donor immune cells followed by target tissue destruction leads to acute GVHD (aGVHD) [11, 12], which most commonly occurs within the first 100 days post-HSCT and involves the skin, gastrointestinal tract, and liver [11, 12]. The pathophysiology of chronic GVHD (cGVHD) is more complex and is thought to involve thymic dysregulation leading to alloreactivity of T-cells, B-cells, and innate immune cells [13]. Chronic GVHD typically occurs later in the transplant course and can affect nearly all organ systems of the body [13]. Additionally, GVHD can cause direct damage to thymic epithelial cells due to their high levels of MHC expression which can lead to decreased thymic output of naïve CD4+ T-cells and further disruption of the T-cell repertoire [6]. It also inhibits appropriate negative selection and regulatory T-cell development, which contributes to the development of cGVHD [6].

Prevention of GVHD includes the use of medications such as T-cell-depleting agents (antithymocyte globulin, alemtuzumab), calcineurin inhibitors (tacrolimus, cyclosporine), inhibitors of cellular proliferation (mycophenolate mofetil, methotrexate), and mammalian target of rapamycin (mTOR) inhibitors (sirolimus). The standard first-line treatment for patients with GVHD is corticosteroid therapy [14].

Glucocorticoids are also known to cause thymic damage and atrophy and can therefore further impede the ability to reconstitute the immune system when used post-HSCT by leading to decreased production of naïve CD4+ T-cells [6]. There is no preferred second-line treatment regimen for GVHD that is unresponsive to steroids [11]. Immunosuppressive therapies most commonly utilized include extracorporeal photopheresis, rituximab, infliximab, etanercept, basiliximab, alemtuzumab, tocilizumab, pentostatin, low-dose interleukin-2, alpha-1 anti-trypsin, and imatinib [12, 15]. Recently, two agents have gained the first-ever FDA approvals for steroid-refractory GVHD. The Bruton's tyrosine kinase inhibitor, ibrutinib, was approved in 2017 for steroid-refractory cGVHD, and the Janus kinase JAK1/JAK2 inhibitor, ruxolitinib, was approved in 2019 for steroid-refractory aGVHD [16, 17]. Both agents are increasingly being utilized in practice. Therefore, both the immune mechanisms underlying GVHD and the treatment modalities for GVHD predispose post-HSCT patients to infections. Many of the agents used for treatment of GVHD increase the susceptibility to infections due to viruses and opportunistic pathogens.

Special Donor Populations

Due to delays in finding suitable donors and the limited donor pool for non-Caucasian and mixed ethnicity patients, alternate donor strategies have been developed and studied. These include allogeneic HSCT using umbilical cord blood units and HLA haplotype mismatched or haploidentical donors. Each strategy is associated with distinct patterns of immune reconstitution post-HSCT and, therefore, patient susceptibility to infection.

Umbilical Cord Blood Transplantation

Umbilical cord blood transplantation (UCBT) utilizes preserved umbilical cord blood (UCB) as the source of hematopoietic cells. Initial studies were performed in pediatric patients using a single UCB unit. Later studies found using a single UCB unit for adult allogeneic HSCT provided insufficient numbers of hematopoietic cells to achieve engraftment, so techniques such as ex vivo UCB expansion and use of double UCB units were developed to afford adequate amounts of hematopoietic cells for adults [18, 19].

Adult recipients of UCB transplants have been found to have longer median days to neutrophil engraftment, ranging from 27 to 29 days post-HSCT [20, 21]. Also noted are slow thymic T-cell recovery extending beyond 1 year post-HSCT [21, 22] and B-cell immune reconstitution starting at approximately 6 months post-HSCT [23]. UCB also contains regulatory T-cells which are potent suppressors of the immune response [24, 25].

Patients who have undergone UCB transplant are considered immunologically naïve and are susceptible for prolonged periods to both bacterial infections as a result of longer durations to neutrophil recovery and viral infections due to delays in T-cell immune reconstitution [3, 20].

Haploidentical Transplantation

Haploidentical donors are available for the majority of patients without significant delays in donor identification and therefore are a highly convenient donor source. Due to HLA haplotype mismatches, patients undergoing haploidentical HSCT are at an increased risk of T-cell-mediated processes such as rejection and GVHD [26]. Transplantation strategies have focused on modulating the T-cell content and activity of haploidentical grafts, specifically, with the use of T-cell-depleted grafts or unmanipulated grafts with post-HSCT alteration of T-cells using the administration of medications such as cyclophosphamide [26, 27].

In both strategies, NK and B-cell immune reconstitution demonstrate similar durations compared to traditional allogeneic HSCT. T-cell reconstitution is slower for T-cell-depleted haploidentical HSCT compared to unmanipulated haploidentical HSCT, with published reports of low to normal numbers of CD4+ T-cells being detected 180 days post-T-cell-depleted haploidentical HSCT [28]. This effect is attributed to the permissive effects cyclophosphamide has on naïve and memory T-cells which are able to reconstitute the immune system more efficiently as compared to T-cell-depleted grafts which rely solely on T-cell reconstitution through de novo generation of bone marrow lymphoid progenitors [29]. Consequently, while significant increases in both fungal diseases and viral infections have been found in patients receiving T-cell-depleted HSCT [28], only an increase in viral infections until day 100 post-HSCT has been noted in patients undergoing T-cell-replete haploidentical HSCT [30]. Additionally, time to neutrophil engraftment has been found to be significantly longer in haploidentical HSCT as compared to matched related and matched unrelated donor allogeneic HSCT, though shorter as compared to UCB transplantation [31, 32].

Pre-HSCT Recipient Infectious Disease Evaluation

The pre-HSCT recipient should be evaluated for evidence of protective antibody and exposure to several infections outlined in Table 2 [5, 33]. The results of these laboratory tests enable clinicians to risk stratify the recipient for development of specific infections post-HSCT. Current guidelines recommend pretransplant hepatitis B immunization for HSCT recipients from donors who are anti-hepatitis B core antibody positive. Revaccination is recommended starting at 6 months post-HSCT for recipients who lost detectable pre-HSCT hepatitis B surface antibody and for those who had no evidence of pre-HSCT hepatitis B surface antibody (please see section "Post-HSCT Vaccinations") [5, 34].

Table 2 Recipient pretransplant infection assessment

Infection	Pretransplant assessment
Cytomegalovirus (CMV)	CMV IGG[a]
Epstein-Barr virus (EBV)	EBV IGG
Hepatitis B virus (HBV)	HBV surface antigen HBV surface antibody HBV core antibody
Hepatitis C virus (HCV)	HCV antibody
Herpes simplex 1/2 (HSV)	HSV 1 and 2 IGG
Human immunodeficiency virus (HIV)	HIV antibody
Syphilis	Treponema antibody
Tuberculosis	Purified protein derivative (PDD) Quantiferon gold test T-spot test
Toxoplasma	Toxoplasma IGG
Varicella zoster virus (VZV)	VZV IGG

[a]*IGG* immunoglobulin G

Prophylaxis and Management of Infection Post-HSCT

Bacterial Infections

Bloodstream Infections

Bacterial infection is a common and life-threatening complication of HSCT, particularly in the pre-engraftment phase. General risk factors for development of bacterial infections after HSCT include the source of stem cells, use of myeloablative conditioning regimens, duration of neutropenia, underlying medical comorbidities, presence of central venous catheters (CVCs), and the presence of GVHD. Compared to autologous HSCT, pre-engraftment neutropenia lasts significantly longer among allogeneic HSCT recipients who receive myeloablative conditioning regimens, thus placing this group at higher risk of bacterial infections with reports of twice the cumulative risk for gram-negative bacteremia in allogeneic HSCT compared to autologous HSCT recipients (17.3% vs. 9.0%) [35]. Patients who receive UCB transplantation and haploidentical HSCT experience longer pre-engraftment neutropenia compared to those who receive peripheral blood grafts, so are at higher risk of bacterial infections. CVCs have considerably improved the management of patients undergoing HSCT by facilitating the administration of chemotherapy, stem cell infusion, intravenous medications, electrolyte supplementation, nutritional support, and blood products [36]. The incidence of CVC-related infections in this patient population is particularly high, ranging as high as 21%, and is associated with significant morbidity [37].

Bloodstream infections are the most commonly documented types of bacterial infection after HSCT (20–30%), followed by pneumonia, skin and soft tissue infections, and enterocolitis [35]. The epidemiology of bacterial pathogen etiology

has changed over time. In the 1960s–1970s, infections due to gram-negative bacteria were most common; however, beginning in the 1980s, gram-positive bacteria have predominated. Within the last decade, many institutions have reported a shift back toward gram-negative bacteria, particularly with multidrug-resistant isolates [38–40]. Increased severity of underlying pre-HSCT hematologic malignancy with prolonged durations of neutropenia, use of antibiotic prophylaxis, and protracted use of broad-spectrum antibiotics have contributed to this shift.

The use of prophylactic antibiotics, fluoroquinolones most commonly, during periods of neutropenia in HSCT patients remains the standard of care for prevention of bacterial infections and is endorsed by multiple guidelines [5, 33, 41]. Routine prophylaxis has been shown to decrease the incidence of febrile neutropenia and all-cause mortality [41]. However, data continues to emerge regarding adverse effects, increased antimicrobial resistance, and disruptions of the gut microbiome associated with fluoroquinolone prophylaxis, and efforts have been made to limit their use whenever possible [42]. In particular, there has been a recent focus in the literature on loss of diversity of the gut microbiome in HSCT patients as a result of fluoroquinolone prophylaxis and broad-spectrum antibiotic treatment. Outside of periods of neutropenia, bacterial prophylaxis with penicillins is also recommended in patients with cGVHD, as they are at an increased risk for *Streptococcus pneumoniae* infection [5, 43]. This loss of diversity has been correlated with increased rates of *Clostridioides difficile* infection, intestinal inflammation, and gastrointestinal GVHD [42].

Diagnosis

Obtaining blood cultures is a crucial determinant of detecting a bloodstream infection. It is recommended that an evaluation of fever in neutropenic patients with cancer should consist of blood culture sets from all CVC lumens (if present) and one set from a peripheral vein [44]. Multiple blood culture sets from different sites are recommended based on studies which found that two blood culture sets detect 80–90% of bloodstream infections in critically ill patients, whereas three or more sets of blood cultures are required to increase detection to greater than 96% [45, 46]. Positive blood cultures drawn from CVCs and a peripheral vein can aid in the determination of source of the infection based on differential time to positivity. A time difference for blood culture positivity of greater than 120 min between peripheral vein and CVC blood cultures, with CVC blood culture turning positive first, is suggestive of a central line-associated bloodstream infection [44].

The implementation of rapid diagnostic tests (RDTs) for bloodstream infections, particularly when paired with antimicrobial stewardship practices, has demonstrated significant decreases in mortality and time to effective antibiotic therapy, even among patients with cancer [47, 48]. Available RDTs for bloodstream infections may detect both bacterial and fungal pathogens from blood cultures within 20–150 min after blood cultures turn positive [49]. Additionally, some RDTs may also detect the genetic presence of certain resistance mechanisms among bacteria, such as

mecA, *vanA/B*, CTX-M, or even NDM, which often aids in the appropriateness of antibiotic therapy and resultant decreases in the time to effective therapy and risk of mortality.

Management

Given the inter-institutional variability in the incidence and epidemiology of bacterial infections, HSCT centers should emphasize the importance of infection control measures and antimicrobial stewardship practices. Hand hygiene, environmental cleaning, screening for colonization with resistant bacteria (e.g., MRSA, VRE, or ESBL-producing gram-negative bacteria), and isolation or cohorting of patients with evidence of colonization, active infection, or a history of resistant bacteria are important infection control measures. Antimicrobial stewardship should be a collaborative effort between hematology physicians and pharmacists, infectious disease providers, nursing staff, and members of the antimicrobial stewardship team to provide optimal empiric therapy for patients with febrile neutropenia. Current therapy generally consists of antibiotics active against most gram-negatives, including *P. aeruginosa*, as well as MRSA in patients who meet defined risk factors. However, an individualized approach to defining an optimal empiric antibiotic regimen may be implemented which accounts for local epidemiology including bacterial resistance patterns, individual patient risk factors, and severity of infection.

Gram-Positive Bacterial Infections

In addition to vancomycin or teicoplanin, antibiotics effective in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection are outlined in Table 3.

Fewer treatment options exist for infections due to vancomycin-resistant *Enterococcus* (VRE) (Table 3). Doses of daptomycin in the range of 8–12 mg/kg are recommended for the treatment of VRE bacteremia when exhibiting higher daptomycin MICs, particularly those defined as susceptible dose-dependent per

Table 3 Gram-positive infection treatment

Etiology and site of infection	Treatment
MRSA bacteremia	Vancomycin, teicoplanin, daptomycin, telavancin, ceftaroline
Persistent MRSA bacteremia salvage therapy	Ceftaroline +/− daptomycin
MRSA pneumonia and skin and soft tissue	Linezolid, tedizolid, oritavancin, dalbavancin
VRE bacteremia	Daptomycin, linezolid, quinupristin-dalfopristin[a], tigecycline, oritavancin[b], dalbavancin[c], and fosfomycin IV

[a] *E. faecium* only
[b] VanA phenotype
[c] VanB phenotype

updated CLSI breakpoints. Linezolid has been extensively studied for the treatment of VRE, including bacteremia; however, bacteriostatic activity, drug-drug interactions, thrombocytopenia, and leukopenia may limit its use in HSCT patients. Data regarding the use of lipoglycopeptides, oritavancin and dalbavancin, have demonstrated variable efficacy. Synergistic combinations, such as daptomycin plus ampicillin or daptomycin plus ceftaroline, have been effective against persistent VRE bacteremia and for VRE with decreased susceptibility to daptomycin and/or linezolid [50, 51].

Multidrug-Resistant Gram-Negative Bacterial Infections

Carbapenems remain the drugs of choice for systemic extended spectrum beta-lactamase (ESBL)-producing gram-negative bacteria [52]. Piperacillin-tazobactam, nitrofurantoin, and oral fosfomycin are therapeutic options for treatment of ESBL cystitis. Tigecycline may be a useful carbapenem-sparing option for the treatment of complicated intra-abdominal infections due to ESBL-producing pathogens. New carbapenem-sparing beta-lactam-beta-lactamase inhibitors (BL-BLIs) ceftolozane-tazobactam and ceftazidime-avibactam, and carbapenem BL-BLIs such as meropenem-vaborbactam and imipenem-relebactam, have in vitro activity against ESBL-producing organisms. The novel siderophore cephalosporin, cefiderocol, currently in the development pipeline has a similar chemical structure to both ceftazidime and cefepime but with increased stability against ESBL production.

Carbapenem-resistant gram-negative bacteria pose a growing challenge. Combinations of antibiotics often including aminoglycosides, polymyxins, and/or tigecycline are frequently used empirically in order to increase the probability of adequate therapy. Depending on the mechanism of carbapenem resistance, treatment with a single agent such as ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam has efficacy against carbapenem-resistant gram-negative organisms. Cefiderocol has also demonstrated in vitro activity. Additionally, tigecycline possesses activity against carbapenem-resistant gram-negative bacteria with the exception of *P. aeruginosa.* Additionally, this antibiotic should not be used for the treatment of bacteremia or urinary tract infections due to limited drug concentrations achieved in these areas.

Multidrug-resistant *P. aeruginosa* has limited treatment options. Empiric regimens often include combinations of a carbapenem plus polymyxin or aminoglycosides. Fosfomycin has also demonstrated activity and may be a treatment option. Among the new BL-BLIs, ceftolozane-tazobactam has demonstrated the most potent activity.

Bacterial Pneumonia

The incidence of bacterial pneumonia after autologous and allogeneic HSCT has been reported to range between 15 and 25% in retrospective studies [53, 54]. One large prospective study of 427 allogeneic HSCT patients reported bacteria being the most common etiology of pneumonia in their cohort, with 44% of all pneumonias

attributed to bacterial pathogens [55]. *Escherichia coli* and *Pseudomonas aeruginosa* were the most frequently isolated bacteria in this multicenter study. Factors that may influence the bacterial etiology include prior bacterial colonization of the recipient, post-HSCT neutropenia, administration of post-HSCT antibacterial prophylaxis, and receipt of vaccinations post-HSCT. This leads to some variability post-HSCT regarding which bacteria may be causing pneumonia, but bacteria such as *E. coli*, *P. aeruginosa*, *Streptococcus* species, and *Staphylococcus aureus* remain significant pathogens.

Clostridioides Difficile

The incidence of *C. difficile* infection (CDI) in HSCT patients is reported to be up to ninefold higher than for hospitalized patients without cancer [56] and twofold higher in allogeneic versus autologous HSCT patients [57, 58]. The severity of CDI in HSCT patients is generally mild to moderate, occurring most frequently in the early posttransplant period, with conflicting mortality data [59, 60]. Due to myeloablative conditioning regimens and slow immune reconstitution, HSCT patients have lower levels of protective antibodies, and CDI in this patient population has been associated with decreased antibody responses to *C. difficile* toxins A and B [61, 62].

Risk factors for CDI such as age $>$ 65 years and proton pump inhibitor use also apply to HSCT patients; however, risk factors such as immunosuppression, antibiotic exposure, and healthcare contact are more frequent in this patient population. Other reported risk factors for CDI in HSCT patients include aGVHD, mucositis, CMV reactivation, HSV or VZV reactivation, and intestinal dysbiosis [60, 63, 64]. Intestinal microbiome diversity is a protective host factor against *C. difficile* and other invasive bacteria. Receipt of antibiotics early in the posttransplant period is associated with decreased microbial diversity and increased transplant-related mortality [65]. In the setting of intestinal dysbiosis, there is loss of gastrointestinal microbial diversity, leading to alterations in intestinal metabolism, allowing for colonization by pathogenic bacteria such as *C. difficile*.

Diagnosis

Current guidelines recommend *C. difficile* testing within 24 h for patients who have greater than or equal to three unformed stools new in onset and no attributable etiology.

The most frequently used laboratory methods for detection of CDI include enzyme-linked immunosorbent assays (EIA) which target glutamate dehydrogenase (GDH) or toxins A and B or nucleic acid amplification tests (NAAT) which are most often polymerase-based reaction (PCR) tests. The EIA for GDH is highly sensitive but not specific, and the NAAT is highly sensitive and moderately specific. Given the high rate of NAAT sensitivity, detection of *C. difficile* colonization is common. Diagnosis of *C. difficile* post-HSCT can be complicated by other causes of diarrhea in this setting, including conditioning regimen-related toxicity, dietary supplementation, and gastrointestinal GVHD.

Table 4 *Clostridioides difficile* testing algorithm for HCT patients

Glutamate dehydrogenase + toxin A and B OR
Glutamate dehydrogenase + nucleic acid amplification test OR
Nucleic acid amplification test + toxin A and B

Table 5 *Clostridioides difficile* management

Initial episode	Vancomycin 125 mg PO or Fidaxomicin 200 mg PO BID
First and subsequent recurrence	Vancomycin 125 mg PO q6h or Vancomycin taper or Fidaxomicin 200 mg PO BID
Second and subsequent recurrence	Fecal microbiota transplant
Fulminant infection	Vancomycin 500 mg PO q6h + intravenous metronidazole 500 mg q8h +/− vancomycin 500 mg q6h per rectum or Surgical intervention
Infection complicated by ileus	Rectal vancomycin instillation 500 mg q6h
Secondary prophylaxis	Vancomycin 125 mg PO daily or Fidaxomicin 200 mg PO daily

Due to data suggesting poor performance of toxin assays in immunocompromised patients [66], guideline-recommended methods for detection of CDI include the use of a stool toxin test as part of a multistep algorithm for HSCT patients (Table 4) [67].

Management

In the United States, management of patients with CDI involves discontinuation of the inciting antibiotic(s) when possible, and treatment modalities are outlined in Table 5.

Oral vancomycin and fidaxomicin are recommended preferentially over metronidazole for initial non-severe and severe episodes due to superior efficacy [67, 68]. Although one small retrospective review of patients with hematologic malignancies who received oral vancomycin or metronidazole for the treatment of CDI showed no difference in treatment outcomes between the two groups [69], subsequent studies have demonstrated superior efficacy of oral vancomycin over metronidazole and reductions in mortality [70, 71].

Initial treatment responses to oral vancomycin and fidaxomicin have been shown to be equivalent [72, 73]. In a post hoc analysis, lower overall treatment responses (79% vs. 87%), prolonged durations of diarrhea, and overall cure rates did not differ among patients with cancer compared to the general study population (89% vs. 89%,

respectively). Although not statistically significant, fidaxomicin demonstrated higher cure rates compared to oral vancomycin in patients with cancer (85% vs. 74%, respectively), and the odds of a sustained response were 2.6-fold higher in the fidaxomicin group ($P = 0.003$) [74]. Other treatment modalities for clinical presentations associated with CDI are shown in Table 5 [67].

Recurrent CDI

Rates of recurrent CDI are similar among immunocompetent patients and those post-HSCT [67, 75]. As outlined in Table 5, several strategies may be considered for the treatment of recurrent CDI. In a retrospective, propensity-matched study of patients with first or second CDI recurrences, there was no difference in the combined outcome of clinical failure or 90-day recurrence between fidaxomicin and oral vancomycin [76]. Of note, only 18% of the population in this study had a hematologic malignancy. Fidaxomicin has been studied as an extended-pulsed dosing regimen compared to standard doses of oral vancomycin as treatment for initial episodes of CDI. The extended-pulsed fidaxomicin regimen significantly improved sustained clinical cure in patients over age 60 years and reduced CDI recurrence rates up to 90 days after treatment initiation versus standard dose oral vancomycin [77].

Fecal microbiota transplantation (FMT) is a guideline-recommended option for the treatment of second and subsequent recurrences of CDI. FMT in HSCT patients has been associated with restoration of gastrointestinal microbiome diversity [78]. Clinically, FMT following HSCT has been reported to be a successful treatment option for recurrent CDI without FMT-related adverse events in several retrospective studies [79].

Bezlotoxumab, an intravenous monoclonal antibody directed against toxin B *C. difficile*, may be a treatment option to prevent cases of recurrent CDI. In pooled data from two large randomized trials, the addition of bezlotoxumab to antibiotic treatment for CDI reduced recurrent CDI rates from 27% in the antibiotic treatment plus placebo arm to 17% in the adjunctive bezlotoxumab arm ($P < 0.0001$) but did not affect clinical cure. The benefits of bezlotoxumab were most pronounced in patients most at risk for recurrence, including those who were immunocompromised, with a 36% rate of recurrence in patients who received antibiotic treatment plus placebo compared to 19% in patients treated with adjunctive bezlotoxumab [80].

Secondary Prophylaxis

The receipt of antibiotics after an episode of CDI is a significant risk factor for recurrent CDI and one that is oftentimes unavoidable early post-HSCT. Therefore, recommendations for the use of prophylaxis are outlined in Table 5. Prophylaxis with oral vancomycin or fidaxomicin versus placebo for HSCT patients has been associated with significantly lower rates of CDI recurrence [81, 82]. Oral vancomycin prophylaxis has also been studied in the setting of multiple CDI recurrences compared to placebo. These studies have raised concern that oral vancomycin prophylaxis may not be as effective for multiple CDI recurrences (> 1 prior episode) versus its use following one episode of CDI [83, 84].

Fungal Infections

Medical advances in the treatment of cancer, notably new chemotherapeutic agents and HSCT, have increased the number of patients susceptible to invasive fungal disease (IFD). The use of broad-spectrum antibiotics and antifungals in this population is common and has led to changes at the patient level as well as the global microbiome, leading to the emergence of drug-resistant fungal pathogens. In parallel, advances in technologies used to identify fungi have led to earlier detection of fungal infections as well as recognition of new species of fungi and novel susceptibility patterns.

The gold standard diagnosis of invasive fungal disease relies on identification of the fungus by histopathology, cytopathology, or direct microscopy or fungal growth in culture of specimen obtained by sterile needle aspiration or biopsy of an abnormal site [85]. Several limitations prohibit obtaining sterile material in the HSCT population, including risk of bleeding due to coagulopathy or thrombocytopenia, pneumothorax in cases of isolated pulmonary disease, and potentially death in critically ill patients. Therefore, clinicians sometimes rely on indirect tests for diagnosis.

Yeasts

Disease Epidemiology

Candida species have been the most common cause of IFD in patients who have undergone HSCT. Colonization of the gastrointestinal tract with *Candida* followed by administration of cytotoxic chemotherapy leading to injury of the gastrointestinal mucosal barrier is one of the most important risk factors for invasive candidiasis during neutropenia [86, 87]. Ninety percent (90%) of invasive human disease is caused by *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei. C. albicans*, which is generally susceptible to fluconazole, has long been the most prevalent species implicated in invasive candidiasis. Widespread use of antifungal prophylaxis targeting *Candida* has led to a decrease in overall rates of *Candida albicans* infections, with an increase in infections caused by more fluconazole-resistant species such as *C. glabrata* and *C. krusei* [88, 89]. The TRANSNET surveillance system, which performed prospective surveillance of 22 HSCT centers, found *Candida glabrata* to be the most common cause of invasive candidiasis (33%), followed by *Candida albicans* (20%) [90]. While *Cryptococcus* remains a significant fungal infection after solid-organ transplant, its impact post-HSCT is not as considerable, with the TRANSNET surveillance system reporting only a 0.4% incidence of *Cryptococcus* in their HSCT cohort [90].

Candida auris

One of the most alarming changes in the epidemiology of invasive candidiasis has been the global emergence of the multidrug-resistant healthcare-associated fungal pathogen *Candida auris*. Since initial isolation in 2009 from the external ear canal of

a patient in Japan, *C. auris* has been reported from 35 countries from 6 continents, and 685 confirmed cases of *C. auris* have been reported in the United States [91]. *C. auris* infections have been associated with nosocomial outbreaks within intensive care units in patients with serious underlying medical conditions such as those with underlying hematologic malignancy and immunocompromised, requiring invasive procedures and receipt of prior antifungal therapy [92–94].

C. auris most commonly causes candidemia, with mortality rates over 50% reported in the United States, but has also been isolated from other sites in the body [92, 93, 95]. *C. auris* infection poses many challenges including difficulties in identification, unknown population prevalence, propensity for environmental spread, virulence factors, and inherent antifungal resistance.

Diagnosis

Blood Cultures

A large multinational prospective cohort study reported a 1.6% incidence of fungemia in HSCT recipients [96]. In many cases, initial fungal blood cultures may be negative, with reports of sensitivity as low as 30% for the diagnosis of invasive candidiasis in neutropenic patients and missed diagnoses of disseminated candidiasis in more than 50% of patients [97–99].

Fungal Antigen Testing: Serum 1,3-Beta-D-Glucan

1,3-Beta-D-glucan (BG) is a polysaccharide in the cell wall of most fungi with the exception of *Mucorales* and *Cryptococcus* [100]. Commercially available tests rely on activation of the coagulation cascade of the horseshoe crab in the presence of BG, with a positive test detecting concentrations of BG in the picogram per milliliter (pg/mL) range. The Fungitell assay, which is FDA approved, reports a positive result as a concentration greater than 80 pg/mL [101].

The reported pooled sensitivity and specificity of one positive BG test in the setting of proven or probable IFD are 76.8% and 85.3%, respectively [100], while two consecutive positive BG tests had a sensitivity of 49.6% and specificity of 98.9% in a cohort of hematology-oncology patients with proven or probable IFD [102].

Invasive fungal infections due to *Candida* or *Aspergillus* species, two of the most common fungal pathogens which cause invasive infection during febrile neutropenia, as well as with *Pneumocystis*, can produce a positive glucan, so a positive result does not help identify the exact species of fungus causing infection. False-positive test results can occur in patients who have been treated with IV albumin, IV immunoglobulin, and hemodialysis with a cellulose-containing membrane [103].

Nanodiagnostics

Magnetic resonance technology has been used to create a panel that detects the DNA of five different species of *Candida*, including *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, and *Candida glabrata*, in the blood. The FDA-approved T2Candida panel has a sensitivity of 90% and specificity

of 98% for candidemia [104]. A 98% negative predictive value of the T2Candida panel for candidemia in neutropenic patients has been reported [104]. The T2Candida panel may be more useful than standard blood cultures when de-escalating empiric antifungal therapy, with reports of patients receiving fewer days of antifungal therapy when using the T2Candida panel [105–107]. Further studies utilizing this technology in the neutropenic and HSCT patient population are needed.

Candida auris-Specific Diagnostics

C. auris is most phylogenetically related to the *C. haemulonii* species complex. Using conventional phenotypic and biochemical diagnostic methods, *C. auris* has often been misidentified as *C. haemulonii* or other fungal species such as *C. famata*, *C. sake*, *Rhodotorula glutinis*, or *R. mucilaginosa* [93, 95, 108, 109]. The CDC recommends reporting *Candida* isolates from sterile site cultures to the species level. The use of chromogenic agar to differentiate between *C. auris* and *C. haemulonii* isolates has been suggested as a low-cost method for identification [109]. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a potentially accurate and rapid method for identification; however, *C. auris* has only been added to libraries for research use thus far [110]. PCR assays have been developed and show promise for rapid and accurate identification of *C. auris* [111].

Management

Candida Species

An echinocandin is recommended as the first-line therapeutic agent to treat candidemia in neutropenic patients and as initial treatment for *Candida krusei* and *glabrata*, until susceptibilities are known. Fluconazole is an acceptable alternative treatment for those patients who are not critically ill or do not have suspected azole-resistant *Candida* infection (i.e., no prior prophylaxis or other major fluconazole exposures). Liposomal amphotericin B is an alternative agent but has more potential for toxicity, and risks versus benefits of administration should be considered [112]. Removing central lines is essential. Ophthalmologic exams for endophthalmitis are also recommended, optimally when not neutropenic [44].

Candida auris

The treatment of *C. auris* is complicated by the lack of species-specific antifungal breakpoints and the organism's ability to harbor and develop multidrug resistance. To date, most isolates have displayed elevated fluconazole MICs ($\geq$ 16 μg/mL) and variable susceptibilities to other azoles. Resistance rates to voriconazole have ranged between 3 and 73%. Among the triazoles, posaconazole, isavuconazole, and itraconazole are generally considered the most potent in vitro. Although less common, elevated MICs have also been demonstrated for amphotericin and echinocandins [94, 113–115]. According to the CDC, the majority of *C. auris*

isolates are susceptible to echinocandins, and treatment with an antifungal from this class, along with infectious diseases consultation, is recommended. Liposomal amphotericin B could be considered for patients clinically unresponsive to echinocandin therapy or who have persistent *C. auris* fungemia for greater than 5 days [116].

Of concern, about 40% of isolates have been resistant to ≥2 antifungal drug classes, and a smaller percentage (4–10%) have been resistant to most antifungals [114]. The novel oral 1,3-β-D-glucan synthesis inhibitor ibrexafungerp (formerly SCY-078) has demonstrated potent in vitro activity against *C. auris* [115]; ibrexafungerp has been an effective treatment for *C. auris* candidemia, even in the setting of high-level echinocandin resistance [113].

Cryptococcus

HSCT patients who have mild to moderate pulmonary cryptococcal infection may be treated with 6–12 months of fluconazole therapy, depending on their level of immunosuppression and clinical course. Cryptococcus infections involving the central nervous system (CNS), causing severe pulmonary disease, or involving sites outside of the CNS or lungs should be treated with at least 2 weeks of liposomal or lipid complex amphotericin B and flucytosine, followed by fluconazole consolidation and maintenance [117]. Lumbar punctures are often recommended so as to help with planning of treatment. The duration of fluconazole therapy may be at least 6 months or more, with longer durations of treatment for those HSCT patients who remain on immunosuppressive therapy for GVHD.

Molds

Disease Epidemiology

Over the last two decades, invasive fungal disease (IFD) secondary to opportunistic molds has become more prevalent than *Candida* in HSCT recipients. The incidence of IFD after allogeneic HSCT has been reported to range from 6 to 8% [90, 118]. Infection secondary to *Aspergillus* species is the most common, followed by *Mucorales* and *Fusarium* species [1, 36, 118, 119].

Clinical Impact

Receipt of a T-cell-depleted allogeneic HSCT, degree and duration of neutropenia after HSCT, and development and treatment of acute and chronic GVHD with corticosteroids are important risk factors for the development of opportunistic mold infection [120]. Invasive mold infections have varied symptoms depending

upon the site/s of infection, with the lungs being the most common site. Symptoms of invasive pulmonary mold infection can range from isolated fever with no respiratory symptoms to pleuritic chest pain with or without cough. Once the diagnosis of IFD is made, careful examination of the patient for other foci of infection (i.e., brain, skin, other organs) is important since dissemination of mold infections can occur, especially during neutropenia. Extrapulmonary sites of IFD should be assessed for feasibility for surgical debridement in an attempt to control the infection.

Diagnosis

Culture

Diagnosis of IFD using standard culture methods may be challenging due to the risks of obtaining specimen from affected sterile sites in critically ill patients and a delay in IFD diagnosis due to slow growth of mold in culture [121]. Current guidelines provide detailed microbiologic criteria for IFD diagnosis [85].

Galactomannan

Galactomannan (GM) is a polysaccharide found in the cell walls of *Aspergillus* and *Penicillium* species and is released into the blood during fungal growth. The detection of GM in serum and/or BAL is a diagnostic tool for invasive aspergillosis.

The Platelia assay, an enzyme immunoassay, can be used to measure GM in serum and bronchoalveolar lavage fluid (BAL). A large meta-analysis found an overall sensitivity of 71% and specificity of 89% when using serum GM in cases of proven invasive aspergillosis [122]. Biweekly GM testing has been found to increase the diagnostic sensitivity for invasive aspergillosis (IA) in high-risk HSCT patients but may be associated with higher costs and fewer additional diagnoses of IA [123–125]. Two large meta-analyses evaluating BAL GM reported a pooled sensitivity of 86–87% and specificity of 89% in cases of proven or probable IA [126, 127].

The sensitivity of the test decreases in patients receiving anti-mold therapy. Several published reports described false-positive GM results in patients receiving piperacillin-tazobactam [128–131], though this problem has been attributed to contamination of the drug lot [131, 132]. False-positive serum GM test results have also been reported in patients with underlying *Histoplasma* infection due to cross-reactivity in the assay [133].

Aspergillus PCR

Several commercial *Aspergillus* PCR tests which target the 18S rDNA and/or the 28S rDNA are available, with reported sensitivity and specificity in bronchoalveolar lavage fluid greater than 70% and 80%, respectively [134]. The *Aspergillus* PCR in conjunction with the *Aspergillus* galactomannan may be a useful combination test for earlier diagnosis of pulmonary aspergillosis [134].

Radiographic Studies

Computed tomography (CT) of the chest may be useful in diagnosing IFD in neutropenic patients due to molds such as *Aspergillus* species or *Mucorales*. The radiographic morphology of a pulmonary opacity on a CT scan of the chest can be suggestive of a potential fungal etiology.

Invasive pulmonary aspergillosis classically manifests radiographically as a "halo sign" (Fig. 2), which is a mass-like infiltrate with a surrounding halo of ground-glass attenuation [135–137]. Other radiographic findings in the setting of invasive pulmonary aspergillosis at various stages of evolution include cavitary lesions, infarct-shaped nodules or consolidations, and air-crescent signs [138].

Invasive pulmonary mucormycosis has been linked to a different radiographic sign, the "reversed halo sign" (Fig. 3), which is a central ground-glass opacity surrounded by a denser airspace consolidation [139].

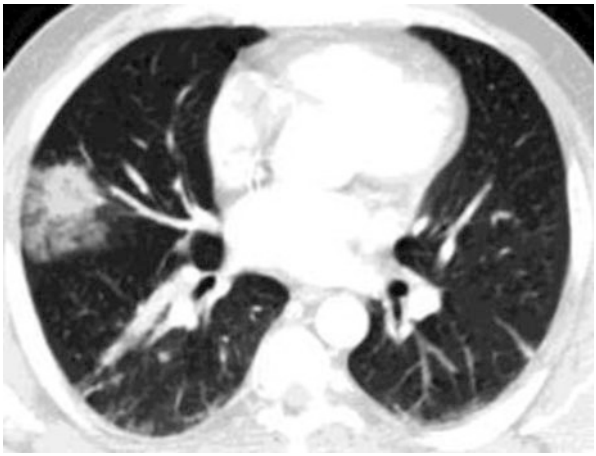


Fig. 2 Halo sign on chest computerized tomography. (Raveendran, S and Lu Zhiyan. *Radiology of Infectious Diseases*. Volume 5, Issue 1, March 2018, pp. 14–25, Fig. 3. Creative common license. Open access)

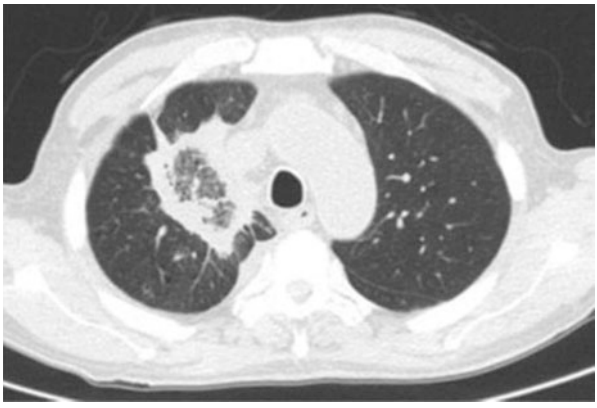


Fig. 3 Reversed halo sign. (Cornely et al. 2004 [150]. License: 4647870816622)

Fungal Metabolite Testing

Thermal desorption gas chromatography/mass spectrometry technology to detect fungal volatile metabolites found in breath specimens is a noninvasive diagnostic tool currently in development for detection of mold disease in HSCT patients [140, 141]. This technology may eventually be a rapid tool to diagnose fungal disease.

Management

Prophylaxis

Prevention of invasive fungal disease using antifungal prophylaxis has been studied in a subset of HSCT recipients [142]. This clinical trial found posaconazole to be non-inferior to fluconazole in prevention of invasive fungal disease in patients with GVHD, with the most significant impact in patients with Grade III–IV GVHD. The incidence of proven or probable IA was lower in patients who received posaconazole and mortality due to IFD was less in the posaconazole arm; however, there was no difference in overall mortality between the two groups.

Treatment

Historically, for cases of suspected or proven invasive *Aspergillus* infection, voriconazole has been recommended as first-line therapy and liposomal amphotericin as alternate therapy for patients who cannot receive voriconazole [120, 143]. A phase III double-blind non-inferiority study found a novel triazole, isavuconazole, to be non-inferior to voriconazole for the treatment of proven or probable IA [144] and led to the addition of isavuconazole as a first-line therapeutic option for *Aspergillus* infection [120]. Of note, there have been reports of breakthrough fungal infections due to *Candida* species, *Aspergillus* species, mucormycosis, and *Fusarium*, in patients on isavuconazole treatment [145, 146].

Of note, with the use of azoles in pesticides and agricultural demethylase inhibitors, there are reports of voriconazole-resistant *Aspergillus fumigatus* in Europe and azole-resistant *Aspergillus fumigatus* in the United States [147–149].

Use of amphotericin B deoxycholate and the more recent, less toxic lipid formulations and surgical debridement remain the mainstays of treatment for mucormycosis [150]. Posaconazole has been used as salvage therapy in patients who could not receive liposomal amphotericin B, with a reported success rate of 61–70% [151, 152], and remains a potential treatment option. In 2016, data were published showing isavuconazole had activity against several species of mucormycosis with comparable treatment outcomes to a matched historical group of patients who received amphotericin B for treatment of mucormycosis [153]. This study led to the approval of isavuconazole for the treatment of mucormycosis. In some cases of mucormycoses, isavuconazole might be an efficacious treatment option. An important consideration is variable susceptibility of the agents of mucormycosis to posaconazole and isavuconazole [154].

There are no recommendations for the optimal antifungal therapy for *Fusarium* infection, but initial treatment with voriconazole or lipid formulations of

amphotericin B has been suggested. In many cases, successful treatment has required varying combinations of antifungal medications while awaiting antifungal susceptibility testing results [155].

Pneumocystis Jiroveci

Pneumocystis jiroveci (PJP) is a fungal pathogen that can cause acute or subacute pulmonary infection in HSCT patients. Current guidelines recommend administration of prophylaxis directed again *Pneumocystis jiroveci* for 3–6 months in those autologous HSCT patients who have received intensive chemotherapy treatment or conditioning regimens or treatment with high-dose steroids post autologous HSCT and for a minimum of 6 months after allogeneic HSCT. The duration of prophylaxis post-allogeneic HSCT should be extended if the patient is receiving immunosuppressive therapy. Trimethoprim-sulfamethoxazole (TMP-SMX) is the preferred agent, but dapsone, aerosolized pentamidine, and oral atovaquone are alternative options.

Viral Infections

Herpes Simplex Virus

Given the ubiquity of herpes simplex virus 1/2 (HSV) seropositivity in the general population, there is an approximately 80% reactivation rate in the early post-HSCT period, particularly in the setting of mucositis [33]. All patients should be tested for serum anti-HSV IgG prior to transplant. Acyclovir prophylaxis for HSV seropositive (IgG) patients is recommended for a minimum of 30 days post-HSCT and is frequently extended to at least 1 year or longer post-HSCT to prevent HSV or VZV infection, especially in those patients who remain on intensive immunosuppression [5, 33]. Valacyclovir is presumed to be an equivalent substitute for acyclovir for HSV prophylaxis; however, data regarding safety and efficacy in the HSCT population is limited [5, 33, 156].

Varicella Zoster

All patients should be tested for serum anti-VZV IgG prior to transplant. Reactivation of VZV typically occurs within 3–12 months of transplantation, and a high proportion of patients will experience reactivation without adequate prophylaxis [5, 33, 157]. Therefore, long-term prophylaxis against VZV is recommended for all autologous and allogeneic HSCT recipients through the first year following transplantation or longer for patients with cGVHD remaining on immunosuppressive therapy [5, 21, 33]. Acyclovir and valacyclovir are the preferred agents for VZV prophylaxis, with valacyclovir having superior bioavailability over acyclovir [5, 33]. Due to the high morbidity associated with VZV disease, it is imperative that HSCT recipients avoid VZV exposure and that

seronegative caregivers and other household contacts receive VZV vaccination at least 4–6 weeks prior to HSCT [5, 33].

Cytomegalovirus

Although patients are at risk for many viral infections post-allogeneic HSCT, cytomegalovirus (CMV) infection remains the most clinically significant. CMV serologic status (IgG) of the recipient and donor is the most important risk factor for development of CMV reactivation, with CMV seropositive recipients of allogeneic HSCT from seronegative donors being at highest risk [158, 159]. Other risk factors for development of CMV infection include undergoing non-matched related donor HSCT, treatment with corticosteroids, and receipt of T-cell-depleting therapy. CMV reactivation occurs in 80% of seropositive recipients who do not receive prophylaxis [159], and mortality as high as 60% has been reported in patients who develop CMV disease [158]. Use of ruxolitinib for treatment of steroid-refractory acute GVHD has also recently emerged as a new risk factor for CMV reactivation post-HSCT [14]. The initial trial describing ruxolitinib's activity in the management of GVHD reported a CMV reactivation rate of 33% in aGVHD patients and 15% in cGVHD patients [160].

CMV reactivation post-allogeneic HSCT can result in detectable virus in the blood, CMV infection, and progression to end-organ disease. The majority of organs can be involved in CMV disease, with published definitions of specific end-organ diseases [161]. CMV has also been associated with indirect effects, specifically an increased risk of bacterial infections such as CDI and fungal diseases post-HSCT [161].

Diagnosis

Initial tests used CMV antigen testing (CMV pp65), with the majority of transplant centers now using quantitative CMV DNA polymerase chain reaction (PCR) and fewer using mRNA nucleic acid sequence-based amplification. International guidelines recommend use of real-time quantitative PCR for the diagnosis and monitoring of CMV viremia [162].

Assessment of CMV-specific immune reconstitution using CMV-specific enzyme-linked immunospot (ELISPOT) assay may help identify those patients at risk for developing CMV infection and disease after allogeneic HSCT, allowing clinicians to administer targeted antiviral prophylaxis and treatment [163].

Preemptive Management

The traditional approach post-HSCT was to monitor patients at risk for CMV reactivation and end-organ disease with weekly CMV DNA PCR until at least day 100 for evidence of CMV virus replication. An institution would determine the threshold viral load above which initiation of CMV treatment was started and patients

were initiated on anti-CMV therapy with an agent such as ganciclovir, valganciclovir, or foscarnet. Patients were generally treated until resolution of CMV viremia and possibly longer in cases of end-organ disease. Uses of preemptive screening and treatment strategies have reduced the risk of death from CMV to less than 10% [164].

Prophylaxis: Shift in Management

Over the last three decades, studies have attempted to identify a safe and effective CMV prophylactic strategy in allogeneic HSCT recipients [165]. Limitations to finding a suitable prophylactic agent included non-efficacious compounds and significant drug side effects. In 2017, an antiviral which inhibits the CMV viral terminase complex, letermovir, was approved for CMV prophylaxis in allogeneic HSCT recipients. Data from a phase III, randomized, double-blind, placebo-controlled study showed that CMV seropositive recipients who received letermovir prophylaxis by day 28 post-allogeneic HSCT had significantly less CMV infection than those receiving placebo or standard of care therapy with no significant differences in side effects between the two groups [166]. The identification of an effective and well-tolerated prophylactic agent has changed the landscape of HSCT, leading to a shift in management from a preemptive to prophylaxis strategy in many centers (Table 6).

Letermovir is an effective prophylaxis only against CMV and lacks prophylactic activity against other human herpes viruses. Therefore, additional prophylaxis for herpes simplex virus (HSV) and varicella zoster virus (VZV) with acyclovir or valacyclovir is recommended for HSCT patients. Mutations in the UL56 terminase gene has been shown to lead to letermovir resistance [167] with numerous such reports in clinical practice [168, 169].

Drug-Resistant CMV Infection

Foscarnet and cidofovir remain potential therapeutic options for HSCT patients who develop ganciclovir-resistant CMV infection. Abnormal renal function, significant electrolyte depletion, and inability to administer long-term intravenous antiviral therapy limit the use of these antivirals. Several phase II clinical trials have shown safety and efficacy of maribavir, an inhibitor of the pUL97 viral kinase, for treatment of patients with CMV viremia and drug-resistant CMV disease [170–172]. Currently, there are two phase III clinical trials evaluating maribavir treatment for CMV infections in HSCT patients and drug-resistant CMV infection in transplant patients.

Table 6 Cytomegalovirus management post-HCT

Preemptive therapy	Letermovir prophylaxis
Weekly cytomegalovirus viral load	Initiate prophylaxis post-HCT Initiate HSV/VZV prophylaxis post-HCT
Institution viral load threshold	Continue until at least day +100
Start antiviral therapy directed at CMV	Stop letermovir and start antiviral therapy directed at CMV for patients with infection

The only method to assure sustained control of CMV infection is to achieve immune reconstitution with CMV-specific CD4+ and CD8+ T-cells [130]. However, this is often delayed in the HSCT population for reasons highlighted throughout this chapter. Therefore, adoptive T-cell therapy is currently an active area of investigation for management of refractory CMV infection [173]. This can be done through initial HSCT donor-derived or third-party donor-derived T-cell engineering, with the former generally proving to be more successful [173].

Epstein-Barr Virus

Epstein-Barr virus (EBV) in the HSCT population most commonly arises from reactivation from the patient or donor, but primary infection can also occur [5, 40]. The primary concern associated with EBV reactivation and infection following HSCT is the development of posttransplant lymphoproliferative disorder (PTLD) which is associated with significant morbidity and mortality [174]. Therefore, serial monitoring of EBV DNA via PCR testing is recommended to allow for preemptive management of reactivation and is particularly important in high-risk patients [33, 174, 175]. Patients at highest risk of EBV reactivation or primary infection are those who received a T-cell-depleted graft or T-cell-depleting serotherapy as well as haploidentical HSCT and UCB HSCT recipients due to profound lymphopenia [5, 33, 174]. No standard threshold exists for initiation of treatment of EBV viremia, and currently available antiviral agents do not have significant activity against EBV, so prophylaxis or treatment with antivirals is not recommended [5, 33, 174]. Preemptive treatment approaches include reduction in immunosuppression, when possible, and B-cell depletion with rituximab, as these have been shown to prevent progression to PTLD, particularly in high-risk patients [5, 33, 176]. Adoptive T-cell therapy using donor or third-party-derived EBV-specific T-cells also has demonstrated efficacy in controlling EBV viremia but is currently only available in clinical trial settings [177, 178].

Adenovirus

In HSCT patients, adenovirus (AdV) reactivation or primary infection can cause pneumonitis, colitis, and hepatitis associated with significant morbidity and mortality. Rates of AdV infection are approximately two times higher in pediatric HSCT patients than adult patients due to persistent viral circulation and therefore is of particular concern in that population [179]. Control of AdV reactivation and infection is highly dependent on cellular immunity following T-cell immune reconstitution, and therefore recipients of UCB, haploidentical, or T-cell-depleted HSCT or patients receiving T-cell-depleting serotherapy, such as ATG, or on intensive immunosuppression for GVHD are at highest risk for disease [5]. A preemptive management approach using weekly AdV viral PCR monitoring for high-risk patients can be considered and has been shown to prevent progression to end-organ disease [179, 180]. However, the majority of data supporting preemptive management is in the

pediatric HSCT population, and the toxic effects of AdV treatment can limit the utility of this approach in adults [179, 181]. Therefore, AdV viral PCR screening can also be restricted to symptom-triggered testing as an alternative [179]. Recommended treatment of AdV reactivation or disease is cidofovir 5 mg/kg IV weekly and should be given with hyperhydration and probenecid to limit nephrotoxicity [179, 181]. Cidofovir 1 mg/kg three times weekly can be used as an alternative dosing strategy for preemptive therapy only and is associated with lower rates of nephrotoxicity [179, 181]. Brincidofovir, an oral lipid conjugate of cidofovir with decreased myelosuppressive and nephrotoxic effects, has a growing body of literature supporting its efficacy in AdV reactivation management and may eventually become more useful as a preemptive therapeutic agent [179]. Adoptive T-cell therapy is another management approach that is an area of active investigation and has documented success in treatment of resistant or refractory AdV disease in early trials [173, 179, 182].

BK Virus

Human polyomavirus type I (BK virus) seropositivity has a high prevalence of up to 90% [183]. Therefore, asymptomatic urinary shedding occurs in up to 80% HSCT recipients in the early posttransplant period and is more common in allogeneic HSCT recipients, particularly those receiving myeloablative conditioning regimens and in MUD, UCB, and haploidentical transplants [183, 184]. However, 5–15% of patients will develop BK virus-associated hemorrhagic cystitis (BKV-HC), typically occurring after engraftment [5]. PCR testing for BK virus in urine or blood can be performed to assess for reactivation. Fluoroquinolones are known to inhibit BK virus replication; however, no preemptive or prophylactic strategies have demonstrated efficacy in preventing the occurrence of BKV-HC [5, 183]. Cidofovir has been reported in several retrospective studies to be effective in treatment of BKV-HC with complete response rates ranging from 60 to 100% [183]. It has been used both IV and intravesicularly and at a variety of dosing schedules ranging from 0.5 to 5 mg/kg one to three times per week with and without probenecid [183]. Nephrotoxicity is a major limitation associated with cidofovir therapy and can be mitigated with intravesicular administration and concomitant probenecid [183]. There is no consensus on when to initiate treatment of BKV-HC versus continuation of supportive care alone, but general consideration can be made for high-grade HC or any grade HC with concomitant BK viremia [183].

Parasitic Infections

Toxoplasmosis

The risk of *Toxoplasma gondii* reactivation is 2–6% in patients who are seropositive prior to HCT, with recipients of UCB, or those on extensive immunosuppression being at higher risk for reactivation [185]. Routine PCR screening of blood can be

considered to allow for early detection prior to onset of clinical symptoms of disease. Alternatively, symptom-triggered testing is also a reasonable approach, particularly in patients receiving appropriate prophylaxis, as reactivation in this setting is rare [5, 186]. Patients receiving TMP-SMX for PJP prophylaxis will be appropriately protected against *T. gondii* as well, so no additional preventative therapy is needed [5, 33]. In patients who cannot tolerate TMP-SMX, prophylaxis with agents active against *T. gondii* can be given (such as clindamycin or pyrimethamine plus leucovorin), or no specific prophylaxis can be administered, and PCR monitoring can be performed to allow for preemptive treatment [5, 186].

Strongyloides

Strongyloidiasis is a rare infection after HSCT, with case reports in the literature. Patients from endemic areas and those with gastrointestinal symptoms or unexplained eosinophilia pre-HSCT should be screened with *Strongyloides* species immunoglobulin G enzyme-linked immunosorbent assay or stool screening if serologic testing is unavailable and treated with anti-parasitic therapy before HSCT. The diagnosis of strongyloidiasis post-HSCT can be challenging, as gastrointestinal symptoms may mimic those of GVHD, and a detailed exposure history may be the only significant factor to initiate evaluation for this infection [5, 187].

Post-HSCT Vaccinations

An essential aspect of infection prevention following both autologous and allogeneic HSCT is the administration of immunizations [5, 33, 188, 189]. Vaccinations should be administered starting between 3 and 6 months posttransplant; however, delays may be warranted in patients who remain on significant immunosuppression for GVHD treatment or those who have received rituximab within the past 6 months [188–190]. Due to the many factors that have been outlined throughout this chapter related to the variability in immune reconstitution following HSCT, it is recommended to obtain antibody titers following diphtheria-tetanus-pertussis, VZV, and hepatitis B vaccination administration to ensure adequate response and have been correlated with vaccine efficacy in studies [188, 189, 191]. Revaccination should be performed if titers are insufficient. Table 7 outlines the typical recommended immunization schedule for HSCT patients.

Conclusions

The risk of developing an infection after HSCT is determined by a complex interplay of a variety of factors. These include cumulative pretransplant cytotoxic chemotherapy, depth and duration of posttransplant neutropenia, receipt of a transplant from an umbilical cord blood or haploidentical donor, and development of acute and/or

Table 7 Immunization schedule post-HSCT

Re-vaccination schedule for HSCT recipients[a]								Vaccination of household hontacts of SCT recipients[b]
Inactivated vaccine or toxoid	**6 months**	**8 months**	**10 months**	**12 months**	**14 months**	**16–24 months**	**24 months**	
Tetanus, diphtheria, pertussis (TDaP/DTaP)				**X**	**X**	**X**		
Haemophilus influenzae type b (Hib) conjugate				**X**	**X**	**X**		
23-Valent pneumococcal polysaccharide (PPV23)				**X**			**X**	
13-Valent conjugated vaccine	**X**	**X**	**X**				**X**	
Inactivated polio virus (IPV)				**X**	**X**	**X**		
Influenza	**X**			**Annually**				**Annually**
Hepatitis B				**X**	**X**	**X**		
Meningococcal				**X**				
Human papillomavirus (HPV)				**X**				
Live-attenuated vaccines	**6 months**	**8 months**	**10 months**	**12 months**	**14 months**	**16 months**	**24 months**	**25 months**
Measles/mumps/rubella (MMR)							**X**	**X**
Varicella							**X**	**X**
Recombinant zoster	**X**		**X**					

[a]If eligible to receive vaccination

[b]Household contacts should also be up to date on all regularly recommended vaccinations

chronic GVHD requiring treatment. Distinguishing immune reconstitution pattern between different modalities of HSCT allows for risk stratification of specific infections over time. Improvements in diagnostic technology have enabled earlier detection and identification of new infections in HSCT recipients. Additionally, drug development and clinical trials have led to new prophylactic and therapeutic options for infections which have historically been associated with significant HSCT patient morbidity and mortality.

Key Points

- Risk of infection after hematopoietic cell transplantation is dependent on multiple factors including extent of pretransplant chemotherapy exposure, duration of posttransplant neutropenia, source of allograft, and severity of graft-versus-host disease.
- Understanding the kinetics of posttransplant immune reconstitution allows for infection risk stratification and development of a timeline of infections after allogeneic hematopoietic cell transplantation.
- Patients who have undergone hematopoietic cell transplant are at risk for bacterial, viral, fungal, and parasitic infections.
- Patients who have undergone HSCT are among those at the highest risk for the development of drug-resistant pathogens, often requiring medications with increased toxicities for treatment.
- Special considerations should be made for prophylaxis and treatment of infections in the HSCT population based on risk factor assessment.

Cross-References

- ▶ *Aspergillus* in Solid Organ and Stem Cell Transplant Patients: Emerging Options for Diagnosis and Management
- ▶ *Candida* Infections in Solid Organ Transplantation
- ▶ Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment
- ▶ Emerging Diagnostics for Transplant Infectious Diseases
- ▶ Hospital Epidemiology and Infection Control in the Transplant Center
- ▶ Management of Mucorales Infections in Transplant Patients
- ▶ Multidrug-Resistant Organisms: Posttransplant Management for Extended-Spectrum Beta-Lactamase Producers and Carbapenem-Resistant Enterobacterales
- ▶ Multidrug-Resistant Organisms: Pre-transplant Evaluation and Management
- ▶ *Pseudomonas aeruginosa* Infections in Transplant: Epidemiology and Emerging Treatment Options

References

1. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood. 2002;100(13): 4358–66. Epub 2002/10/24.
2. Schuster MG, Cleveland AA, Dubberke ER, Kauffman CA, Avery RK, Husain S, et al. Infections in hematopoietic cell transplant recipients: results from the organ transplant infection project, a multicenter, prospective, cohort study. Open Forum Infect Dis. 2017;4(2): ofx050. Epub 2017/05/12.
3. Ballen K, Woo Ahn K, Chen M, Abdel-Azim H, Ahmed I, Aljurf M, et al. Infection rates among acute leukemia patients receiving alternative donor hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22(9):1636–45. Epub 2016/06/28.
4. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune reconstitution after allogeneic hematopoietic stem cell transplantation. Front Immunol. 2016;7:507. Epub 2016/12/03.
5. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238. Epub 2009/09/15.
6. Chaudhry MS, Velardi E, Malard F, van den Brink MR. Immune reconstitution after allogeneic hematopoietic stem cell transplantation: time to T up the Thymus. J Immunol. 2017;198(1): 40–6. Epub 2016/12/21.
7. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood. 2010;115(19):3861–8. Epub 2010/03/11.
8. Palmer DB. The effect of age on thymic function. Front Immunol. 2013;4:316. Epub 2013/10/11.
9. Toubert A, Glauzy S, Douay C, Clave E. Thymus and immune reconstitution after allogeneic hematopoietic stem cell transplantation in humans: never say never again. Tissue Antigens. 2012;79(2):83–9. Epub 2012/01/10.
10. van den Brink MR, Velardi E, Perales MA. Immune reconstitution following stem cell transplantation. Hematology Am Soc Hematol Educ Program. 2015;2015:215–9. Epub 2015/12/08.
11. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373(9674): 1550–61. Epub 2009/03/14.
12. Zeiser R, Blazar BR. Acute graft-versus-host disease – biologic process, prevention, and therapy. N Engl J Med. 2017;377(22):2167–79. Epub 2017/11/25.
13. Cooke KR, Luznik L, Sarantopoulos S, Hakim FT, Jagasia M, Fowler DH, et al. The biology of chronic graft-versus-host disease: a task force report from the National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2017;23(2):211–34. Epub 2016/10/08.
14. Martin PJ, Rizzo JD, Wingard JR, Ballen K, Curtin PT, Cutler C, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2012;18 (8):1150–63. Epub 2012/04/19.
15. MacDonald KPA, Betts BC, Couriel D. Reprint of: emerging therapeutics for the control of chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2018;24(3S):S7–S14. Epub 2018/02/10.
16. Jagasia M, Zeiser R, Arbushites M, Delaite P, Gadbaw B, Bubnoff NV. Ruxolitinib for the treatment of patients with steroid-refractory GVHD: an introduction to the REACH trials. Immunotherapy. 2018;10(5):391–402. Epub 2018/01/11.
17. Miklos D, Cutler CS, Arora M, Waller EK, Jagasia M, Pusic I, et al. Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. Blood. 2017;130(21):2243–50. Epub 2017/09/20.

18. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004;351(22):2265–75. Epub 2004/11/27.
19. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med. 2004;351(22):2276–85. Epub 2004/11/27.
20. Hamza NS, Lisgaris M, Yadavalli G, Nadeau L, Fox R, Fu P, et al. Kinetics of myeloid and lymphocyte recovery and infectious complications after unrelated umbilical cord blood versus HLA-matched unrelated donor allogeneic transplantation in adults. Br J Haematol. 2004;124(4):488–98. Epub 2004/02/27.
21. Thomson BG, Robertson KA, Gowan D, Heilman D, Broxmeyer HE, Emanuel D, et al. Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. Blood. 2000;96(8):2703–11. Epub 2000/10/07.
22. Klein AK, Patel DD, Gooding ME, Sempowski GD, Chen BJ, Liu C, et al. T-cell recovery in adults and children following umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2001;7(8):454–66. Epub 2001/09/25.
23. Brown JA, Boussiotis VA. Umbilical cord blood transplantation: basic biology and clinical challenges to immune reconstitution. Clin Immunol. 2008;127(3):286–97. Epub 2008/04/09.
24. Godfrey WR, Spoden DJ, Ge YG, Baker SR, Liu B, Levine BL, et al. Cord blood CD4(+) CD25(+)-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood. 2005;105(2):750–8. Epub 2004/09/18.
25. Li L, Godfrey WR, Porter SB, Ge Y, June CH, Blazar BR, et al. CD4+CD25+ regulatory T-cell lines from human cord blood have functional and molecular properties of T-cell anergy. Blood. 2005;106(9):3068–73. Epub 2005/07/16.
26. Ruggeri A, Peffault de Latour R, Carmagnat M, Clave E, Douay C, Larghero J, et al. Outcomes, infections, and immune reconstitution after double cord blood transplantation in patients with high-risk hematological diseases. Transpl Infect Dis. 2011;13(5):456–65. Epub 2011/04/07.
27. Chang YJ, Zhao XY, Huang XJ. Immune reconstitution after haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20(4):440–9. Epub 2013/12/10.
28. Ciurea SO, Mulanovich V, Saliba RM, Bayraktar UD, Jiang Y, Bassett R, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2012;18(12):1835–44. Epub 2012/07/17.
29. Shabbir-Moosajee M, Lombardi L, Ciurea SO. An overview of conditioning regimens for haploidentical stem cell transplantation with post-transplantation cyclophosphamide. Am J Hematol. 2015;90(6):541–8. Epub 2015/03/03.
30. Crocchiolo R, Bramanti S, Vai A, Sarina B, Mineri R, Casari E, et al. Infections after T-replete haploidentical transplantation and high-dose cyclophosphamide as graft-versus-host disease prophylaxis. Transpl Infect Dis. 2015;17(2):242–9. Epub 2015/02/05.
31. Rashidi A, DiPersio JF, Westervelt P, Vij R, Schroeder MA, Cashen AF, et al. Comparison of outcomes after peripheral blood Haploidentical versus matched unrelated donor allogeneic hematopoietic cell transplantation in patients with acute myeloid leukemia: a retrospective single-center review. Biol Blood Marrow Transplant. 2016;22(9):1696–701. Epub 2016/05/26.
32. Reisner Y, Hagin D, Martelli MF. Haploidentical hematopoietic transplantation: current status and future perspectives. Blood. 2011;118(23):6006–17. Epub 2011/09/17.
33. Ullmann AJ, Schmidt-Hieber M, Bertz H, Heinz WJ, Kiehl M, Kruger W, et al. Infectious diseases in allogeneic haematopoietic stem cell transplantation: prevention and prophylaxis strategy guidelines 2016. Ann Hematol. 2016;95(9):1435–55. Epub 2016/06/25.

34. Cordonnier C, Einarsdottir S, Cesaro S, Di Blasi R, Mikulska M, Rieger C, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European conference on infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(6):e200–e12. Epub 2019/02/13.
35. Girmenia C, Bertaina A, Piciocchi A, Perruccio K, Algarotti A, Busca A, et al. Incidence, risk factors and outcome of pre-engraftment gram-negative bacteremia after allogeneic and autologous hematopoietic stem cell transplantation: an Italian prospective multicenter survey. Clin Infect Dis. 2017;65(11):1884–96. Epub 2017/10/12.
36. Wingard JR, Hsu J, Hiemenz JW. Hematopoietic stem cell transplantation: an overview of infection risks and epidemiology. Hematol Oncol Clin North Am. 2011;25(1):101–16. Epub 2011/01/18.
37. Boersma RS, Jie KS, Verbon A, van Pampus EC, Schouten HC. Thrombotic and infectious complications of central venous catheters in patients with hematological malignancies. Ann oncol. 2008;19(3):433–42. Epub 2007/10/27.
38. Chong Y, Yakushiji H, Ito Y, Kamimura T. Cefepime-resistant gram-negative bacteremia in febrile neutropenic patients with hematological malignancies. Int J Infect Dis. 2010;14 (Suppl 3):e171–5. Epub 2010/05/18.
39. Mikulska M, Del Bono V, Raiola AM, Bruno B, Gualandi F, Occhini D, et al. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of gram-negative rods and increasing antibiotic resistance. Biol Blood Marrow Transplant. 2009;15(1): 47–53. Epub 2009/01/13.
40. Mikulska M, Viscoli C, Orasch C, Livermore DM, Averbuch D, Cordonnier C, et al. Aetiology and resistance in bacteraemias among adult and paediatric haematology and cancer patients. J Infect. 2014;68(4):321–31. Epub 2013/12/29.
41. Taplitz RA, Kennedy EB, Bow EJ, Crews J, Gleason C, Hawley DK, et al. Antimicrobial prophylaxis for adult patients with Cancer-related immunosuppression: ASCO and IDSA clinical practice guideline update. J Clin Oncol. 2018;36(30):3043–54. Epub 2018/09/05.
42. Horton LE, Haste NM, Taplitz RA. Rethinking antimicrobial prophylaxis in the transplant patient in the world of emerging resistant organisms-where are we today? Curr Hematol Malig Rep. 2018;13(1):59–67. Epub 2018/01/28.
43. Tauro S, Dobie D, Richardson G, Hastings M, Mahendra P. Recurrent penicillin-resistant pneumococcal sepsis after matched unrelated donor (MUD) transplantation for refractory T cell lymphoma. Bone Marrow Transplant. 2000;26(9):1017–9. Epub 2000/12/02.
44. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases society of America. Clin Infect Dis. 2011;52(4):e56–93. Epub 2011/01/25.
45. Gaur AH, Flynn PM, Heine DJ, Giannini MA, Shenep JL, Hayden RT. Diagnosis of catheter-related bloodstream infections among pediatric oncology patients lacking a peripheral culture, using differential time to detection. Pediatr Infect Dis J. 2005;24(5):445–9. Epub 2005/05/07.
46. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? J Clin Microbiol. 2007;45(11):3546–8. Epub 2007/09/21.
47. Buss BA, Baures TJ, Yoo M, Hanson KE, Alexander DP, Benefield RJ, et al. Impact of a multiplex PCR assay for bloodstream infections with and without antimicrobial stewardship intervention at a Cancer hospital. Open Forum Infect Dis. 2018;5(10):ofy258. Epub 2018/11/06.
48. Timbrook TT, Morton JB, McConeghy KW, Caffrey AR, Mylonakis E, LaPlante KL. The effect of molecular rapid diagnostic testing on clinical outcomes in bloodstream infections: a systematic review and meta-analysis. Clin Infect Dis. 2017;64(1):15–23. Epub 2016/11/03.
49. Azar MM, Gaston DC, Kotton CN, Malinis MF. Emerging microbiology diagnostics for transplant infections: on the cusp of a paradigm shift. Transplantation. 2020. Epub 2020/01/24.
50. Hindler JA, Wong-Beringer A, Charlton CL, Miller SA, Kelesidis T, Carvalho M, et al. In vitro activity of daptomycin in combination with beta-lactams, gentamicin, rifampin, and

tigecycline against daptomycin-nonsusceptible enterococci. Antimicrob Agents Chemother. 2015;59(7):4279–88. Epub 2015/05/13.
51. Smith JR, Barber KE, Raut A, Aboutaleb M, Sakoulas G, Rybak MJ. Beta-lactam combinations with daptomycin provide synergy against vancomycin-resistant Enterococcus faecalis and *Enterococcus faecium*. J Antimicrob Chemother. 2015;70(6):1738–43. Epub 2015/02/04.
52. Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of Piperacillin-Tazobactam vs. Meropenem on 30-day mortality for patients with E coli or *Klebsiella pneumoniae* bloodstream infection and ceftriaxone resistance: a randomized clinical trial. JAMA. 2018;320(10):984–94. Epub 2018/09/13.
53. Forslow U, Mattsson J, Ringden O, Klominek J, Remberger M. Decreasing mortality rate in early pneumonia following hematopoietic stem cell transplantation. Scand J Infect Dis. 2006;38(11–12):970–6. Epub 2006/12/07.
54. Gentile G, Micozzi A, Girmenia C, Iori AP, Donati PP, Capria S, et al. Pneumonia in allogenic and autologous bone marrow recipients. A retrospective study. Chest. 1993;104(2):371–5. Epub 1993/08/01.
55. Aguilar-Guisado M, Jimenez-Jambrina M, Espigado I, Rovira M, Martino R, Oriol A, et al. Pneumonia in allogeneic stem cell transplantation recipients: a multicenter prospective study. Clin Transpl. 2011;25(6):E629–38. Epub 2011/12/14.
56. Chopra T, Chandrasekar P, Salimnia H, Heilbrun LK, Smith D, Alangaden GJ. Recent epidemiology of *Clostridium difficile* infection during hematopoietic stem cell transplantation. Clin Transpl. 2011;25(1):E82–7. Epub 2010/10/27.
57. Guddati AK, Kumar G, Ahmed S, Ali M, Kumar N, Hari P, et al. Incidence and outcomes of *Clostridium difficile*-associated disease in hematopoietic cell transplant recipients. Int J Hematol. 2014;99(6):758–65. Epub 2014/04/10.
58. Zacharioudakis IM, Ziakas PD, Mylonakis E. *Clostridium difficile* infection in the hematopoietic unit: a meta-analysis of published studies. Biol Blood Marrow Transplant. 2014;20(10):1650–4. Epub 2014/06/11.
59. Kinnebrew MA, Lee YJ, Jenq RR, Lipuma L, Littmann ER, Gobourne A, et al. Early *Clostridium difficile* infection during allogeneic hematopoietic stem cell transplantation. PLoS One. 2014;9(3):e90158. Epub 2014/03/26.
60. Lavallee C, Labbe AC, Talbot JD, Alonso CD, Marr KA, Cohen S, et al. Risk factors for the development of *Clostridium difficile* infection in adult allogeneic hematopoietic stem cell transplant recipients: a single-center study in Quebec, Canada. Transpl Infect Dis. 2017;19(1). Epub 2016/12/13.
61. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. N Engl J Med. 2000;342(6):390–7. Epub 2000/02/10.
62. Leav BA, Blair B, Leney M, Knauber M, Reilly C, Lowy I, et al. Serum anti-toxin B antibody correlates with protection from recurrent *Clostridium difficile* infection (CDI). Vaccine. 2010;28(4):965–9. Epub 2009/11/28.
63. Alonso CD, Treadway SB, Hanna DB, Huff CA, Neofytos D, Carroll KC, et al. Epidemiology and outcomes of *Clostridium difficile* infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2012;54(8):1053–63. Epub 2012/03/14.
64. Willems L, Porcher R, Lafaurie M, Casin I, Robin M, Xhaard A, et al. *Clostridium difficile* infection after allogeneic hematopoietic stem cell transplantation: incidence, risk factors, and outcome. Biol Blood Marrow Transplant. 2012;18(8):1295–301. Epub 2012/03/06.
65. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota disruption induced by early use of broad-Spectrum antibiotics is an independent risk factor of outcome after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2017;23(5):845–52. Epub 2017/02/25.
66. Erb S, Frei R, Stranden AM, Dangel M, Tschudin-Sutter S, Widmer AF. Low sensitivity of fecal toxin A/B enzyme immunoassay for diagnosis of *Clostridium difficile* infection in immunocompromised patients. Clin Microbiol Infect. 2015;21(11):998 e9–e15. Epub 2015/08/02.

67. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):987–94. Epub 2018/03/22.
68. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. Clin Infect Dis. 2007;45(3):302–7. Epub 2007/06/30.
69. Parmar SR, Bhatt V, Yang J, Zhang Q, Schuster M. A retrospective review of metronidazole and vancomycin in the management of *Clostridium difficile* infection in patients with hematologic malignancies. J Oncol Pharm Pract. 2014;20(3):172–82. Epub 2013/06/28.
70. Johnson S, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, et al. Vancomycin, metronidazole, or tolevamer for *Clostridium difficile* infection: results from two multinational, randomized, controlled trials. Clin Infect Dis. 2014;59(3):345–54. Epub 2014/05/07.
71. Stevens VW, Nelson RE, Schwab-Daugherty EM, Khader K, Jones MM, Brown KA, et al. Comparative effectiveness of Vancomycin and metronidazole for the prevention of recurrence and death in patients with *Clostridium difficile* infection. JAMA Intern Med. 2017;177 (4):546–53. Epub 2017/02/07.
72. Cornely OA, Crook DW, Esposito R, Poirier A, Somero MS, Weiss K, et al. Fidaxomicin versus vancomycin for infection with *Clostridium difficile* in Europe, Canada, and the USA: a double-blind, non-inferiority, randomised controlled trial. Lancet Infect Dis. 2012;12(4): 281–9. Epub 2012/02/11.
73. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. N Engl J Med. 2011;364(5):422–31. Epub 2011/02/04.
74. Cornely OA, Miller MA, Fantin B, Mullane K, Kean Y, Gorbach S. Resolution of *Clostridium difficile*-associated diarrhea in patients with cancer treated with fidaxomicin or vancomycin. J Clin Oncol. 2013;31(19):2493–9. Epub 2013/05/30.
75. Alonso CD, Braun DA, Patel I, Akbari M, Oh DJ, Jun T, et al. A multicenter, retrospective, case-cohort study of the epidemiology and risk factors for *Clostridium difficile* infection among cord blood transplant recipients. Transpl Infect Dis. 2017;19(4). Epub 2017/05/26.
76. Tieu JD, Williams RJ 2nd, Skrepnek GH, Gentry CA. Clinical outcomes of fidaxomicin vs. oral vancomycin in recurrent *Clostridium difficile* infection. J Clin Pharm Ther. 2019;44(2):220–8. Epub 2018/10/24.
77. Cornely OA, Watt M, McCrea C, Goldenberg SD, De Nigris E. Extended-pulsed fidaxomicin versus vancomycin for *Clostridium difficile* infection in patients aged >/=60 years (EXTEND): analysis of cost-effectiveness. J Antimicrob Chemother. 2018;73(9):2529–39. Epub 2018/05/26.
78. DeFilipp Z, Peled JU, Li S, Mahabamunuge J, Dagher Z, Slingerland AE, et al. Third-party fecal microbiota transplantation following Allo-HCT reconstitutes microbiome diversity. Blood advances. 2018;2(7):745–53. Epub 2018/03/30.
79. DeFilipp Z, Hohmann E, Jenq RR, Chen YB. Fecal microbiota transplantation: restoring the injured microbiome after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2019;25(1):e17–22. Epub 2018/11/09.
80. Gerding DN, Kelly CP, Rahav G, Lee C, Dubberke ER, Kumar PN, et al. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection in patients at increased risk for recurrence. Clin Infect Dis. 2018;67(5):649–56. Epub 2018/03/15.
81. Mullane KM, Winston DJ, Nooka A, Morris MI, Stiff P, Dugan MJ, et al. A randomized, placebo-controlled trial of Fidaxomicin for prophylaxis of *Clostridium difficile*-associated diarrhea in adults undergoing hematopoietic stem cell transplantation. Clin Infect Dis. 2019;68(2):196–203. Epub 2018/06/13.
82. Van Hise NW, Bryant AM, Hennessey EK, Crannage AJ, Khoury JA, Manian FA. Efficacy of Oral Vancomycin in preventing recurrent *Clostridium difficile* infection in patients treated with systemic antimicrobial agents. Clin Infect Dis. 2016;63(5):651–3. Epub 2016/06/19.

83. Caroff DA, Menchaca JT, Zhang Z, Rhee C, Calderwood MS, Kubiak DW, et al. Oral vancomycin prophylaxis during systemic antibiotic exposure to prevent Clostridioides difficile infection relapses. Infect Control Hosp Epidemiol. 2019;40(6):662–7. Epub 2019/04/30.
84. Chan JD, Bryson-Cahn C, Jain R, Lynch JB, Liu C. Does oral vancomycin prophylaxis during systemic antibiotic exposure prevent Clostridioides difficile infection relapses? Still in search of an answer. Infect Control Hosp Epidemiol. 2019;40(9):1084–6. Epub 2019/07/11.
85. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the mycoses study group education and research consortium. Clin Infect Dis. 2019. Epub 2019/12/06.
86. Blijlevens NM, Donnelly JP, de Pauw BE. Impaired gut function as risk factor for invasive candidiasis in neutropenic patients. Br J Haematol. 2002;117(2):259–64. Epub 2002/04/26.
87. Bow EJ. Considerations in the approach to invasive fungal infection in patients with haematological malignancies. Br J Haematol. 2008;140(2):133–52. Epub 2008/01/05.
88. Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I. The changing epidemiology of invasive candidiasis: Candida glabrata and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. Cancer. 2008;112(11):2493–9. Epub 2008/04/17.
89. Robenshtok E, Gafter-Gvili A, Goldberg E, Weinberger M, Yeshurun M, Leibovici L, et al. Antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem-cell transplantation: systematic review and meta-analysis. J Clin Oncol. 2007;25(34):5471–89. Epub 2007/10/03.
90. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the transplant-associated infection surveillance network (TRANSNET) database. Clin Infect Dis. 2010;50(8):1091–100. Epub 2010/03/12.
91. https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html
92. Calvo B, Melo AS, Perozo-Mena A, Hernandez M, Francisco EC, Hagen F, et al. First report of Candida auris in America: clinical and microbiological aspects of 18 episodes of candidemia. J Infect. 2016;73(4):369–74. Epub 2016/07/28.
93. Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, et al. First three reported cases of nosocomial fungemia caused by Candida auris. J Clin Microbiol. 2011;49(9):3139–42. Epub 2011/07/01.
94. Rudramurthy SM, Chakrabarti A, Paul RA, Sood P, Kaur H, Capoor MR, et al. Candida auris candidaemia in Indian ICUs: analysis of risk factors. J Antimicrob Chemother. 2017;72(6):1794–801. Epub 2017/03/24.
95. Vallabhaneni S, Kallen A, Tsay S, Chow N, Welsh R, Kerins J, et al. Investigation of the first seven reported cases of Candida auris, a globally emerging invasive, multidrug-resistant fungus – United States, May 2013-August 2016. MMWR Morb Mortal Wkly Rep. 2016;65(44):1234–7. Epub 2016/11/11.
96. Cornely OA, Gachot B, Akan H, Bassetti M, Uzun O, Kibbler C, et al. Epidemiology and outcome of fungemia in a cancer Cohort of the Infectious Diseases Group (IDG) of the European Organization for Research and Treatment of Cancer (EORTC 65031). Clin Infect Dis. 2015;61(3):324–31. Epub 2015/04/15.
97. Kozel TR, Wickes B. Fungal diagnostics. Cold Spring Harb Perspect Med. 2014;4(4):a019299. Epub 2014/04/03.
98. Ostrosky-Zeichner L, Pappas PG. Invasive candidiasis in the intensive care unit. Crit Care Med. 2006;34(3):857–63. Epub 2006/03/01.
99. Stevens DA. Diagnosis of fungal infections: current status. J Antimicrob Chemother. 2002;49(Suppl 1):11–9. Epub 2002/01/22.
100. Karageorgopoulos DE, Vouloumanou EK. Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. Beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin Infect Dis. 2011;52(6):750–70. Epub 2011/03/04.
101. Persat F, Ranque S, Derouin F, Michel-Nguyen A, Picot S, Sulahian A. Contribution of the (1→3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J Clin Microbiol. 2008;46(3):1009–13. Epub 2007/12/28.

102. Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, et al. Beta-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the third European conference on infections in leukemia (ECIL-3). Clin Infect Dis. 2012;54(5): 633–43. Epub 2011/12/27.
103. Koo S, Bryar JM, Page JH, Baden LR, Marty FM. Diagnostic performance of the (1→3)-beta-D-glucan assay for invasive fungal disease. Clin Infect Dis. 2009;49(11):1650–9. Epub 2009/10/30.
104. Clancy CJ, Nguyen MH. T2 magnetic resonance for the diagnosis of bloodstream infections: charting a path forward. J Antimicrob Chemother. 2018;73(suppl_4):iv2–5. Epub 2018/04/03.
105. Munoz P, Vena A, Machado M, Gioia F, Martinez-Jimenez MC, Gomez E, et al. T2Candida MR as a predictor of outcome in patients with suspected invasive candidiasis starting empirical antifungal treatment: a prospective pilot study. J Antimicrob Chemother. 2018;73(suppl_4): iv6–iv12. Epub 2018/04/03.
106. Munoz P, Vena A, Machado M, Martinez-Jimenez MC, Gioia F, Gomez E, et al. T2MR contributes to the very early diagnosis of complicated candidaemia. A prospective study. J Antimicrob Chemother. 2018;73(suppl_4):iv13–iv9. Epub 2018/04/03.
107. Patch ME, Weisz E, Cubillos A, Estrada SJ, Pfaller MA. Impact of rapid, culture-independent diagnosis of candidaemia and invasive candidiasis in a community health system. J Antimicrob Chemother. 2018;73(suppl_4):iv27–30. Epub 2018/04/03.
108. Chowdhary A, Sharma C, Duggal S, Agarwal K, Prakash A, Singh PK, et al. New clonal strain of Candida auris, Delhi, India. Emerg Infect Dis. 2013;19(10):1670–3. Epub 2013/09/21.
109. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, et al. Candida auris: a review of the literature. Clin Microbiol Rev. 2018;31(1). Epub 2017/11/17.
110. Spivak ES, Hanson KE. Candida auris: an emerging fungal pathogen. J Clin Microbiol. 2018;56(2). Epub 2017/11/24.
111. Kordalewska M, Zhao Y, Lockhart SR, Chowdhary A, Berrio I, Perlin DS. Rapid and accurate molecular identification of the emerging multidrug-resistant pathogen Candida auris. J Clin Microbiol. 2017;55(8):2445–52. Epub 2017/05/26.
112. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1–50. Epub 2015/12/19.
113. Biagi MJ, Wiederhold NP, Gibas C, Wickes BL, Lozano V, Bleasdale SC, et al. Development of high-level Echinocandin resistance in a patient with recurrent Candida auris Candidemia secondary to chronic Candiduria. Open Forum Infect Dis. 2019;6(7):ofz262. Epub 2019/07/10.
114. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis. 2017;64(2):134–40. Epub 2016/12/19.
115. Larkin E, Hager C, Chandra J, Mukherjee PK, Retuerto M, Salem I, et al. The emerging pathogen Candida auris: growth phenotype, virulence factors, activity of antifungals, and effect of SCY-078, a novel Glucan synthesis inhibitor, on growth morphology and biofilm formation. Antimicrob Agents Chemother. 2017;61(5). Epub 2017/02/23.
116. https://www.cdc.gov/fungal/candida-auris/c-auris-treatment.html
117. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of America. Clin Infect Dis. 2010;50(3):291–322. Epub 2010/01/06.
118. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica. 2006;91(8):1068–75. Epub 2006/08/04.
119. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2002;34(7):909–17. Epub 2002/03/07.
120. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017

ESCMID-ECMM-ERS guideline. Clinical Microbiol Infect. 2018;24(Suppl 1):e1–e38. Epub 2018/03/17.
121. Patterson TF, Donnelly JP. New concepts in diagnostics for invasive mycoses: non-culture-based methodologies. J Fungi (Basel). 2019;5(1). Epub 2019/01/20.
122. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis. 2006;42(10):1417–27. Epub 2006/04/19.
123. Foy PC, van Burik JA, Weisdorf DJ. Galactomannan antigen enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2007;13(4):440–3. Epub 2007/02/09.
124. Ji Y, Xu LP, Liu DH, Chen YH, Han W, Zhang XH, et al. Positive results of serum galactomannan assays and pulmonary computed tomography predict the higher response rate of empirical antifungal therapy in patients undergoing allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2011;17(5):759–64. Epub 2010/11/09.
125. Maertens J, Theunissen K, Verhoef G, Verschakelen J, Lagrou K, Verbeken E, et al. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. Clin Infect Dis. 2005;41(9):1242–50. Epub 2005/10/06.
126. Guo YL, Chen YQ, Wang K, Qin SM, Wu C, Kong JL. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate metaanalysis and systematic review. Chest. 2010;138(4):817–24. Epub 2010/05/11.
127. Zou M, Tang L, Zhao S, Zhao Z, Chen L, Chen P, et al. Systematic review and meta-analysis of detecting galactomannan in bronchoalveolar lavage fluid for diagnosing invasive aspergillosis. PLoS One. 2012;7(8):e43347. Epub 2012/08/21.
128. Adam O, Auperin A, Wilquin F, Bourhis JH, Gachot B, Chachaty E. Treatment with piperacillin-tazobactam and false-positive Aspergillus galactomannan antigen test results for patients with hematological malignancies. Clin Infect Dis. 2004;38(6):917–20. Epub 2004/03/05.
129. Aubry A, Porcher R, Bottero J, Touratier S, Leblanc T, Brethon B, et al. Occurrence and kinetics of false-positive Aspergillus galactomannan test results following treatment with beta-lactam antibiotics in patients with hematological disorders. J Clin Microbiol. 2006;44(2):389–94. Epub 2006/02/04.
130. Sulahian A, Touratier S, Ribaud P. False positive test for aspergillus antigenemia related to concomitant administration of piperacillin and tazobactam. N Engl J Med. 2003;349(24):2366–7. Epub 2003/12/12.
131. Viscoli C, Machetti M, Cappellano P, Bucci B, Bruzzi P, Van Lint MT, et al. False-positive galactomannan platelia Aspergillus test results for patients receiving piperacillin-tazobactam. Clin Infect Dis. 2004;38(6):913–6. Epub 2004/03/05.
132. Mikulska M, Furfaro E, Del Bono V, Raiola AM, Ratto S, Bacigalupo A, et al. Piperacillin/tazobactam (Tazocin) seems to be no longer responsible for false-positive results of the galactomannan assay. J Antimicrob Chemother. 2012;67(7):1746–8. Epub 2012/04/14.
133. Wheat LJ, Hackett E, Durkin M, Connolly P, Petraitiene R, Walsh TJ, et al. Histoplasmosis-associated cross-reactivity in the BioRad Platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol. 2007;14(5):638–40. Epub 2007/03/09.
134. Rath PM, Steinmann J. Overview of commercially available PCR assays for the detection of Aspergillus spp. DNA in Patient Samples. Front Microbiol. 2018;9:740. Epub 2018/05/10.
135. Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. Radiology. 1985;157(3):611–4. Epub 1985/12/01.
136. Orr DP, Myerowitz RL, Dubois PJ. Patho-radiologic correlation of invasive pulmonary aspergillosis in the compromised host. Cancer. 1978;41(5):2028–39. Epub 1978/05/01.
137. Primack SL, Hartman TE, Lee KS, Muller NL. Pulmonary nodules and the CT halo sign. Radiology. 1994;190(2):513–5. Epub 1994/02/01.
138. Greene RE, Schlamm HT, Oestmann JW, Stark P, Durand C, Lortholary O, et al. Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. Clin Infect Dis. 2007;44(3):373–9. Epub 2007/01/06.

139. Wahba H, Truong MT, Lei X, Kontoyiannis DP, Marom EM. Reversed halo sign in invasive pulmonary fungal infections. Clin Infect Dis. 2008;46(11):1733–7. Epub 2008/04/19.
140. Acharige MJT, Koshy S, Ismail N, Aloum O, Jazaerly M, Astudillo CL, et al. Breath-based diagnosis of fungal infections. J Breath Res. 2018;12(2):027108. Epub 2017/11/08.
141. Koo S, Thomas HR, Daniels SD, Lynch RC, Fortier SM, Shea MM, et al. A breath fungal secondary metabolite signature to diagnose invasive aspergillosis. Clin Infect Dis. 2014;59(12):1733–40. Epub 2014/10/25.
142. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med. 2007;356(4):335–47. Epub 2007/01/26.
143. Patterson TF, Thompson GR 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice guidelines for the diagnosis and Management of Aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):e1–e60. Epub 2016/07/02.
144. Maertens JA, Raad II, Marr KA, Patterson TF, Kontoyiannis DP, Cornely OA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. Lancet. 2016;387(10020):760–9. Epub 2015/12/20.
145. Lionakis MS, Lewis RE, Kontoyiannis DP. Breakthrough invasive mold infections in the hematology patient: current concepts and future directions. Clin Infect Dis. 2018;67(10): 1621–30. Epub 2018/06/04.
146. Rausch CR, DiPippo AJ, Bose P, Kontoyiannis DP. Breakthrough fungal infections in patients with leukemia receiving Isavuconazole. Clin Infect Dis. 2018;67(10):1610–3. Epub 2018/05/18.
147. Berkow EL, Nunnally NS, Bandea A, Kuykendall R, Beer K, Lockhart SR. Detection of TR34/L98H CYP51A mutation through passive surveillance for azole-resistant Aspergillus fumigatus in the United States from 2015 to 2017. Antimicrob Agents Chemother. 2018;62(5). Epub 2018/02/22.
148. Buil JB, Snelders E, Denardi LB, Melchers WJG, Verweij PE. Trends in azole resistance in Aspergillus fumigatus, the Netherlands, 1994–2016. Emerg Infect Dis. 2019;25(1):176–8. Epub 2018/12/19.
149. Verweij PE, Te Dorsthorst DT, Rijs AJ, De Vries-Hospers HG, Meis JF. Nationwide survey of in vitro activities of itraconazole and voriconazole against clinical Aspergillus fumigatus isolates cultured between 1945 and 1998. J Clin Microbiol. 2002;40(7):2648–50. Epub 2002/06/29.
150. Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosis 2013. Clin Microbiol Infect. 2014;20(Suppl 3):5–26. Epub 2014/02/01.
151. Greenberg RN, Mullane K, van Burik JA, Raad I, Abzug MJ, Anstead G, et al. Posaconazole as salvage therapy for zygomycosis. Antimicrob Agents Chemother. 2006;50(1):126–33. Epub 2005/12/27.
152. van Burik JA, Hare RS, Solomon HF, Corrado ML, Kontoyiannis DP. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. Clin Infect Dis. 2006;42(7):e61–5. Epub 2006/03/03.
153. Marty FM, Ostrosky-Zeichner L, Cornely OA, Mullane KM, Perfect JR, Thompson GR 3rd, et al. Isavuconazole treatment for mucormycosis: a single-arm open-label trial and case-control analysis. Lancet Infect Dis. 2016;16(7):828–37. Epub 2016/03/13.
154. Sipsas NV, Gamaletsou MN, Anastasopoulou A, Kontoyiannis DP. Therapy of Mucormycosis. J Fungi (Basel). 2018;4(3). Epub 2018/08/02.
155. Tortorano AM, Richardson M, Roilides E, van Diepeningen A, Caira M, Munoz P, et al. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: Fusarium spp., Scedosporium spp. and others. Clin Microbiol Infect. 2014;20(Suppl 3):27–46. Epub 2014/02/20.
156. Tyring SK, Baker D, Snowden W. Valacyclovir for herpes simplex virus infection: long-term safety and sustained efficacy after 20 years' experience with acyclovir. J Infect Dis. 2002;186 (Suppl 1):S40–6. Epub 2002/09/28.

157. Koc Y, Miller KB, Schenkein DP, Griffith J, Akhtar M, DesJardin J, et al. Varicella zoster virus infections following allogeneic bone marrow transplantation: frequency, risk factors, and clinical outcome. Biol Blood Marrow Transplant. 2000;6(1):44–9. Epub 2000/03/09.
158. Camargo JF, Komanduri KV. Emerging concepts in cytomegalovirus infection following hematopoietic stem cell transplantation. Hematol Oncol Stem Cell Ther. 2017;10(4):233–8. Epub 2017/06/24.
159. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. Hematol Oncol Clin North Am. 2011;25(1):151–69. Epub 2011/01/18.
160. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. Leukemia. 2015;29(10):2062–8. Epub 2015/08/01.
161. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis. 2002;34(8):1094–7. Epub 2002/03/27.
162. Ljungman P, de la Camara R, Robin C, Crocchiolo R, Einsele H, Hill JA, et al. Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European conference on infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(8):e260–e72. Epub 2019/06/04.
163. Nesher L, Shah DP, Ariza-Heredia EJ, Azzi JM, Siddiqui HK, Ghantoji SS, et al. Utility of the enzyme-linked Immunospot interferon-gamma-release assay to predict the risk of Cytomegalovirus infection in hematopoietic cell transplant recipients. J Infect Dis. 2016;213(11):1701–7. Epub 2016/02/26.
164. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, et al. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. Blood. 2016;127(20):2427–38. Epub 2016/02/18.
165. Chen K, Cheng MP, Hammond SP, Einsele H, Marty FM. Antiviral prophylaxis for cytomegalovirus infection in allogeneic hematopoietic cell transplantation. Blood Adv. 2018;2(16):2159–75. Epub 2018/08/30.
166. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir prophylaxis for Cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med. 2017;377(25):2433–44. Epub 2017/12/07.
167. Chou S, Satterwhite LE, Ercolani RJ. New locus of drug resistance in the human Cytomegalovirus UL56 gene revealed by in vitro exposure to Letermovir and ganciclovir. Antimicrob Agents Chemother. 2018;62(9). Epub 2018/06/20.
168. Jung S, Michel M, Stamminger T, Michel D. Fast breakthrough of resistant cytomegalovirus during secondary letermovir prophylaxis in a hematopoietic stem cell transplant recipient. BMC Infect Dis. 2019;19(1):388. Epub 2019/05/10.
169. Knoll BM, Seiter K, Phillips A, Soave R. Breakthrough cytomegalovirus pneumonia in hematopoietic stem cell transplant recipient on letermovir prophylaxis. Bone Marrow Transplant. 2019;54(6):911–2. Epub 2018/11/08.
170. Maertens J, Cordonnier C, Jaksch P, Poire X, Uknis M, Wu J, et al. Maribavir for preemptive treatment of Cytomegalovirus reactivation. N Engl J Med. 2019;381(12):1136–47. Epub 2019/09/19.
171. Maertens J, Cordonnier C, Jaksch P, Poiré X, Wu JJ, Wijatyk A, et al. Maribavir versus Valganciclovir for preemptive treatment of Cytomegalovirus (CMV) Viremia: a randomized, dose-ranging, phase 2 study among hematopoietic stem cell transplant (SCT) and solid organ transplant (SOT) recipients. Open Forum Infect Dis. 2016;3(suppl_1).
172. Papanicolaou GA, Silveira FP, Langston AA, Pereira MR, Avery RK, Uknis M, et al. Maribavir for refractory or resistant Cytomegalovirus infections in hematopoietic-cell or solid-organ transplant recipients: a randomized, dose-ranging, double-blind, phase 2 study. Clin Infect Dis. 2019;68(8):1255–64. Epub 2018/10/18.
173. Kaeuferle T, Krauss R, Blaeschke F, Willier S, Feuchtinger T. Strategies of adoptive T-cell transfer to treat refractory viral infections post allogeneic stem cell transplantation. J Hematol Oncol. 2019;12(1):13. Epub 2019/02/08.

174. Rasche L, Kapp M, Einsele H, Mielke S. EBV-induced post transplant lymphoproliferative disorders: a persisting challenge in allogeneic hematopoetic SCT. Bone Marrow Transplant. 2014;49(2):163–7. Epub 2013/07/09.
175. Liu Q, Xuan L, Liu H, Huang F, Zhou H, Fan Z, et al. Molecular monitoring and stepwise preemptive therapy for Epstein-Barr virus viremia after allogeneic stem cell transplantation. Am J Hematol. 2013;88(7):550–5. Epub 2013/04/09.
176. Gruhn B, Meerbach A, Hafer R, Zell R, Wutzler P, Zintl F. Pre-emptive therapy with rituximab for prevention of Epstein-Barr virus-associated lymphoproliferative disease after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2003;31(11):1023–5. Epub 2003/05/30.
177. Clevinger R. When your pediatric patient becomes a crime scene. J Emerg Nurs. 2010;36(1):53–4. Epub 2010/01/30.
178. Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010;115(5):925–35. Epub 2009/11/03.
179. Lindemans CA, Leen AM, Boelens JJ. How I treat adenovirus in hematopoietic stem cell transplant recipients. Blood. 2010;116(25):5476–85. Epub 2010/09/15.
180. Anderson EJ, Guzman-Cottrill JA, Kletzel M, Thormann K, Sullivan C, Zheng X, et al. High-risk adenovirus-infected pediatric allogeneic hematopoietic progenitor cell transplant recipients and preemptive cidofovir therapy. Pediatr Transplant. 2008;12(2):219–27. Epub 2008/03/01.
181. Muller WJ, Levin MJ, Shin YK, Robinson C, Quinones R, Malcolm J, et al. Clinical and in vitro evaluation of cidofovir for treatment of adenovirus infection in pediatric hematopoietic stem cell transplant recipients. Clin Infect Dis. 2005;41(12):1812–6. Epub 2005/11/17.
182. Feucht J, Opherk K, Lang P, Kayser S, Hartl L, Bethge W, et al. Adoptive T-cell therapy with hexon-specific Th1 cells as a treatment of refractory adenovirus infection after HSCT. Blood. 2015;125(12):1986–94. Epub 2015/01/27.
183. Hirsch HH. BK virus: opportunity makes a pathogen. Clin Infect Dis. 2005;41(3):354–60. Epub 2005/07/12.
184. Philippe M, Ranchon F, Gilis L, Schwiertz V, Vantard N, Ader F, et al. Cidofovir in the treatment of BK virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2016;22(4):723–30. Epub 2016/01/01.
185. Martino R, Bretagne S, Einsele H, Maertens J, Ullmann AJ, Parody R, et al. Early detection of toxoplasma infection by molecular monitoring of toxoplasma gondii in peripheral blood samples after allogeneic stem cell transplantation. Clin Infect Dis. 2005;40(1):67–78. Epub 2004/12/23.
186. Costa JM, Munoz C, Kruger D, Martino R, Held TK, Darde ML, et al. Quality control for the diagnosis of toxoplasma gondii reactivation in SCT patients using PCR assays. Bone Marrow Transplant. 2001;28(5):527–8. Epub 2001/10/11.
187. Iori AP, Ferretti A, Gentile G, Gabrielli S, Perrone S, Barberi W, et al. *Strongyloides stercoralis* infection in allogeneic stem cell transplant: a case report and review of the literature. Transpl Infect Dis. 2014;16(4):625–30. Epub 2014/06/04.
188. Ljungman P, Cordonnier C, Einsele H, Englund J, Machado CM, Storek J, et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant. 2009;44(8):521–6. Epub 2009/10/29.
189. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58(3):309–18. Epub 2014/01/15.
190. Eisenberg RA, Jawad AF, Boyer J, Maurer K, McDonald K, Prak ET, et al. Rituximab-treated patients have a poor response to influenza vaccination. J Clin Immunol. 2013;33(2):388–96. Epub 2012/10/16.
191. Kennedy LB, Li Z, Savani BN, Ljungman P. Measuring immune response to commonly used vaccinations in adult recipients of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2017;23(10):1614–21. Epub 2017/06/19.

Evolving Epidemiology of Transplant Infections: Impact of Population Shifts and Climate Change

5

Tirdad T. Zangeneh and Mohanad Al-Obaidi

Contents

Introduction 83
Migration and Population Shift 84
Modern Patterns of Human Migration and Infection 85
Shifting Populations and the Changing Epidemiology of Infectious Diseases 86
Preventative Strategies and Global Demographic Changes 88
Climate Change and Infectious Diseases 89
Climate Change and Parasitic Infections 90
Climate Change and Vector-Borne Infections 91
Climate Change and Fungal Infections 92
Climate Change and Bacterial Infections 93
Conclusion 93
Key Points 94
References 94

Introduction

The emergence and spread of infectious diseases is related to weather and seasonal variability, from the amount of rainfall, flooding, and drought to the rise in local temperatures. Climate change and global warming will impact worldwide temperatures and seasonal variability with different amounts of moisture and heat [1]. Selected infectious diseases affected by climate change including viral, bacterial, parasitic, and fungal infections are expected to rise in frequency [2]. The incidence of emerging infectious diseases is likely to increase in relation to changes in human

T. T. Zangeneh (✉)
Infectious Diseases, University of Arizona College of Medicine, Tucson, AZ, USA
e-mail: tzangeneh@deptofmed.arizona.edu

M. Al-Obaidi
Infectious Diseases, Banner – University Medical Center Tucson (BUMC-T), Tucson, AZ, USA
e-mail: alobaidim@deptofmed.arizona.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_4

activity through commercial globalization, population movement, and global environmental changes [3]. Profoundly immunosuppressed patients are at a greater risk of mortality and morbidity secondary to infections related to population shift and climate change. This is due to this population's underlying suppressed immunity, lack of effective and appropriate response to immunizations, and the absence of unified proper initiatives to face the problem of infectious diseases in the setting of epidemiological shifts. In addition, SOT and HSCT recipients are at risk of donor-derived infections and debilitating infectious diseases through the acquisition of new or reactivation of old infections that they acquired through the environment. Predicting infectious diseases patterns due to epidemiological shifts resulting from climate change will guide the prevention of infectious diseases in transplant recipients [4].

Migration and Population Shift

Geographic shifts in population and global demographic changes have become the norm, as it is estimated that 150 million people lived somewhere outside their country of origin by the end of the twentieth century [3]. In 2015 there were reported 244 million international migrants from Europe, Asia, Africa, and the Middle East [5]. In the same year, close to 65 million people were displaced globally due to wars and natural disasters, generally relocating from regions with limited healthcare systems [6]. In addition, international travel has become more popular. According to the United Nations World Tourism Organization (UNWTO), international tourist arrivals grew by 7.0% in 2017, the greatest increase since 2009. A total of 1,326,000 tourist arrivals were recorded in destinations around the world, 8.5% more than in 2016. It is expected that by the year 2050 close to 70% of the world population will inhabit urban areas with limited resources [7]. There are well-established historical accounts of how colonialism, human migration, and religious conquests led to outbreaks of new infections in susceptible populations. The "Black Death" or the Great Plague pandemic, caused by the bacterium *Yersinia pestis* transmitted by flea-infested rodents, originated in China and spread along the great trade routes to Europe via Italy. The Plague killed between 25 and 30 million people worldwide and, from 1347 to 1352 CE, destroyed 60% of the European population [8, 9]. Today, a rise in the incidence of emerging infectious diseases may be linked to changes in human activity through commercial globalization, population movement, and environmental changes [3]. The increased movement of humans, animals, and food products resulting from changes in global commerce practices has led to infectious disease outbreaks [3]. Transportation used for human migration and travel including the use of ships has led to the spread of influenza, legionellosis, and diarrheal illnesses due to *Vibrio cholerae*. In addition, transportation of goods for human use has introduced diseases and led to the introduction of vectors into new geographic regions as documented by outbreaks of West Nile virus (WNV) transmitted by *Culex* mosquitoes and Chikungunya fever by *Aedes* mosquitoes. The emergence of outbreaks of Zika virus (ZKV) in South America, Central America,

and Florida highlights the link between human movement and emergence of new infectious diseases. This movement of people has intensified in the twenty-first century with the rise in globalization, poverty, environmental disasters, and political unrest resulting in the spread of infections [3]. The devastating 2010 earthquake in Haiti resulted in the death of over 200,000 people and the displacement of over a million people. Ten months following the earthquake, an outbreak of toxigenic *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor, linked to a distant geographic source caused 8,183 deaths [10]. Civil unrest in the Democratic Republic of the Congo (DRC) resulted in 1152 Ebola-related deaths by July 2019. The armed militia hindered the efforts of Ebola response teams and containment efforts resulting in the displacement of at-risk populations [11]. Natural disasters have contributed to outbreaks of fungal infections as well. The displacement of fungi into new environments by the disruption of their habitats has led to clusters of respiratory and cutaneous infections. Coccidioidomycosis, mucormycosis, aspergillosis, and other pathogenic fungal infections have been implicated in such disasters. During the recovery phase, population displacement, lack of access to healthcare, and conditions of poor sanitation and hygiene may result in the rise of infections [12].

Diseases can either emerge in new environments independent of certain climate requirements or adapt specifically to tropical and subtropical regions where certain vectors are present. In addition, non-zoonotic means of transmission such as person-to-person transmission are linked to population density, sexual practices, and lack of immunity to vaccine-preventable diseases [3]. Political instability in Venezuela has resulted in the breakdown of its public health system. Much of the population has limited access to food and healthcare. This has resulted in new cases of measles, diphtheria, and malaria [13]. According to the World Health Organization (WHO), immigration and forced displacement have led to an increase in imported and autochthonously transmitted cases of leishmaniasis in Europe and other non-endemic regions where there is a limited healthcare provider experience in the control and management of this disease [14]. Global cholera epidemics most recently in Yemen have resulted from international spread of the infection due to mass migration, war, famine, and lack of infrastructure with limited access to medical care [15].

Modern Patterns of Human Migration and Infection

Modern patterns of human migration are complex and linked to changes in global trade and travel, advancements in communication technology, socioeconomic inequality, wars and other interpersonal conflicts, and lack of access to jobs and education. These factors coupled with changes in climate result in rising migration and immigration in order to avoid rising sea levels, extreme weather changes, droughts, and resulting decreased access to food [16]. Human migration due to weather-related disasters leads to forced displacement, often resulting in disease outbreaks. Slower migration patterns due to a gradual decline in environmental conditions force communities to move into crowded and poverty-stricken urban

areas with increased exposure to infectious diseases. Finally, planned resettlement and labor-related migration may increase infectious disease exposure risk [16].

As military, business, and pleasure travel increase in the twenty-first century, travel-associated illnesses are reported in regions where healthcare providers may be unfamiliar with these illnesses [17]. For example, patients who have visited the southwestern United States may experience significant delays in the diagnosis of coccidioidomycosis after returning home. International adoption practices must include early screening of children on arrival for latent or active infections and provide appropriate immunization for diseases such as polio, varicella, and measles [17]. Recent outbreaks of Ebola have raised international awareness of infection control measures. Infection control may be compromised in settings where access to hand sanitizers, soap, masks, gowns, isolation rooms, and other hygiene practices and education are limited or even nonexistent [17].

As increased tourism and global commercial exchanges have contributed to the emergence of some infectious diseases, institutions such as the Centers for Disease Control and Prevention (CDC) have been focusing on affected travelers, immigrants, and refugees who may become transplant donors or recipients. In a study of local international travelers attending a travel and migration center for travel counseling and immunization in Southern Italy between 2015 and 2017, at least 15% had specific antibodies to one or more vector-borne diseases including dengue, WNV, chikungunya, and ZKV [18]. In another study investigating imported malaria cases through the use of social networks in Swaziland, 73% of imported cases included laborers and self-employed contractors who traveled frequently to Mozambique to visit their families and conduct business while working in Swaziland. Interviews revealed common congregation points, such as urban markets and bus stations, where people with similar travel patterns and malaria risk behaviors could be located and tested. The study highlighted the use of social networking methods to provide interventions in the prevention of vector-borne infections including malaria among at-risk populations [19]. An increase in reported cases of endemic mycoses including histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis has recently been reported in Spain, where infections are not endemic. The study documented an increase in imported endemic mycoses between 1997 and 2014 due to migration. Of the total cases, 65% with histoplasmosis, 25% with coccidioidomycosis, and 32% with paracoccidioidomycosis occurred in immunocompromised patients. Many diseases encountered outside endemic areas are also underdiagnosed due to the unfamiliarity of healthcare providers and the lack of mandatory reporting [20]. This raises the risk for unrecognized infectious diseases in the pool of transplant donors and recipients with a history of migration or relocation.

Shifting Populations and the Changing Epidemiology of Infectious Diseases

Organ, cell, and tissue transplant donors and recipients are exposed to tropical diseases through receipt of blood products, infected grafts, de novo infections,

reactivation of latent infections, and functionally healthy grafts that are reinfected posttransplant. Although most acquired parasitic infections resolve in 3–5 years, infections such as strongyloidiasis and Chagas disease can endure for decades and may result in catastrophic infections after transplantation. Additionally, commercial and transplant tourism may further fuel this process [21]. The transmission of *Trypanosoma cruzi* through blood and blood products and organ and bone marrow transplantation has been well documented in the literature. The changing epidemiology of Chagas disease with increased migration from endemic regions to Europe, the United States, and Australia has been recognized [22]. The CDC has estimated that 1.31% of Latin American immigrants in the United States have been exposed to Chagas disease. However, these estimates are based on test results from blood donors [23]. In a large-scale study, Meymandi et al. sought to assess the prevalence of Chagas disease in a US population of Latin American immigrants through *T. cruzi* antibody testing and reported a prevalence of 1.24%, implying that greater than 30,000 individuals were infected in Los Angeles County [23]. In Europe, most patients with Chagas disease have been diagnosed in Spain, Italy, France, the United Kingdom, and Switzerland where pooled seroprevalence studies report an overall prevalence of 4.2%. Efforts are underway to improve screening programs and raise physician awareness in order to recognize the serious complications of chronic Chagas disease. In addition, the risk of autochthonous transmission of *T. cruzi* and other infectious diseases is of concern for the transplant community [22]. Changes in the policies for blood donor screening in Europe and North America have been implemented for *T. cruzi* and *Plasmodium* species to prevent cases of transfusion-transmitted infections [24]. The first reported case of transfusion-associated babesiosis was reported in 1979; in 2010 blood donation centers in the United States began screening blood in endemic regions. In July 2018, the FDA issued a draft guidance to help reduce the risk of transfusion-transmitted babesiosis [25]. In 2016, in response to the Zika epidemic, the US Food and Drug Administration (FDA) required universal ID-NAT testing in the 50 states and Washington, DC [26]. Simkins et al. have since reported a low prevalence of ZIKV among their deceased donors in Florida despite local ZIKV transmission; infections were associated with donors who traveled to ZIKV-endemic countries [27]. By 2018, 23 transplant recipients had received organs from WNV-positive donors. US blood banks provide year-round screening for WNV, and living donor screening is recommended by Organ Procurement and Transplantation Network (OPTN) in endemic areas. However, for deceased donors there are no formal recommendations, and practices differ by organ procurement organizations (OPO) [28].

It is estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* (TB) and transplant recipients have a higher risk of developing life-threatening complications with TB. International travel and immigration have changed the epidemiology and donor populations; currently TB has become one of the common donor-derived bacterial infections in the United States and Europe where a reported 0.35–6.6% of recipients develop TB [29]. The diagnosis of donor-derived and reactivated latent tuberculosis (TB) in transplant recipients is challenging in low TB prevalence regions despite the availability of sensitive imaging modalities and improved diagnostic assays [29].

The global public health threat of antimicrobial resistance is linked directly to travel and/or immigration. This may have a significant negative impact for patients undergoing HSCT or SOT [3]. Numerous studies have linked the acquisition of multidrug-resistant organisms (MDROs) including multidrug-resistant *Enterobacteriaceae* (MRE) with international travel. The CDC has issued warnings concerning infections with nontuberculosis mycobacteria related to cosmetic surgery in countries such as the Dominican Republic. Most studies note that acquisition occurs through contact with local food, water, colonized individuals, antimicrobial use during travel, and nosocomial transmission during medical care. Many travelers and migrants are asymptomatic carriers of MDROs, and studies have documented that 6–24% continue to harbor MRE 6 months post travel [30]. Increased travel coupled with a global increase in the number of transplant procedures facilitates the emergence and transmission of infectious diseases in previously unaffected populations [7]. Transplant tourism places recipients at risk for medical and surgical complications. There is also a reported threefold increase in the risk of acquiring infections, including antibiotic-resistant bacteria such as New Delhi metallo-beta-lactamase-1 (NDM-1)-producing *Enterobacter cloacae* and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* [31, 32]. Infections are not limited to drug-resistant bacteria; at certain centers invasive fungal infections (IFIs) have been reported in up to 8% of commercial transplant recipients [33].

Preventative Strategies and Global Demographic Changes

Prevention of infectious diseases related to geographic shifts in population requires a multidisciplinary approach. Efforts are needed in the development of surveillance systems, vector control programs, educational initiatives, access to healthcare and immunizations, and humane political systems that do not demonize travelers, immigrants, and refugees [3]. In order to prevent the spread of infectious diseases, it is important to intervene early and provide appropriate public health measures and preventative services for immigrants and travelers. Awareness of travel history is key for evaluating infectious disease exposure and critical when addressing infectious disease risk for organ donors and recipients. Screening for communicable diseases such as tuberculosis, foodborne illness, and vaccine-preventable diseases encountered during prolonged travel or during residence in refugee camps is important. The testing and management of HIV, hepatitis B, hepatitis C, malaria, and chronic helminthiasis such as strongyloidiasis are a crucial component of the transplant screening process. Appropriate access to care is critical in this time of high human mobility and is an important factor in the prevention of infectious diseases. Access to such services should be independent of one's legal status [5]. Immigrants and refugees have faced discrimination through unfounded claims of infection transmission to new populations such as the baseless polio threat posed by Syrian refugees into Europe after a 2013–2014 outbreak [6, 34]. With the rise in human migration, infectious disease specialists and other healthcare providers have the additional task

of encouraging and educating society on the importance of humane and compassionate care for our fellow human beings who are fleeing unfavorable conditions [6].

Healthcare provider awareness of certain infectious diseases is also limited in developed countries. For example, the differential diagnosis for conditions associated with splenomegaly may be different than when encountered in developing countries where malaria, leishmaniasis, and schistosomiasis are more common [35]. Various studies have stressed the importance of research and public health policy in the development of surveillance systems for vector-borne diseases. In addition, calls have been made to the scientific community and policy makers to provide strategies that promote sharing of experience and data, pooling resources in order to respond promptly to the threat of vector-borne diseases [7].

Transplant screening guidelines, including those from the American Society for Transplantation (AST), the CDC, the Infectious Diseases Society of America, and the American Society for Blood and Marrow Transplantation, have addressed the prevention of infection in transplant recipients [21]. Screening and selection protocols can assist transplant care providers with the early detection of infections in donors and recipients from tropical and other geographic regions, which are usually not encountered at a transplant center [21]. In addition, recently published guidelines from the Infectious Disease Community of Practice of the AST have updated recommendations for prevention and management of travel-related infection in solid organ transplant (SOT) recipients and transplant tourists. The guidelines recommend counseling regarding travel, evaluation by travel medicine specialists, advice on vaccine-preventable illnesses, and antimicrobial prophylaxis. These guidelines and a chapter in the current book address the risks associated with transplant tourism [36].

Climate Change and Infectious Diseases

Over the past 3.5 billion years, the earth's climate went through a multitude of changes that led to environmental pressure on living organisms, species selection, and extinction. Most of the changes were naturally driven by the earth's geological activity, which resulted in the rise of new species while others perished [37]. The current planetary period during which humans have manipulated their surrounding natural habitats and affected species evolution is referred to as Anthropocene. Indeed, such changes were observed since Darwin's time and led to the inception of the theory of evolution. The recent rise in global CO_2 concentration, through its greenhouse effect, leads to increases in the global temperature and rising sea levels [1]. This phenomenon will impose variable selection pressure on different species, leading to the extinction of certain species [1]. According to the Intergovernmental Panel on Climate Change (IPCC), the anthropogenic climate change is expected to increase the global temperature by 1.5 °C above the pre-industrial era by the middle of the twenty-first century [1]. As predicted, global warming and climate change will affect the biodiversity and life on earth and will also have a direct effect

on human health, particularly impacting the spread of infectious diseases. This rise in global temperature and change in seasonal variation may lead to widespread endemics of vector-borne diseases including parasitic, viral, fungal, and bacterial infections [2].

Infections caused by respiratory viruses are known for their seasonal variation and their association with climate change. To give an example, a study of H7N9 and H5N1 viruses in China showed that temperature variation played a role in the distribution of both strains; however, interestingly humidity played a factor in H7N9, while atmospheric pressure was more associated with H5N1 infections [38]. Surgical site infections (SSIs) are among other infections found to be affected by seasonal variation. Incident of SSIs is found to be at the highest rates in August, although this may have been related to the "July effect" where new staffing turnaround occurs. However, the study concluded that the seasonal effect persisted after adjusting for hospital settings (academic vs. private) [39].

The predicted spread of infectious diseases with climate change will carry devastating consequences to human health and the immunocompromised population in particular. This is expected to carry significant health risks to the SOT/HSCT population in particular as it can lead to higher rates of organ rejection, morbidity, and mortality. As the prevalence of immunosuppressed population is expected to rise in the following years, studying this aspect of infectious diseases will help design plans to tackle this emerging problem in this vulnerable population.

Climate Change and Parasitic Infections

Climate change is expected to alter the current epidemiological trend of parasitic infections, by affecting the host and vector interplay secondary to population shift of vectors, hosts, and reservoirs. It is believed that the current epidemiology of *Schistosoma* spp. may suffer changes in its geography. Such trends can be unpredictable and pose a risk for the emergence of donor-derived infections as current donor travel history screening methods lack the understanding and ability to predict future changes in the environment [40]. Similar to the epidemiological change of *Schistosoma* spp., the geographic distribution of other parasitic infections will be affected. A study from Hawaii suggests that there will be a shift in the *Angiostrongylus cantonensis* epidemiology, the agent of eosinophilic meningitis, as it is expected to expand to higher altitudes within the Hawaiian Islands [41]. Global warming is thought to cause an increase in the incidence of helminthic infections in specific geographical regions. At the same time, other regions will have a possible reduction in the rates of such infections, as there will be a shift favoring certain environments from one area to another [42]. The human data needed to predict the future trends of other parasitic infections known to affect SOT recipients such as *Strongyloides stercoralis* are currently lacking [4]. However, a veterinary study from Ireland suggests that warmer and more humid weather driven by climate change may increase the rates of gastrointestinal sheep nematode infections [43].

Climate Change and Vector-Borne Infections

It is estimated that 17% of all infectious diseases are due to vector-borne diseases including dengue, chikungunya, Zika virus, malaria, leishmaniasis, and lymphatic filariasis in tropical and subtropical regions [7]. Solid organ transplant patients are at risk of acquiring infections through donors who are infected with vector-borne pathogens, such as chikungunya, *Eastern equine encephalitis virus*, WNV, and *Leishmania* [42, 44–47]. Vectors such as *Aedes aegypti* and *Aedes albopictus* are observed to follow humans' movement; however, the long-term prediction models suggest global warming will primarily drive future spread. It is expected that global warming will provide a suitable medium for both *Ae. aegypti* and *Ae*. *albopictus* to expand into new territories in Europe and North America. This will facilitate the spread of chikungunya, dengue, yellow fever, and ZKV infections [48]. WNV has established its presence in the United States with reported cases of transmission to organ recipients [47]. It is expected that with the increase of global temperatures and changes in the weather driven by climate change, dengue, chikungunya, and ZKV will consolidate their current foothold in the globe's tropical and subtropical regions around many seasons and will likely spread to more temperate areas within the continent of North America [49]. These viruses are reported to infect SOT and HSCT recipients; however, the extent of their impact on such populations requires more study.

Experimental mosquito models revealed that increases in temperature toward the optimum of 29 °C would lead to higher rates of infected mosquitos with ZKV, which in turn will lead to the higher transmission of this virus. This suggests the spread of ZKV from current areas to high-latitude temperate regions [50]. Atypical clinical presentations of ZKV infections have been observed in solid organ transplantation recipients during the posttransplantation period; however, donor-derived infection rates and outcomes are yet to be studied. Following new trends in the spread of such viruses will help healthcare providers and organ procurement organizations to screen donors and educate recipients properly [45].

Multiple mathematical models have been utilized to assess the effect of climate change and the spread of tick-borne illnesses. Such models predicted the spread of tick-borne illness through the dissemination of associated vectors driven by the changes in climate in different parts of the world. One model predicted the rise in temperatures might lead to the spread of *Amblyomma americanum* (lone star tick) to more northern and eastern parts of the continental United States. Such expected expansion will lead to the spread of *Rickettsia* spp. (e.g., *Rickettsia rickettsii*, *Rickettsia parkeri*), *Ehrlichia* spp. (e.g., *Ehrlichia chaffeensis*, *Ehrlichia ewingii*), *Francisella tularensis*, and Heartland virus [51]. Surveillance data from Canada showed expansion of the *Ixodes scapularis* and non-scapularis ticks in the Ontario and Quebec regions of Canada which can be related directly or indirectly to climate change and its effect on host animals [52, 53]. Such vectors carry infectious agents including Lyme disease and *Powassan virus*. Additionally, an experimental model *Rhodnius prolixus* at different temperatures showed that an increase in temperature from 26 °C to 30 °C led to decreases in the development time of *Rhodnius prolixus*

accompanied by the increase of infective forms of *Trypanosoma cruzi* associated with this vector [54]. Tick-borne and insect vector-borne illnesses can be transmitted through blood transfusion, tissue, cell, and organ donation.

Similar approaches are being taken to better understand future changes in parasitic infections. Use of the Maxent machine learning to study lymphatic filariasis in Africa has helped predict a possible spread of lymphatic filariasis to higher altitudes in the continent of Africa affected by global warming [55]. In another study, human population growth and population shift are both predicted to play a significant role in the expected increase of the spread of these infections. However, it is also believed that climate change will favor the development of *Simulium* species (black fly), which is responsible for the spread of *Onchocerca volvulus* [43].

More than 200 million people around the globe are at risk of contracting malaria including SOT and HSCT recipients [56–58]. Malaria is one of the parasitic infections affected by climate change with a shift of geographical distributions of its vector (*Aedes* species) toward higher altitudes. In a study exploring the epidemiology of malaria in Ethiopia and Colombia, researchers observed the expansion of malaria infection to higher elevations with rising temperatures [59]. Such trends impact the incidence of donor-derived parasitic infections in organ transplantation. This will require monitoring of such patterns and vigilance as unconventional manifestations may occur in the immunocompromised patient and are associated with high morbidity and mortality.

Climate Change and Fungal Infections

SOT and HSCT recipients are at increased risk of developing invasive mycoses, and among fungal infections, endemic mycoses pose a special risk to this vulnerable population [60]. Interseasonal variability is observed in endemic mycoses, and as climate change is expected to result in increases in seasonal temperatures around the globe, the spread of certain fungal infections into areas not previously affected is possible. Indeed, the current pattern of climate change is expected to shift the geographical distribution of endemic fungal infections [61]. A study of coccidioidomycosis in Arizona throughout 1998–2001 showed evident seasonal variability with an increase in incidence of infections during the months of November through February. Additionally, it was also revealed that there was an increase in the annual incidence of this endemic mycosis over the studied period [62].

The relationship between environment and fungal organisms is complex as it is driven by various factors among which climate and available nutrients play an important role. Fungal pathogens from both *Ascomycetes* and *Basidiomycetes* pose a risk to humans and other mammals. Pathogens within *Ascomycetes* like *Coccidioides* spp., *Histoplasma* spp., and *Candida* spp. are found to be more thermotolerant and therefore more likely to cause infections, in contrast to *Basidiomycetes* which are less thermotolerant. However, selected *Basidiomycetes* such as *Cryptococcus* spp. are known thermotolerant pathogens. Thermotolerant *Basidiomycetes* such as *Cryptococcus laurentii* have caused infections in immunocompromised individuals

[63]. Therefore, the rise in global temperatures may select for certain fungi, which are thermotolerant and pathogenic in mammals. The rise in temperatures worldwide could facilitate new fungal pathogens likely to present initially in the immunocompromised patient population.

Climate Change and Bacterial Infections

Climate change and global warming are expected to cause changes in the spread and emergence of bacterial infections [2]. In a recent study of antimicrobial resistance, geographical patterns of temperature increase within the United States led to a rise in the rates of antibiotic resistance among different bacterial species, and the trend persisted despite adjusting for variables including human population growth [64]. In another study using an experimental model of *Vibrio coralliilyticus* that infects coral reefs, an increase of temperature to 27 °C increased the organism's virulence and caused an upregulation in efflux mechanisms. This was associated with antimicrobial resistance; such findings may be extrapolated to other microorganisms that could infect humans. However more studies are required to confirm these findings [65]. Although climate change is thought to cause the spread of certain bacterial infections, the effect on the expansion of *Vibrio* species is uncertain [66]. In a study of El Niño/Southern Oscillation (ENSO) relationship to *Vibrio cholera* spread in Bangladesh, ENSO prolonged the duration of warmer seasons which created a favorable environment for the bacterium to thrive. A similar trend is expected in non-cholera *Vibrio* spp. with the predicted warming of waters worldwide [67, 68]. This was observed in an outbreak of *V. parahaemolyticus* in northwestern Spain, where it was suggested that the outbreak resulted from regional seawater warming following the introduction of the organism to the area [69].

Conclusion

Geographic shifts in population and global demographic changes have resulted in significant changes in the epidemiology of common and emerging infectious diseases. The incidence and prevalence of infectious diseases is directly linked to human movement, placing communities at risk of exposure in new geographic locations, during travel, and upon return to their original communities [16]. Climate change has resulted in the displacement of large populations and is linked to the increase in infectious diseases. Addressing these challenges requires a multidisciplinary, humane, and nondiscriminatory approach that includes public health, economic, policy-making, environmental, and medical expertise [16]. These changes also directly impact the risk of infection in organ, cell, and tissue donors and recipients. It is imperative that transplant centers provide appropriate screening and testing and maintain surveillance and transmissibility data that can be shared. In addition, centers should provide risk mitigation and reporting strategies, conduct

research and quality improvement projects, and insure that transplant providers are familiar with the changing epidemiology of infectious diseases [4].

Finally, anthropogenic climate change will significantly alter many features of life on earth. The emergence of infectious diseases through changes in the geographical distribution of pathogens and their vectors will likely have major impact on human health. Knowledge of this phenomenon and its negative consequences on public health is crucial for proper preparation and initiation of targeted plans to combat new emerging and re-emerging infectious diseases impacting immunocompromised populations most significantly.

Key Points

- Solid organ and hematopoietic stem cell transplantation recipients are vulnerable populations at risk for the infectious complications relating to climate change.
- It is expected that population growth, migration, and shift will introduce new challenges within the field of infectious diseases.
- Anthropogenic climate change and global warming will affect many aspects of human life and alter the epidemiology and emergence of infectious diseases.
- Knowledge of the epidemiological changes in infectious diseases is imperative in the management of transplant recipients.

References

1. Global Warming of 1.5 °C. 2019. https://www.ipcc.ch/sr15/
2. WHO | COP24 Special report: health & climate change. World Health Organization; 2019 [updated 2019-02-08 11:31:46]. https://www.who.int/globalchange/publications/COP24-report-health-climate-change/en/
3. Soto SM. Human migration and infectious diseases. Clin Microbiol Infect. 2009; 15(Suppl 1):26–8.
4. Greenwald MA, Kuehnert MJ, Fishman JA. Infectious disease transmission during organ and tissue transplantation. Emerg Infect Dis. 2012;18:e1.
5. Castelli F, Sulis G. Migration and infectious diseases. Clin Microbiol Infect. 2017;23(5):283–9.
6. Diseases TLI. Migration and health. Lancet Infect Dis. 2016;16(8):867.
7. Otmani del Barrio M, Simard F, Caprara A. Supporting and strengthening research on urban health interventions for the prevention and control of vector-borne and other infectious diseases of poverty: scoping reviews and research gap analysis. Infect Dis Poverty. 2018;7:94.
8. History of the Plague | Plague | CDC 2018 [updated 2018-11-27T07:32:22Z/]. https://www.cdc.gov/plague/history/index.html
9. Cartwright, M. Black Death. 2018. Retrieved from https://www.ancient.eu/Black_Death/
10. CDC – Centers for Disease Control and Prevention. Cholera outbreak – Haiti, October 2010. MMWR Morb Mortal Wkly Rep. 2010;59(43):1411.
11. Jephcott FL. Holding back Ebola. BMJ. 2019;366:l4566.
12. Benedict K, Park BJ. Invasive fungal infections after natural disasters. Emerg Infect Dis. 2014;20(3):349–55.
13. News. Clin Infect Dis. 2019;68(1).

14. World Health Organization. Global leishmaniasis surveillance update, 1998–2016. Wkly Epidemiol Rec. 2018;40:521–30.
15. News. Clin Infect Dis. 2019;68(7).
16. McMichael C. Climate change-related migration and infectious disease. Virulence. 2015;6(6): 548–53.
17. McFee DR. Selected epidemics & emerging pathogens. Dis Mon. 2017;63(9):240–6.
18. Loconsole D, Metallo A, De Robertis AL, Morea A, Quarto M, Chironna M. Seroprevalence of Dengue virus, West Nile virus, Chikungunya virus, and Zika virus in international travelers attending a travel and migration center in 2015–2017, Southern Italy. Vector Borne Zoonotic Dis. 2018;18(6):331–4.
19. Koita K, Novotny J, Kunene S, Zulu Z, Ntshalintshali N, Gandhi M, et al. Targeting imported malaria through social networks: a potential strategy for malaria elimination in Swaziland. Malar J. 2013;12(1):219.
20. Molina-Morant D, Sanchez-Montalva A, Salvador F, Sao-Aviles A, Molina I. Imported endemic mycoses in Spain: evolution of hospitalized cases, clinical characteristics and correlation with migratory movements, 1997–2014. PLoS Negl Trop Dis. 2018;12(2):e0006245.
21. Martin-Davila P, Fortun J, Lopez-Velez R, Norman F, Montes de Oca M, Zamarron P, et al. Transmission of tropical and geographically restricted infections during solid-organ transplantation. Clin Microbiol Rev. 2008;21(1):60–96.
22. Antinori S, Galimberti L, Bianco R, Grande R, Galli M, Corbellino M. Chagas disease in Europe: a review for the internist in the globalized world. Eur J Intern Med. 2017;43:6–15.
23. Meymandi SK, Forsyth CJ, Soverow J, Hernandez S, Sanchez D, Montgomery SP, et al. Prevalence of Chagas disease in the Latin American-born population of Los Angeles. Clin Infect Dis. 2017;64(9):1182–8.
24. Machado CM, Levi JE. Transplant-associated and blood transfusion-associated tropical and parasitic infections. Infect Dis Clin North Am. 2012;26(2):225–41.
25. Villatoro T, Karp JK. Transfusion-transmitted babesiosis. Arch Pathol Lab Med. 2019; 143(1):130–4.
26. Russell WA, Stramer SL, Busch MP, Custer B. Screening the blood supply for Zika virus in the 50 U.S. States and Puerto Rico: a cost-effectiveness analysis. Ann Intern Med. 2019;170:164.
27. Simkins J, Anjan S, Morillas-Rodriguez JA, Greissman SR, Abbo LM, Camargo JF, et al. Screening for Zika virus in deceased organ donors in Florida. Am J Transplant. 2018;18(3):731–6.
28. Anesi JA, Silveira FP. Arenaviruses and West Nile virus in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13576.
29. Morris MI, Daly JS, Blumberg E, Kumar D, Sester M, Schluger N, et al. Diagnosis and management of tuberculosis in transplant donors: a donor-derived infections consensus conference report. Am J Transplant. 2012;12(9):2288–300.
30. Murray CK, Blyth DM. Acquisition of multidrug-resistant gram-negative organisms during travel. Mil Med. 2017;182(S2):26–33.
31. AlBugami MM, AlOtaibe FE, Alabadi AM, Hamawi K, Bel'eed-Akkari K. Transplant tourism following the declaration of Istanbul: poor outcomes and nephrologist dilemma. Nephrology (Carlton). 2018;23(12):1139–44.
32. Stewart J, Snoeyenbos Newman G, Jain R, Bryan A, Berger H, Montenovo M, et al. Transplant tourism complicated by life-threatening New Delhi metallo-beta-lactamase-1 infection. Am J Transplant. 2019;19(4):1224–8.
33. Al Salmi I, Metry AM, Al Ismaili F, Hola A, Al Riyami M, Khamis F, et al. Transplant tourism and invasive fungal infection. Int J Infect Dis. 2018;69:120–9.
34. Khan MS, Osei-Kofi A, Omar A, Kirkbride H, Kessel A, Abbara A, et al. Pathogens, prejudice, and politics: the role of the global health community in the European refugee crisis. Lancet Infect Dis. 2016;16(8):e173–7.
35. Marano N, Wojno AE, Stauffer WM, Weinberg M, Klosovsky A, Ballew JD, et al. Partnerships that facilitate a refugee's journey to wellbeing. Am J Trop Med Hyg. 2016;95(5):985–7.

36. Buchan CA, Kotton CN. Travel medicine, transplant tourism, and the solid organ transplant recipient – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13529.
37. Nogues-Bravo D, Rodriguez-Sanchez F, Orsini L, de Boer E, Jansson R, Morlon H, et al. Cracking the code of biodiversity responses to past climate change. Trends Ecol Evol. 2018; 33(10):765–76.
38. Li J, Rao Y, Sun Q, Wu X, Jin J, Bi Y, et al. Identification of climate factors related to human infection with avian influenza A H7N9 and H5N1 viruses in China. Sci Rep. 2015;5:18094.
39. Anthony CA, Peterson RA, Polgreen LA, Sewell DK, Polgreen PM. The seasonal variability in surgical site infections and the association with warmer weather: a population-based investigation. Infect Control Hosp Epidemiol. 2017;38(7):809–16.
40. Stensgaard AS, Vounatsou P, Sengupta ME, Utzinger J. Schistosomes, snails and climate change: current trends and future expectations. Acta Trop. 2019;190:257–68.
41. Kim JR, Wong TM, Curry PA, Yeung NW, Hayes KA, Cowie RH. Modelling the distribution in Hawaii of *Angiostrongylus cantonensis* (rat lungworm) in its gastropod hosts. Parasitology. 2019;146(1):42–9.
42. Carrasco-Antón N, López-Medrano F, Fernández-Ruiz M, Carrillo E, Moreno J, García-Reyne A, et al. Environmental factors as key determinants for visceral leishmaniasis in solid organ transplant recipients, Madrid, Spain. Emerg Infect Dis. 2017;23:1155–9.
43. Blum AJ, Hotez PJ. Global "worming": climate change and its projected general impact on human helminth infections. PLoS Negl Trop Dis. 2018;12:e0006370.
44. Pouch SM, Katugaha SB, Shieh WJ, Annambhotla P, Walker WL, Basavaraju SV, et al. Transmission of eastern equine encephalitis virus from an organ donor to three transplant recipients. Clin Infect Dis. 2018;69(3):450–458.
45. Nogueira ML, Estofolete CF, Terzian AC, Mascarin do Vale EP, da Silva RC, da Silva RF, et al. Zika virus infection and solid organ transplantation: a new challenge. Am J Transplant. 2017; 17(3):791–5.
46. Chalghaf B, Chemkhi J, Mayala B, Harrabi M, Benie GB, Michael E, et al. Ecological niche modeling predicting the potential distribution of Leishmania vectors in the Mediterranean basin: impact of climate change. Parasit Vectors. 2018;11:461.
47. Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003;348(22):2196–203.
48. Kraemer MUG, Reiner RC Jr, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nat Microbiol. 2019;4:854.
49. Mordecai EA, Cohen JM, Evans MV, Gudapati P, Johnson LR, Lippi CA, et al. Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models. PLoS Negl Trop Dis. 2017;11(4):e0005568.
50. Tesla B, Demakovsky LR, Mordecai EA, Ryan SJ, Bonds MH, Ngonghala CN, et al. Temperature drives Zika virus transmission: evidence from empirical and mathematical models. Proc Biol Sci. 2018;285(1884). https://doi.org/10.1098/rspb.2018.0795
51. Springer YP, Jarnevich CS, Barnett DT, Monaghan AJ, Eisen RJ. Modeling the present and future geographic distribution of the lone star tick, *Amblyomma americanum* (Ixodida: Ixodidae), in the continental United States. Am J Trop Med Hyg. 2015;93(4):875–90.
52. Gasmi S, Bouchard C, Ogden NH, Adam-Poupart A, Pelcat Y, Rees EE, et al. Evidence for increasing densities and geographic ranges of tick species of public health significance other than *Ixodes scapularis* in Quebec, Canada. PLoS One. 2018;13(8):e0201924.
53. Clow KM, Leighton PA, Ogden NH, Lindsay LR, Michel P, Pearl DL, et al. Northward range expansion of *Ixodes scapularis* evident over a short timescale in Ontario, Canada. PLoS One. 2017;12(12):e0189393.
54. Tamayo LD, Guhl F, Vallejo GA, Ramírez JD. The effect of temperature increase on the development of *Rhodnius prolixus* and the course of *Trypanosoma cruzi* metacyclogenesis. PLoS Negl Trop Dis. 2018;12:e0006735.

55. Slater H, Michael E. Predicting the current and future potential distributions of lymphatic filariasis in Africa using maximum entropy ecological niche modelling. PLoS One. 2012; 7(2):e32202.
56. Centers for Disease Control and Prevention. CDC – malaria – malaria worldwide – impact of malaria. 2019 [updated 2019-01-29T05:12:33Z/]. https://www.cdc.gov/malaria/malaria_worldwide/impact.html
57. WHO | Climate change and human health – risks and responses. World Health Organization; 2012 [updated 2012-10-25 18:04:33]. https://www.who.int/globalchange/publications/cchhbook/en/
58. Pierrotti LC, Levi ME, Di Santi SM, Segurado AC, Petersen E. Malaria disease recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102(2S Suppl 2): S16–26.
59. Siraj AS, Santos-Vega M, Bouma MJ, Yadeta D, Ruiz Carrascal D, Pascual M. Altitudinal changes in malaria incidence in highlands of Ethiopia and Colombia. Science (New York, NY). 2014;343(6175):1154–8.
60. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2019;50(8):1101–11.
61. Brown EM, McTaggart LR, Dunn D, Pszczolko E, Tsui KG, Morris SK, et al. Epidemiology and geographic distribution of blastomycosis, histoplasmosis, and coccidioidomycosis, Ontario, Canada, 1990–2015. Emerg Infect Dis. 2018;24(7):1257–66.
62. Park BJ, Sigel K, Vaz V, Komatsu K, McRill C, Phelan M, et al. An epidemic of coccidioidomycosis in Arizona associated with climatic changes, 1998–2001. J Infect Dis. 2005;191(11): 1981–7.
63. Garcia-Solache MA, Casadevall A. Global warming will bring new fungal diseases for mammals. mBio. 2010;1(1):e00061–10. https://doi.org/10.1098/rspb.2018.0795
64. MacFadden DR, McGough SF, Fisman D, Santillana M, Brownstein JS. Antibiotic resistance increases with local temperature. Nat Clim Chang. 2018;8(6):510–4.
65. Kimes NE, Grim CJ, Johnson WR, Hasan NA, Tall BD, Kothary MH, et al. Temperature regulation of virulence factors in the pathogen *Vibrio coralliilyticus*. ISME J. 2012;6:835–46.
66. Urquhart EA, Zaitchik BF, Waugh DW, Guikema SD, Del Castillo CE. Uncertainty in model predictions of *Vibrio vulnificus* response to climate variability and change: a Chesapeake Bay case study. PLoS One. 2014;9(5):e98256.
67. Baker-Austin C, Trinanes J, Gonzalez-Escalona N, Martinez-Urtaza J. Non-cholera vibrios: the microbial barometer of climate change. Trends Microbiol. 2017;25(1):76–84.
68. Rodo X, Pascual M, Fuchs G, Faruque AS. ENSO and cholera: a nonstationary link related to climate change? Proc Natl Acad Sci USA. 2002;99(20):12901–6.
69. Martinez-Urtaza J, Trinanes J, Abanto M, Lozano-Leon A, Llovo-Taboada J, Garcia-Campello M, et al. Epidemic dynamics of *Vibrio parahaemolyticus* illness in a hotspot of disease emergence, Galicia, Spain. Emerg Infect Dis. 2018;24:852–9.

Hospital Epidemiology and Infection Control in the Transplant Center

6

Gopi Patel and Sarah Hochman

Contents

Introduction 100
Hand Hygiene 100
Central Line-Associated Bloodstream Infections 101
Catheter-Associated Urinary Tract Infections 102
Clostridioides difficile 103
Surgical Site Infections 105
Preventing Infections Associated with the Healthcare Environment 106
  Environmental Mold 106
  Waterborne Pathogens 107
Colonization with Multidrug-Resistant Organisms 111
Respiratory Viruses 113
  Influenza 114
  Respiratory Syncytial Virus 115
  Adenovirus 116
  Other Respiratory Viruses 116
Varicella-Zoster Virus 118
Measles 119
Healthcare Personnel Vaccination 119
Conclusion 119
Key Points 120
Cross-References 120
References 120

G. Patel (✉)
Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, USA
e-mail: gopi.patel@mountsinai.org

S. Hochman
Department of Medicine, Division of Infectious Disease and Immunology, New York University School of Medicine, New York, NY, USA
e-mail: sarah.hochman@nyulangone.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_5

Introduction

Despite significant advancements in immunosuppression, prophylactic strategies, and pre- and posttransplant management, candidates and recipients of both solid organ and hematopoietic stem cell transplants continue to be at higher risk for healthcare-associated infections when compared to other patients. Prolonged wait times and new allocation systems leave some patients reliant on indwelling devices like central venous catheters, ventilators, and urinary catheters both before and after transplantation, and the hospital environment itself serves as a potential source for infections with opportunistic pathogens. Improved access to molecular diagnostics and molecular typing has improved the ability to identify outbreaks and address source control. In addition, application of process improvement techniques and partnering between institutional and transplant leadership and infection prevention and control have led to a remarkable improvement in outcomes that were once considered inherent to transplant including healthcare-associated infections.

Here we review select infection prevention strategies that are applicable to transplant patients and review specific pathogens that pose risk to both candidates and recipients. General infection prevention practices are applicable to these vulnerable patient populations and include hand hygiene, respiratory hygiene, vaccination promotion, adherence to care bundles, and antimicrobial stewardship. In addition, patient placement and targeted surveillance for specific pathogens and syndromes may have a pivotal role in the prevention of infection with certain infections.

Hand Hygiene

Hand hygiene is one of the most basic yet important aspects of infection prevention and control. Viruses, bacteria, and fungi can all be spread to and from the environment, staff, and patients via the hands of healthcare personnel (HCP). Hand hygiene prevents horizontal transmission of multidrug-resistant organisms (MDROs), including *Clostridioides difficile*, and reduces the risk of healthcare-associated infections (HAI). Despite this knowledge, HCP hand hygiene is poor even when working with vulnerable transplant recipients [1], highlighting the need for targeted interventions. Clinical staff and patients should have easy access to sinks and alcohol-based hand rub (ABHR) as part of a comprehensive strategy to improve compliance [2]. Prospective monitoring through direct observation or electronically assisted monitoring with real-time feedback can increase hand hygiene compliance [3, 4].

Patient hand hygiene is also important for infection prevention. Patient hand contamination with MDROs is common, and this can be transmitted to and from their environment [5]. An observational study of transplant recipients confirmed that hand hygiene compliance among patients is low, possibly contributing to pathogen transmission [6]. Visitor hand hygiene is important because visitors frequently touch surfaces in the healthcare environment that are also touched by both patients and

HCP, thereby increasing the risk of transmission of nosocomial pathogens [7]. Thus, patient and visitor education encouraging hand hygiene and instructing the proper way to perform hand hygiene should be incorporated into a multimodal program addressing infection prevention.

Central Line-Associated Bloodstream Infections

Central venous catheters (CVCs) are used frequently in the pre- and posttransplant period for hemodynamic monitoring, blood sampling, and administration of medications and blood products. In addition to the convenience of direct venous access, they confer a risk of infection due to bypassing of the protective barrier of the skin. Central line-associated bloodstream infections (CLABSI) are a dangerous complication of CVCs, conferring increased length of stay, increased healthcare costs, and increased mortality risk [8, 9].

Transplant recipients and those awaiting transplant are at high risk for CLABSI, due to high prevalence of CVCs, prolonged hospital lengths of stay, prolonged duration of CVCs, and compromised immunity. Patients awaiting heart transplant frequently have pulmonary artery catheters, which allow for continuous monitoring of left ventricular filling pressures and confer a high urgency status for transplantation. At one institution, the CLABSI rate for patients with pulmonary artery catheters was five times higher than the CLABSI rate for intensive care unit (ICU) patients [10].

Patients who are pre- or immediately post-hematopoietic stem cell transplantation (HSCT) are at additional risk for bloodstream infections secondary to mucosal barrier injuries due to prolonged neutropenia. Single-center studies focusing on HSCT patients report a 20–60% incidence of bloodstream infections in the immediate posttransplant period and higher incidence in patients with acute graft-versus-host disease (GVHD) compared with rates prior to development of GVHD [11, 12]. HSCT candidates undergoing conditioning and HSCT recipients should perform oral hygiene throughout the day to reduce the risk of oral mucositis and secondary bacteremia [13].

A care bundle is a group of evidence-based practices which when performed together are associated with improved outcomes. Each included intervention is considered good practice. Compliance with care bundles should be audited, and feedback should be given to improve compliance and address barriers to compliance. Bundled interventions have proved to decrease CLABSI in ICUs and prompted a paradigm shift. CLABSI now were considered avoidable, and in many centers transplant populations are highlighted for increased incidence of CLABSI [14].

Indeed the majority of CLABSI are likely preventable. In a pivotal statewide trial, the implementation of a CVC insertion bundle including hand hygiene, full barrier precautions, cleaning the insertion site with chlorhexidine gluconate (CHG), avoiding the femoral site, and removing unnecessary CVCs resulted in a 66% sustained decrease in CLABSI in the ICU setting [15]. Additional strategies

to prevent CLABSI focus on maintenance of the necessary CVC. These include daily CHG bathing to reduce the bacterial load on the skin and daily patient hygiene [12, 16]; disinfection of catheter hubs prior to access, needleless connectors, and injection ports; dressing changes with CHG-impregnated dressings every 5–7 days or sooner if visibly soiled, wet, or loose; use of antibiotic-impregnated catheters; and use of ethanol-impregnated disinfection caps [17–22].

Compliance with central line insertion and maintenance bundles is associated with lower CLABSI rates [23–25]. In order to ensure CVC bundled care, compliance is sustained on a patient, unit, and health system level; audit and reminders of bundle elements may increase consistency and subsequently reduce the risk of bloodstream infection [26].

Bundled CVC care has been shown to reduce CLABSI rates in transplant recipients as well. Implementation of process improvement strategies including standardization, monitoring with feedback, and plan-do-study-act cycles contributed to a 61% decrease (4.2–1.8 per 1000 CVC days) in a liver transplant ICU [14]. Implementation of a CVC maintenance bundle resulted in a significant CLABSI reduction in pediatric HSCT patients [27]. Identification of drivers for a rising CLABSI rate in a pediatric hematology-oncology unit and rapid development of interventions, such as identification of patients at high risk for CLABSI and involvement of senior nurses to assist less experienced nurses in dressing changes, resulted in a CLABSI rate reduction from 2.03 to 0.39 per 1000 CVC days [12].

Catheter-Associated Urinary Tract Infections

Catheter-associated urinary tract infection (CAUTI) is a common HAI, resulting in increased cost [8] and increased length of stay [28]. Like CLABSI, CAUTI are largely preventable. The greatest risk factor for CAUTI is prolonged duration of indwelling urinary catheterization. Urinary catheters are often placed unnecessarily and remain in place longer than needed. Prevention measures include limiting insertion of indwelling urinary catheters, use of aseptic technique for insertion by a trained provider, regular perineal care, maintaining unobstructed flow of urine, and removal of the catheter as soon as it is no longer necessary.

CAUTI disproportionately affect solid organ transplant (SOT) recipients, with the most data available for kidney transplant recipients. In an analysis of kidney transplant recipients in the United States from 2002 to 2012, 7.5% of kidney transplant recipients developed CAUTI [29]. Patient-specific risk factors for CAUTI in kidney transplant include age, female sex, and pre-transplant loss of function. Several studies confirm that prolonged duration of indwelling urinary catheter in kidney transplant recipients is a risk factor for both CAUTI and bacteremia [30–32].

Hospitals can reduce urinary catheter utilization by developing and implementing criteria for acceptable indications for indwelling urinary catheter use, such as when there is acute urinary obstruction, to assist in healing of pressure ulcers, or for select surgical procedures like kidney transplantation [33]. There are several

approaches to facilitate compliance with facility-specific utilization guidelines. A nurse-led approach incorporating multidisciplinary rounds on inpatient units that focused on appropriate indications for insertion and timely removal of urinary catheters was effective in reducing the number of catheter days on several units [34]. Reminders for indwelling urinary catheter removal and stop orders embedded within the electronic health record have been effective in decreasing catheter use and significantly reducing CAUTI rates in multiple studies [35]. Since limiting duration of urinary catheters is recommended, the risk of early catheter removal in kidney transplant must be balanced with the potential risk of perioperative urologic complications. The optimal duration of catheterization in this population remains controversial; however early removal should be considered in the appropriate patient [36, 37]. A multidisciplinary team focused on CAUTI-prevention education of nurses and other clinical staff led to a reduction in the CAUTI rate in the transplant care unit where rates had been higher than in the general hospital population [38].

There are no specific differences in the recommendations for maintenance of indwelling urinary catheters in transplant recipients compared with other patient populations. The Centers for Disease Control and Prevention (CDC) recommends use of aseptic technique with sterile equipment for urinary catheter insertion [39]. In a recent randomized controlled trial in Australia, use of chlorhexidine solution for meatal cleaning at the time of catheter insertion resulted in a decreased incidence of CAUTI compared with use of sterile saline [40]. For maintenance after insertion, however, meatal care with antiseptic solutions has shown no demonstrable benefit over routine perineal hygiene [41].

In addition to routine hygiene, maintenance efforts to reduce CAUTI focus on ensuring unobstructed flow of urine. Preventing kinking in catheter tubing allows for free flow of urine from the urethra to the collection bag. Lastly, keeping the collection bag below the level of the bladder and regular emptying of the collection bag prevent reflux of urine from the unsterile bag to the urethra [39]. Involving ancillary HCP (e.g., transporters and physical therapists) in an interdisciplinary promotion of urinary catheter maintenance is important as all members of the healthcare team may be involved in the maintenance of necessary urinary catheters [42].

Clostridioides difficile

Clostridioides difficile infection (CDI) causes a spectrum of disease ranging from asymptomatic colonization or diarrhea due to mild infection to ileus, megacolon, bowel perforation, and septic shock. It is the most common HAI and poses unique infection control risks due to the environmental persistence of infectious spores.

Risk factors for CDI include frequent contact with healthcare settings, receipt of antimicrobials, hospitalization on inpatient units with high rates of overall antimicrobial use [43], immune compromise, poor hand hygiene compliance, and lapses in environmental disinfection. Thus it is not surprising that SOT and HSCT

recipients have significantly higher rates of CDI than do general medical or surgical inpatients [44, 45].

Up to 29% of hospitalized patients are colonized with *C. difficile* [46]. This colonization rate may be higher in HSCT patients and is not well described in SOT recipients [47]. Patients who are colonized are more likely to subsequently develop symptomatic CDI [48]. Antimicrobial stewardship, hand hygiene, and environmental disinfection are important strategies to reduce rates of both symptomatic infection and transmission.

Recommended commercial testing modalities for *C. difficile* include a stool toxin test as part of a multistep algorithm or nucleic acid amplification test (NAAT) alone. There are drawbacks to each type of test; two- or three-step algorithms that include a stool toxin test have relatively low sensitivity, while NAAT without patient-specific symptoms to guide testing does not differentiate between colonization and symptomatic disease. Diagnostic testing should be considered in patients with three or more unformed stools in 24 h or less, who have not had prior testing within the preceding 7 days and who do not have alternative causes of diarrhea, such as laxative use. Testing of asymptomatic patients is not recommended [49], and there are no recommendations to use contact precautions for patients who are asymptomatically colonized. Test of cure should not be performed to guide either treatment duration or cessation of transmission-based precautions.

For patients with diarrhea, contact precautions including gowns and gloves should be initiated preemptively pending *C. difficile* test results and continued if positive until 48 h after diarrhea resolution to prevent transmission to other patients from HCP hands, clothing, and mobile medical equipment. It is preferable to have patients with CDI in single-patient rooms with separate toileting facilities. In the setting of space limitations, incontinent patients should be prioritized for single-patient rooms. If cohorting is required, patients with the same strain of CDI (e.g., two patients with the *C. difficile* B1/NAP1/027 strain) rather than different strains can be cohorted [49]. This information, however, may not be readily available. Shedding of *C. difficile* spores in the stool may be prolonged even after symptom resolution. In centers where there are high rates of CDI, maintaining contact precautions until discharge may be warranted.

Use of ABHR or handwashing with soap and water is recommended when caring for patients with CDI. Compliance with ABHR is higher than with traditional handwashing; however ABHR is not sporicidal and thus may pose a higher risk of transmission of *C. difficile* spores despite good compliance. In settings where there are outbreaks or sustained high rates of CDI, hand hygiene with soap and water is preferred.

Bleach or other sporicidal surface disinfectants are needed to eliminate environmental persistence of spores. Daily and terminal room cleaning with a sporicidal agent may help reduce the risk of transmission of *C. difficile* to patients, especially when there are high baseline rates of CDI [49]. The addition of ultraviolet light terminal disinfection may reduce the risk of *C. difficile* transmission, although studies have shown mixed results [50, 51]. Despite environmental disinfection interventions that result in objective measures of improved environmental cleaning,

a multicenter randomized study found no effect of this on the incidence of healthcare-associated CDI [52]. This highlights that there are many other important factors that affect the incidence of CDI, including pre-existing *C. difficile* asymptomatic colonization, antimicrobial use and overuse, testing stewardship, hand hygiene, disinfection of shared mobile medical devices, and compliance with contact precautions.

Surgical Site Infections

Despite improvements in perioperative management, surgical site infections (SSIs) continue to threaten SOT recipients early after transplantation. Healthcare and antimicrobial exposures are ubiquitous in SOT, and with increased wait times, there is a parallel increased risk for MDROs. The highest rates of SSI are reported in intestinal and multivisceral abdominal organ transplants [53].

Evidence-based strategies aimed toward SSI reduction in the general surgical population can be applied to SOT [54]. These include close attention to maintenance of sterility, surgical technique, minimizing operative time, and minimizing transfusion requirements. The role of the anesthesiologist has been defined and includes reducing environmental contamination, catheter and airway management, as well as perioperative temperature, oxygenation, and glycemic management [54, 55]. Organ-specific risk factors for SSI have been recently reviewed [53].

In centers with increased incidence of *S. aureus* infections, there may be a role for active surveillance and decolonization with mupirocin and/or CHG. Decrease in *S. aureus* SSIs has been demonstrated in cardiac and lower joint surgeries when decolonization is included as part of a care bundle [56]. Although not specific to SSI prevention, predictive models suggest utility in thoracic transplantation with the goal of decreasing posttransplant *S. aureus* infections [57]. Decolonization of SOT candidates can be problematic as durability is incomplete [58], and unless a live donor is available, the timing of transplant remains unpredictable.

Perioperative antimicrobial selection should be determined by the transplanted organ. Preferred antimicrobial regimens tend to be heterogeneous and transplant center-specific [59]. Organ-specific risk factors may require modification or augmentation of regimens (i.e., addition of antifungal prophylaxis) [53]. Although not systematically studied, alteration in prophylaxis based on donor and recipient microbiology as well as institutional antibiograms is likely warranted. Societal recommendations for dosage, including weight-based dosing, and timing including re-dosing are applicable [60]. Extended durations are rarely warranted and should be discouraged.

Recently, the need for patient engagement in HAI prevention has been highlighted [61]. The role of the patient and caregiver may be paramount in SSI prevention [62]. Smoking cessation, diabetes management, and hygiene including compliance with hand hygiene and bathing can influence infection risk. Both patients and their caregivers should be encouraged to promote HCP hand hygiene and appropriate wound care. In addition, educating patients about signs and

symptoms of infection can prompt early identification of potential SSIs that may prevent morbidity.

Preventing Infections Associated with the Healthcare Environment

Transplant recipients are not only at risk for the same wide variety of infections that affect patients with intact immune systems; they are also at a unique risk for infections related to bacteria, mycobacteria, and molds found in the healthcare environment. While HSCT guidelines outline specific infection prevention and control practices [13], there are no standard infection prevention and control strategies among SOT programs [63]. Limiting exposure to environmental molds, such as *Aspergillus*, and water-related opportunistic pathogens, such as *Legionella* species and nontuberculous mycobacteria (NTM), should be a focus of infection prevention and control efforts in the healthcare settings. In addition, access to molecular typing may aid in the identification of potential sources and inform mitigation strategies.

Environmental Mold

Nosocomial mold infections among transplant recipients result from respiratory exposure to fungal spores. Air and water can be sources of pathogenic fungi. Opportunistic molds such as *Aspergillus* and *Fusarium* can be present in a hospital's water supply and may be ingested or inhaled when in water droplets formed from hospital showers or sinks. Water damage to sheetrock from leaks can lead to mold growth and subsequent aerosolization of mold spores within the hospital. Plants, unwashed fruits and vegetables, and fresh or dried flowers can harbor *Aspergillus*. Elevator shafts may pose an additional risk, as shaft surfaces are hard to clean, air moving within the shaft can harbor mold spores, and patients may be exposed during transit within the hospital.

There have been several published reports of environmental mold infections in immune-compromised patients, including transplant recipients, related to hospital linens. Outbreaks of *Rhizopus* have been linked to hospital linens that have been washed in low temperatures, stored in humid and/or dusty conditions, or stored in bins exposed to dust [64, 65]. Facilities supplying linens to the healthcare setting should wash, store, and transport linens in a clean environment, use high wash temperatures, and ensure linens are completely dry prior to packaging. Hospitals should prevent dust contamination of linens during transport, keep linen storage bins clean, and not top off linens prior to patient use (first in, first out premise) [66].

Hospitalized HSCT recipients should be placed in protective environment (PE) rooms [13, 67]. Use of PE rooms for SOT recipients, including lung transplant recipients, is variable among transplant centers [63]. PE rooms are designed to protect severely immune-compromised patients from human and environmental

airborne pathogens. These rooms have positive air pressure in relation to the hallway, with high-efficiency particulate air (HEPA) filtration to remove particles $\geq$0.3 μm in diameter that can pose an infectious risk and $\geq$12 air exchanges per hour to bring in fresh air and remove stale air. In a tertiary care hospital setting, patients with HSCT or hematologic malignancy who had significant exposure outside of the PE room during their hospitalization had higher risk for invasive mold infections [68].

The differential in air pressure should be monitored continuously, ideally by automated systems that alert appropriate staff when the pressure difference is lost. In order to prevent air containing spores from outside the room getting in, and to maintain positive pressure, PE rooms should be sealed, with filling in of gaps between walls, floors, ceilings, windows, and doors [13].

Use of HEPA filtration is especially important when construction is conducted within or near the hospital. A heightened risk to immune-compromised patients comes from the aerosolization of mold spores during construction within or in proximity to a hospital, and outbreaks of healthcare-associated environmental mold infections have occurred in relation to adjacent construction in hospitalized immune-compromised patients [69].

Construction plans should include measures to control mold and should be made in conjunction with the hospital's infection prevention and control department, who can develop an infection control risk assessment (ICRA). ICRAs in this scenario focus on measures to control and minimize airborne dissemination of fungal spores, such as resealing of hospital windows, use of portable HEPA filters in rooms of susceptible patients, use of N95 particulate respirators for patients during travel within and outside of the hospital, and planning for staff caring for susceptible patients to enter and exit the hospital away from the site of construction [66, 70].

Due to multiple potential environmental sources, hospitals should perform surveillance for healthcare-associated mold infections particularly during and after construction events and in highly susceptible patients. This can include utilization of the electronic health record or surveillance software to identify patients with radiographic changes consistent with invasive mold infections; regular review of microbiology laboratory reports for growth of *Aspergillus* and other opportunistic molds; monitoring of therapeutic antifungal use; and location tracing of infected patients to identify high-risk environmental exposures [71]. Additionally, scheduled environmental assessments for water damage and dust, and environmental sampling for identification of mold, can be used to target mitigation efforts [66].

Waterborne Pathogens

In addition to invasive mold infections, transplant recipients and other immune-compromised patients are at higher risk for infections due to waterborne pathogens. The hospital water supply is increasingly identified as a source of nosocomial acquisition [72], likely related to the size and complexity of healthcare facility water systems and the vulnerability of patients treated in these facilities. Outbreaks

of infections related to water exposure within a hospital have included *Legionella* species, nontuberculous mycobacteria, *Pseudomonas aeruginosa*, and other Gram-negative bacteria. These organisms are often referred to as opportunistic premise plumbing pathogens (OPPP). Sources identified in outbreak investigations include potable water, sinks, aerators, showers, immersion tubs, ice and ice machines, and decorative fountains. Transmission of pathogens occurs through both direct and indirect contacts, ingestion, aspiration, and/or aerosolization.

Legionella, atypical mycobacteria, as well as *Pseudomonas aeruginosa* and other Gram-negative bacteria are normal inhabitants of drinking water. These bacteria may be chlorine- and heat-resistant and can form biofilms on plumbing surfaces, making them difficult to eradicate from a hospital's water supply once detected [73]. Older water systems can promote stagnation, corrosion, and the biofilm formation, thus propagating the growth of OPPP.

Water management programs targeting conditions conducive to growth of *Legionella* and other OPPP can reduce their growth in water and prevent transmission to susceptible patients. Mitigation strategies (Table 1) for potential sources have been previously published [72]. Copper-silver ionization of water is one disinfection method used by healthcare facilities to control growth of *Legionella* spp.; it reduces the risk of *Legionella* positivity in environmental samples, and it may have effects on *Pseudomonas* and other Gram-negative bacteria by binding to bacterial cell walls and destroying biofilms that can harbor bacteria [74, 75]. Monochloramine, a combination of chlorine and ammonia, is also used to limit growth of *Legionella* and other opportunistic pathogens in hospital water supplies [76]. However, neither disinfection system is perfect. *Legionella* may still be detected in hospital water systems despite use of copper-silver ionization [77], and there is the potential for an increase in detection of mycobacteria in water systems with use of monochloramine disinfection [78].

Plumbing design to limit dead-end pipes and subsequent water stagnation can help limit OPPP overgrowth [66]. Other interventions include use of reverse osmosis filters on faucets and shower heads, maintaining circulating water at high temperatures with addition of cold water at the point of use (sinks, showers), or periodic superheating of the water system to limit bacterial growth in circulation [79, 80].

Legionella pneumophila and other *Legionella* species are opportunistic pathogens that can cause a life-threatening pneumonia in susceptible patients. Transmission occurs via inhalation of aerosols and can come from showers, faucets, or cooling towers in the community or healthcare setting. An outbreak of *L. pneumophila* serotype 3 among HSCT patients in a new bone marrow transplant unit in an Israeli hospital was linked to heavy contamination of potable water with the same serotype [81]. Interventions implemented included restrictions on drinking tap water and showers for HSCT patients, in addition to superheating and hyperchlorination of the water system. An outbreak of *Legionella micdadei* pneumonia among kidney and heart transplant recipients at a US hospital was linked molecularly to *L. micdadei* isolated from hot water sources, including showers and sinks in patient rooms and the hospital-wide hot water recirculation loop [82]. Mitigation strategies included superheating and subsequent chlorination of the

Table 1 Healthcare-associated reservoirs for opportunistic premise plumbing pathogens and potential risk mitigation strategies in the setting of healthcare-associated cases or isolation of potential pathogens during routine sampling

Reservoir	Potential pathogens	Mitigation strategies for high-risk units
Potable (tap) water	*Legionella* species *Pseudomonas aeruginosa* Gram-negative bacteria Nontuberculous mycobacteria	Ensure that semicritical devices including respiratory devices are not rinsed in tap water (use sterile water) During water disruptions refrain from using or drinking tap water (e.g., showering, rinsing, or ingesting) Periodic sampling of water for *Legionella* spp. especially in high-risk units including organ transplant units and hematopoietic stem cell transplant units Remove aerators or develop an appropriate maintenance and disinfection plan for high-risk units especially in the setting of detected *Legionella* spp. in water samples or evidence of hospital-onset disease Discourage automated faucets due to the inability to achieve high outlet temperatures and inability to flush Discourage decorative water features (e.g., fountains) Discourage showers or develop an appropriate maintenance and disinfection plan of shower heads If basins are used for bathing, a plan should be in place for disinfection of basins or the basins should not be reused Potential disinfection methods include copper ionization, hyperchlorination, superheating, and filtration – a water management plan should be in place and reviewed periodically with infection prevention and control and facilities experts Heighten awareness and surveillance for legionellosis, unusual nontuberculous mycobacterial infections, and increased incidence of hospital-onset respiratory infections with Gram-negative bacteria
Sinks	Gram-negative bacteria including carbapenem-resistant *Enterobacteriaceae*	Ensure separation of handwashing sinks from sinks where contaminated waste may be disposed (e.g., hopper or utility sink) Ensure a maintenance and disinfection plans for sinks (including drains) located in patient rooms where aerosolization or splashing may occur
Heater-cooler devices	*Legionella* species *Pseudomonas aeruginosa* Gram-negative bacteria Nontuberculous mycobacteria	Ensure that maintenance and disinfection are occurring as per manufacturer's instructions Heighten awareness and surveillance for nontuberculous mycobacteria after cardiac surgery including transplant Position the device to allow vent to exhaust away from the surgical field

water system. *Legionella* outbreaks have also been linked to decorative water fountains in the healthcare setting [77, 83]. Mortality from Legionnaire's disease is 25% based on national surveillance data; however rates specific to transplant recipients are not known [84].

Nontuberculous mycobacteria can be found in soil, water, and dust, and these environmental sources can lead to community-acquired or nosocomial infection. NTM have been linked to multiple outbreaks in healthcare settings, often related to the hospital or municipal water supply. One such outbreak occurred among pediatric HSCT patients after the opening of a new hospital building. An increase in isolation of the rapidly growing mycobacteria *M. chelonae* from clinical cultures was traced to the new building's water supply. Water samples collected from hospital faucets, showers, and ice grew *M. chelonae* and other NTM. Infection prevention and control efforts included use of only bottled water for consumption in HSCT patients and reinforcement of a policy to run showers for 2 minutes prior to patients entering to reduce the mycobacteria density in water [85]. This policy had been put in place several years earlier in response to an outbreak of *M. mucogenicum* bacteremia linked to exposure of patients with central venous catheters to *M. mucogenicum*-contaminated shower water [86].

Another US hospital noted an increase in the incidence of *M. abscessus* isolated from respiratory sample cultures after opening a new hospital building, disproportionately affecting lung transplant recipients [87]. Environmental cultures from biofilms of water sources in the hospital addition grew *M. abscessus*, and it was hypothesized that micro aspirations from tap water led to lung colonization and subsequent infection. Interventions to prevent and control further exposure to *M. abscessus* included use of sterile water instead of tap water for oral care, respiratory therapy, consumption, and enteral tube flushes for all lung and heart transplant recipients, ICU patients, and patients with disrupted GI tracts [87]. The authors noted that low flow rates in the hospital addition's water distribution system, put in place to conserve water, may have facilitated higher water concentrations of *M. abscessus*.

Disseminated NTM infections, specifically *M. chimaera*, have been associated with the use of extracorporeal circulation in cardiac surgeries [88]. These heater-cooler devices (HCDs) are used ubiquitously in heart transplantation. Identification of the potential for these devices to aerosolize NTM as well as other waterborne pathogens led to several control efforts. These included revisions and a request for adherence to the manufacturers' instructions for maintenance and disinfection of these devices; use of sterile or filtered water; and positioning the device in order to vent to exhaust away from the operative field. Although not previously routine practice, NTM should be considered in the differential diagnoses of otherwise unexplained infections in patients after cardiac surgery including SSI. Legionellosis and infections with Gram-negative bacteria have also been linked to HCDs.

An outbreak of *Pseudomonas fluorescens*-related febrile neutropenia in HSCT recipients was linked by molecular typing to a contaminated drinking water dispenser in a hospital in the United Kingdom. This hospital performed routine

weekly pharyngeal screening for *Pseudomonas* carriage at baseline in hematology patients, allowing for identification of pharyngeal colonization in the absence of other positive clinical cultures [89]. A hospital in Finland used pulsed-field gel electrophoresis to link 11 cases of *Pseudomonas aeruginosa* bacteremia in HSCT recipients to isolates from showers and sinks with the same PFGE pattern in their bone marrow transplant unit [90]. No further cases of *Pseudomonas aeruginosa* infection with the same PFGE pattern occurred after implementation of infection control measures, which included hand disinfection, disinfection of shower pipes and shower heads, and use of hot water when cleaning. Similarly, other outbreaks due to other multidrug-resistant Gram-negative bacteria including carbapenem-resistant *Enterobacteriaceae* (CRE) have been linked to contaminated sinks using molecular typing including whole genome sequencing [91–93]. Separating handwashing sinks from areas used for disposal of contaminated waste and disinfection and/or reconfiguration of existing pipes or water systems are important strategies to address identified risks.

Similar to surveillance for other HAI such as CLABSI or CAUTI, use of the electronic health record to identify patients with nosocomial pneumonia can guide targeted interventions, such as testing for *Legionella* infection and other waterborne opportunistic pathogens in susceptible patients. Additionally, targeted environmental surveillance that includes culture of the hospital water supply in areas housing high-risk patients can identify the affected areas and guide eradication efforts [77]. Special attention should be given to the performance of institutional hazard analyses and revisions of water management plans to ensure ongoing attention to the potential environmental risk to transplant populations and other susceptible hosts.

Colonization with Multidrug-Resistant Organisms

Multidrug-resistant organisms (MDROs) include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and Gram-negative organisms including extended-spectrum β-lactamase (ESBL) or CRE. *Candida auris*, an emerging healthcare-associated yeast, is also considered a MDRO because of its antifungal resistance profiles, its unique ability to colonize the skin, and its persistence in the healthcare environment [94].

Enterococci are gastrointestinal common commensals. Gastrointestinal VRE carriage is the major reservoir of the organism in the healthcare setting. VRE can survive on surfaces, and transmission can occur via healthcare workers, mobile medical equipment, or shared toileting facilities [95]. Similar to VRE, ESBL-producing and carbapenem-resistant Gram-negative bacteria can persist through gastrointestinal carriage.

Like VRE and multidrug-resistant Gram-negative organisms, patients tend to be the reservoir for transmission of *S. aureus*. *S. aureus* colonizes the nares and skin in up to 30% of healthy people. Patients with MRSA colonization are more likely to develop MRSA bacteremia than patients who are not colonized [96, 97]. Nosocomial MRSA infections are often associated with breaks in skin and mucosal barriers

including intravascular catheters, endotracheal tubes, or recent surgery and confer significant morbidity and mortality risk [98, 99]. The epidemiology of MRSA in organ transplantation has recently been reviewed [100].

The prevalence of MDRO in the healthcare setting has increased globally over the last several decades [101–103]. In data reported to the CDC's National Healthcare Safety Network, MRSA and VRE are the most commonly identified MDRO associated with device-related infections or SSI [104, 105]. SOT recipients in particular have a high prevalence of CRE [106–109], and VRE colonization is common in HSCT recipients [110] and organ transplant candidates and recipients [95]. In terms of CRE risk, international travel and healthcare exposures including SOT and HSCT in other countries may potentially introduce novel mechanisms of resistance to transplant units [109].

Risk factors for MDRO acquisition are ubiquitous in transplantation. These include high rates of antimicrobial use for both prophylaxis and treatment, frequent and/or prolonged hospitalizations, invasive procedures, high device utilization rates, and medical comorbidities such as kidney failure [100].

As with MRSA, MDRO colonization often precedes invasive disease. In the setting of active surveillance, liver transplant candidates and recipients with asymptomatic MRSA or VRE colonization were at increased risk for invasive MRSA or VRE infection, respectively, when compared with patients who were not colonized, and VRE colonization was associated with an increased risk of mortality [111]. A meta-analysis of 23 studies looking at the effect of MRSA and VRE colonization in liver and other SOT recipients found a similarly increased risk of infection with colonization in the pre- and posttransplant periods [112]. Additionally, a prospective study of liver transplant recipients with active surveillance for rectal MDRO colonization found that even intermittent colonization was significantly associated with subsequent MDRO infection [113]. In a retrospective study of liver recipients, carbapenem-resistant *Klebsiella pneumoniae* colonization was a significant risk factor for invasive carbapenem-resistant *K. pneumonia*e [114].

Interventions such as antimicrobial stewardship; monitoring of environmental disinfection – including disinfection of mobile medical equipment – monitoring of hand hygiene compliance; active surveillance; antiseptics applied to the skin, nares, and oral mucous membranes; and standard and contact precautions can help reduce MDRO colonization and prevent transmission to other patients [115]. Measures to lower the risk of invasive infection include the instating and monitoring compliance with the aforementioned evidence-based bundles to insert and maintain vascular and urinary catheters and oral care and ventilator management to prevent lower respiratory tract infections in ventilated patients.

The CDC and European guidelines recommend the use of standard and contact precaution for patients with MDRO colonization or infection [115–117]. In select settings the role of contact precautions and the duration of contact precautions may require reexamination especially for ESBL producers [109, 118], MRSA and VRE. Two large trials found these precautions were no more effective than standard precautions in reducing MRSA or VRE transmission [119, 120]. A systematic review and meta-analysis of 14 studies found that discontinuing contact precautions

for MRSA and VRE did not lead to increased infection rates [121]. An additional meta-analysis of nine studies looking at infection prevention and control measures to reduce transmission of VRE found that improved hand hygiene compliance, but not contact precautions, decreased the VRE transmission rate [122]. Additionally, contact precautions are associated with fewer bedside visits from healthcare personnel and more preventable adverse events [123]. In the setting of outbreaks, the use of contact precautions may be warranted [124].

Active surveillance for MDROs in asymptomatic patients can identify patients at higher risk for invasive infection and facilitate targeted interventions, such as skin and mucous membrane antisepsis to reduce MDRO colonization or broader empiric antimicrobial management for sepsis. Recent publications have shown that active surveillance with implementation of standard plus contact precautions for carbapenem-resistant *Enterobacteriaceae* (CRE) reduces CRE colonization and infection [125, 126]. However, there is a lack of strong evidence that universal screening for CRE in asymptomatic pre- and posttransplant recipients is of benefit [127].

In a multicenter cluster randomized trial, daily chlorhexidine bathing of patients in ICUs and HSCT units reduced the risk of MDRO acquisition and subsequent bloodstream infection [16]. A large cluster randomized trial of ICU patients in multiple hospitals found that universal decolonization with nasal mupirocin and CHG bathing and, to a lesser degree, targeted MRSA screening and decolonization were more effective than active MRSA surveillance with standard and contact precautions in reducing rates of MRSA colonization and bloodstream infection [128]. In a single center, active surveillance for MRSA colonization in liver transplant recipients with targeted infection control interventions (i.e., contact precautions, cohorting, intranasal mupirocin for decolonization) reduced MRSA colonization and invasive infection [129].

Respiratory Viruses

Respiratory viral infections can contribute to significant morbidity in the immune-compromised host [130]. During periods of intense immunosuppression, early after transplantation or in the setting of GVHD, both SOT and HSCT recipients are at increased risk of viral infection-related complications. These potential complications include progression from upper respiratory tract infections to lower tract infections and pneumonia, bacterial and fungal superinfections, and respiratory failure [131]. In addition, respiratory viral infections have been associated with bronchiolitis obliterans in lung transplant recipients and long-term airflow decline in HSCT recipients.

Molecular diagnostic platforms provide both rapid and accurate results and should be utilized early. Staff should be encouraged to adhere to standard precautions including hand hygiene, disinfection of shared equipment, and respiratory hygiene and cough etiquette. Due to the high rates of morbidity observed in HSCT recipients, some centers recommend universal masking either year-round or during periods of increased community activity of respiratory syncytial virus (RSV) or

influenza [132]. Systematic evaluations have not been performed supporting universal masking, and controversy exists regarding the preferred mask [133, 134]. Moreover, contact precautions (use of gowns and gloves) are recommended for some respiratory viruses in addition to standard precautions [115].

Persistent and asymptomatic viral shedding is common in immunosuppressed populations [131]. Thus, defining the optimal duration of transmission-based precautions in the inpatient setting is difficult, and variability exists between centers.

Influenza

Although traditionally influenza season commences in the Fall and can persist well into Spring, novel influenza viruses with pandemic potential may emerge at any time. Indeed, many lessons were learned in 2009 with the emergence of H1N1 in both the community and healthcare settings [135, 136].

Nosocomial acquisition and healthcare-associated outbreaks among transplant recipients (both SOT and HSCT) have been reported. In general, an outbreak investigation is initiated in the setting of a single hospital-onset case. Updated guidance regarding outbreak control is available and is applicable to both the SOT and HSCT patients [137]. In order to avoid nosocomial and/or sustained transmission, institutions are recommended to encourage vaccination, screen for symptoms, emphasize appropriate patient placement and early diagnostics, initiate early treatment, and establish a procedure for addressing ill healthcare personnel.

Annual vaccination with inactivated influenza vaccine for patients and household contacts is strongly encouraged. It is important to note that both antibody- and cell-mediated immune responses are likely suboptimal early posttransplantation [138]. However, vaccination of SOT and HSCT patients has been associated with improved outcomes [139]. Potential strategies to improve immune response in the SOT population include offering booster doses or high-dose vaccine [140, 141]. Current recommendations include initiating annual influenza vaccine 6 months after HSCT. In the setting of increased community activity, vaccination can be offered as early as 3–4 months after HSCT [138].

During influenza season aggressive symptom screening of HCP, patients, and visitors is recommended. Many institutions limit nonessential visitors and enforce visitor age restrictions on high-risk units (i.e., HSCT units). Patients and visitors should be educated regarding symptoms, respiratory hygiene, and cough etiquette, and ill visitors should be excluded.

Influenza is transmitted through large droplets generated through talking, coughing, and sneezing. Transmission can occur through indirect contact with a contaminated environment and inoculation of mucosal surfaces. Airborne transmission can also occur especially in the setting of aerosolizing procedures, but the superiority of using respirators over surgical masks has not been established [142]. Currently, in addition to standard precautions, droplet precautions are recommended to reduce the risk of transmission. Patients should be prioritized to a single room when influenza is suspected or confirmed. Cohorting of

symptomatic cases based on strain type may be prudent; however cohorting strategies should be discussed with infection preventionists or public health authorities.

In the setting of sustained transmission, nonessential admissions should be delayed, and consideration should be given to redirecting new admissions. Patient transport and movement should be minimized. Symptom screening of staff should be performed daily, and ill staff should immediately mask and be removed from direct patient care. Current recommendations are to exclude these individuals from working with patients until afebrile for 24 h without antipyretics and no earlier than 5 days after illness onset. In the setting of HSCT, the CDC recommends work exclusion or temporary reassignment for as long as 7 days from symptom onset.

Chemoprophylaxis with a neuraminidase inhibitor (i.e., oseltamivir) should be offered to roommates and ward mates of patients with influenza, irrespective of vaccination status [137]. Monitoring and audit of both hand hygiene and environmental disinfection should be prioritized. Patients should be empirically treated when influenza is suspected pending diagnostic results [139]. HCP who are not vaccinated should be vaccinated and offered chemoprophylaxis for 2 weeks after vaccination. In the setting of antigenic drift of circulating virus from vaccine strains, chemoprophylaxis should be considered for all influenza-exposed HCP irrespective of vaccination status.

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) has been associated with morbidity in both SOT and HSCT [143]. The incidence of nosocomial RSV infections often mirrors that of increased community activity. Frequently increased activity is seen during the Fall and early Winter. Transmission is due to direct inoculation of mucous membranes and inhalation of droplets or by indirect inoculation by fomites from a contaminated environment. Outbreaks have occurred in both inpatient and ambulatory settings [144].

Despite evidence of droplet transmission, the routine use of masks is controversial. There has not been conclusive evidence suggesting masks decrease transmission even in the setting of an outbreak. The conjunctiva and nasal mucosa are major portals of entry, and the addition of masks and eye protection should be considered as part of standard precautions if exposure to respiratory secretions is anticipated [115]. A recent systematic review suggested that eye protection was more effective at reducing transmission than the addition of a mask as part of a multiple component outbreak response [145]. Patients with known or suspected RSV should be prioritized for single-patient rooms and placed in contact precautions. Cohorting, if necessary, should be coordinated with an institution's infection prevention and control department.

In the absence of an effective vaccine or chemoprophylaxis, primary prevention of nosocomial transmission is paramount. Prophylaxis with palivizumab, a RSV-specific monoclonal antibody, is variable among surveyed SOT centers [146, 147]. The American Academy of Pediatrics offers guidance regarding the use of

palivizumab in pediatric populations during RSV season [148–150]. Off-label use in adults in an outbreak setting has been described [151].

Like influenza, in the setting of an outbreak of RSV, aggressive screening of visitors, HCP, and patients should occur. Visitor limitations and age restrictions may be required. Cohorting of both patients and staff and restricting patient movement as well as augmented monitoring of hand hygiene and environmental disinfection are recommended. In addition, HCP and visitors are encouraged to practice respiratory and cough etiquette. Ill HCP should be immediately removed from patient care; however, the optimal duration of exclusion or reassignment remains unclear. It should be noted that often individuals can be asymptomatic and can serve as a source for ongoing transmission [144]. Active laboratory surveillance in the absence of symptoms cannot be categorically recommended but can be considered in settings of sustained transmission [144].

Adenovirus

Contrary to both RSV and influenza, adenovirus infections do not demonstrate seasonality [152]. In immunocompetent hosts adenovirus causes a variety of infections depending on serotype. Infections can range from the asymptomatic or mild upper respiratory tract infection to epidemic keratoconjunctivitis and gastroenteritis. More serious infections that more often plague the immune-compromised host include pneumonia, hepatitis, hemorrhagic cystitis, encephalitis, and disseminated disease [153, 154].

Adenovirus is transmitted through direct contact with a patient's environment or via fomites and respiratory secretions. Serotypes associated with gastroenteritis can be transmitted through the fecal-oral route. Adenovirus requires both contact and droplet precautions in addition to standard precautions. Due to prolonged shedding, it is recommended that patients remain in precautions for the duration of the hospitalization [115].

Like RSV, there are no proven prophylactic strategies to prevent adenovirus transmission in the setting of an outbreak. The strategies outlined for both influenza and RSV including symptom screening, visitor restriction, increased audit of hand hygiene, PPE compliance, and disinfection are recommended. Adenovirus can be quite tenacious, and evaluating the role of the environment and potentially contaminated shared equipment is vital in stemming a potential outbreak [155]. The live vaccine currently available only to US military recruits is contraindicated in SOT and HSCT.

Other Respiratory Viruses

Other respiratory viruses (Table 2) can cause morbidity in both SOT and HSCT recipients. Partnering with infectious diseases and infection prevention and control is vital to controlling and preventing outbreaks. Like RSV and adenovirus,

Table 2 Respiratory viruses frequently encountered in the healthcare setting and recommended transmission-based precautions and prophylaxis strategies

Respiratory virus	Seasonality	Transmission-based precautions in addition to standard precautions	Recommended duration of precautions	Primary prophylaxis	Postexposure prophylaxis
Adenovirus	None	Contact and droplet precautions	Duration of hospitalization	None	None
Coronavirus[b]	None	Standard	N/A	None	None
Seasonal influenza[c]	Fall to Winter	Droplet precautions	24 h after resolution of fever and respiratory symptoms or 7 days after symptom onset whichever is longer – viral shedding can be prolonged	Vaccination of HCP, patients, and household contacts with inactivated influenza vaccine Neuraminidase inhibitor (e.g., oseltamivir) prophylaxis in the setting of increased incidence or contraindication to vaccination	Vaccination Neuraminidase inhibitor (e.g., oseltamivir) prophylaxis in the setting of exposure irrespective of vaccination status
Human metapneumovirus	None	Contact precautions[a]	Duration of illness	None	None
Parainfluenza	Depends on subtype, PIV3 more common in Spring and Summer but can be year-round	Contact precautions[a]	Duration of illness – viral shedding can be prolonged	None	None
Respiratory syncytial virus	Fall to early Winter	Contact precautions[a]	Duration of illness – viral shedding can be prolonged	Palivizumab recommended for high-risk patients as per AAP guidelines	None

[a]Masks can be added as part of standard precautions
[b]Excludes severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and 2019 novel coronavirus (2019-nCoV)
[c]Excludes pandemic or novel influenza viruses

there are no proven prophylactic measures available; thus adherence to standard and recommended transmission-based precautions is crucial. In the setting of nosocomial acquisition, a multicomponent approach to outbreak control is recommended.

Varicella-Zoster Virus

Varicella may be transmitted through direct contact with the rash or through airborne transmission during primary or disseminated infection. Additional transmission-based precautions may be recommended for patients on minimal immunosuppression with uncomplicated dermatomal zoster that be covered; however standard precautions can be used once disseminated disease has been excluded. Patients with primary VZV or herpes zoster involving more than two adjacent dermatomes should be placed in airborne and contact precautions. Patients should remain in airborne and contact precautions until lesions are dry and crusted, which can take longer in the profoundly immune compromised.

The advent of the varicella zoster vaccine has been pivotal in the prevention of primary varicella infections (chickenpox), and the timing of vaccination in transplant candidates has been reviewed [140, 156, 157]. In HSCT, patients can be vaccinated if no longer on immunosuppression and if over 2 years from HSCT without evidence of GVHD [157, 158]. Household contacts should receive age-appropriate VZV vaccinations. Transplant recipients should be isolated from contacts recently vaccinated with live-attenuated vaccines who develop a varicella-like rash. HCP are considered immune to VZV with laboratory confirmed immunity or written documentation of receipt of two doses of varicella vaccine [159].

In the setting of an exposure to varicella, nonimmune household contacts are encouraged to seek immunization. In terms of herpes zoster vaccination, household contacts should preferentially be offered the inactivated subunit vaccine rather than the live-attenuated vaccine. A categorical recommendation regarding subunit herpes zoster vaccine in SOT cannot be given at this time; however safety and efficacy data are emerging and trials are ongoing [140].

In the setting of nonimmunity, administration of Varicella zoster immune globulin (VZIG) within 10 days of an exposure is recommended [160]. Immunosuppressed patients with significant exposure may be required to furlough or be placed into airborne and contact precautions for 8–21 days postexposure. This period can extend to up to 28 days in the setting of receipt of VZIG. This recommendation is based on the average incubation of VZV being 2 weeks and transmission occurring 5–6 days prior to onset of the rash.

Use of antivirals for postexposure prophylaxis has not been systematically evaluated. It can be considered in the setting of patients unable to receive passive prophylaxis prior to 10 days postexposure. Current recommendations include a 7-day course of acyclovir or valacyclovir beginning 7–10 days after exposure. Many recommend longer durations.

Measles

In 2018–2019, the resurgence of measles worldwide after eradication in many countries has led to a refocus on the prevention of measles in the healthcare setting [161, 162]. Use of the live vaccine is contraindicated in SOT. In the setting of HSCT, vaccination is recommended in the absence of ongoing immunosuppression and GVHD. Current recommendations are to consider vaccination over 2 years from transplant. Irrespective of timing of SOT or HSCT, however, vaccination of household contacts is encouraged [140, 163].

Measles is highly contagious and requires immediate recognition and placement in airborne precautions. Patients are infectious 4 days prior to and 4 days after onset of the rash. The classic prodrome of cough, coryza, and conjunctivitis can be subtle and may be missed. In the setting of potential exposure and ongoing transmission, HCP should remain vigilant and err on the side of caution.

In the setting of an exposure where patients do not have documented immunity (defined as receipt of two doses of MMR vaccine or laboratory confirmation of immunity), transplant patients should receive immunoglobulin within 6 days of exposure [164]. In most cases patients with exposure who do not have evidence of immunity will be asked to quarantine with monitoring for symptoms for 21 days after exposure if no receipt of postexposure prophylaxis and 28 days after exposure with receipt of immunoglobulin.

Outbreaks of measles have occurred within healthcare institutions involving both HCP and patients [163, 165, 166]. Currently HCP vaccination and assessment for immunity are recommended [159].

Healthcare Personnel Vaccination

HCP, like household contacts, are encouraged to receive all age-appropriate vaccinations including those where inactivated vaccines are not available. Currently, vaccination against influenza, varicella, measles, mumps, rubella, pertussis, and hepatitis B is recommended [159]. In the setting of inadvertent exposures to communicable diseases, healthcare providers should contact their institutional infection prevention and control department and occupation health providers in order to determine the role of prophylaxis, reassignment, or furlough in the case of documented nonimmunity and significant exposure.

Conclusion

Recommendations for infection prevention and control practices in the general inpatient and ambulatory population are applicable to both SOT and HSCT populations. It should be noted, however, that these populations do pose additional infection prevention challenges due to endogenous and exogenous

immunosuppression, frequent exposure to healthcare, and antimicrobial exposures. Promotion of vaccination of patients, families, and HCP affords some protections from viruses and bacteria; however, vaccine hesitancy continues to exist in some communities. It is important for institutional leadership, transplant leadership, and infection prevention and control experts to partner and ensure that these unique populations are included in process improvement initiatives including those directed toward HAI prevention.

Key Points

- Hand hygiene compliance plays a pivotal role in preventing healthcare-associated infections.
- Care bundles and interdisciplinary teamwork have been associated with reduction in device-associated infections like central line-associated bloodstream infections and catheter-associated urinary tract infections in transplant patients.
- Transplant candidates and recipients are at increased risk for infections with environmental and waterborne pathogens, and mitigation strategies are available including patient placement, infection control risk assessments at the time of construction, and comprehensive water management plans.
- The use of molecular typing can aid in identifying the potential sources of outbreaks and inform mitigation strategies.
- Vaccination of healthcare personnel, household contacts, and patients can prevent severe illness from communicable diseases like influenza and reemerging infections like measles.

Cross-References

► Clostridioides Difficile: Where Should the Management Strategies in Transplant Vary?
► Respiratory Viral Pathogens in Solid Organ and Hematopoietic Stem Cell Transplant Recipients
► Slow-Growing Nontuberculous Mycobacteria in Transplant

References

1. Graf K, Ott E, Wolny M, Tramp N, Vonberg RP, Haverich A, et al. Hand hygiene compliance in transplant and other special patient groups: an observational study. Am J Infect Control. 2013;41(6):503–8.
2. World Health Organization. WHO guidelines on hand hygiene in health care: first global patient safety challenge clean care is safer care. Geneva: WHO Guidelines Approved by the Guidelines Review Committee; 2009.

3. Ellison RT 3rd, Barysauskas CM, Rundensteiner EA, Wang D, Barton B. A prospective controlled trial of an electronic hand hygiene reminder system. Open Forum Infect Dis. 2015;2(4):ofv121.
4. Dyson J, Madeo M. Investigating the use of an electronic hand hygiene monitoring and prompt device: influence and acceptability. J Infect Prev. 2017;18(6):278–87.
5. Mody L, Washer LL, Kaye KS, Gibson K, Saint S, Reyes K, et al. Multidrug-resistant organisms in hospitals: what is on patient hands and in their rooms? Clin Infect Dis. 2019;67:1837–44.
6. Srigley JA, Furness CD, Gardam M. Measurement of patient hand hygiene in multiorgan transplant units using a novel technology: an observational study. Infect Control Hosp Epidemiol. 2014;35(11):1336–41.
7. Cheng VC, Chau PH, Lee WM, Ho SK, Lee DW, So SY, et al. Hand-touch contact assessment of high-touch and mutual-touch surfaces among healthcare workers, patients, and visitors. J Hosp Infect. 2015;90(3):220–5.
8. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, et al. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. JAMA Intern Med. 2013;173(22):2039–46.
9. Ziegler MJ, Pellegrini DC, Safdar N. Attributable mortality of central line associated bloodstream infection: systematic review and meta-analysis. Infection. 2015;43(1):29–36.
10. Yetmar ZA, Lahr B, O'Horo J, Behfar A, Sampathkumar P, Beam E. Pulmonary artery catheter epidemiology of risk in pre-heart-transplant recipients. Infect Control Hosp Epidemiol. 2019;40(6):632–8.
11. Dandoy CE, Alonso PB. MBI-LCBI and CLABSI: more than scrubbing the line. Bone Marrow Transplant. 2019;54(12):1932–9. https://doi.org/10.1038/s41409-019-0489-1. Epub 2019 Feb 26.
12. Dandoy CE, Hausfeld J, Flesch L, Hawkins D, Demmel K, Best D, et al. Rapid cycle development of a multifactorial intervention achieved sustained reductions in central line-associated bloodstream infections in haematology oncology units at a children's hospital: a time series analysis. BMJ Qual Saf. 2016;25(8):633–43.
13. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.
14. Russell TA, Fritschel E, Do J, Donovan M, Keckeisen M, Agopian VG, et al. Minimizing central line-associated bloodstream infections in a high-acuity liver transplant intensive care unit. Am J Infect Control. 2019;47(3):305–12.
15. Pronovost P, Needham D, Berenholtz S, Sinopoli D, Chu H, Cosgrove S, et al. An intervention to decrease catheter-related bloodstream infections in the ICU. N Engl J Med. 2006;355(26):2725–32.
16. Climo MW, Yokoe DS, Warren DK, Perl TM, Bolon M, Herwaldt LA, et al. Effect of daily chlorhexidine bathing on hospital-acquired infection. N Engl J Med. 2013;368(6):533–42.
17. Marschall J, Mermel LA, Fakih M, Hadaway L, Kallen A, O'Grady NP, et al. Strategies to prevent central line-associated bloodstream infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol. 2014;35(Suppl 2):S89–107.
18. Kagan E, Salgado CD, Banks AL, Marculescu CE, Cantey JR. Peripherally inserted central catheter-associated bloodstream infection: risk factors and the role of antibiotic-impregnated catheters for prevention. Am J Infect Control. 2019;47(2):191–5.
19. O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, et al. Summary of recommendations: guidelines for the prevention of intravascular catheter-related infections. Clin Infect Dis. 2011;52(9):1087–99.
20. Chong HY, Lai NM, Apisarnthanarak A, Chaiyakunapruk N. Comparative efficacy of antimicrobial central venous catheters in reducing catheter-related bloodstream infections in adults: abridged cochrane systematic review and network meta-analysis. Clin Infect Dis. 2017;64(suppl_2):S131–S40.

21. Kamboj M, Blair R, Bell N, Son C, Huang YT, Dowling M, et al. Use of disinfection cap to reduce central-line-associated bloodstream infection and blood culture contamination among hematology-oncology patients. Infect Control Hosp Epidemiol. 2015;36(12):1401–8.
22. Merrill KC, Sumner S, Linford L, Taylor C, Macintosh C. Impact of universal disinfectant cap implementation on central line-associated bloodstream infections. Am J Infect Control. 2014;42(12):1274–7.
23. Furuya EY, Dick AW, Herzig CT, Pogorzelska-Maziarz M, Larson EL, Stone PW. Central line-associated bloodstream infection reduction and bundle compliance in intensive care units: a national study. Infect Control Hosp Epidemiol. 2016;37(7):805–10.
24. Tang HJ, Lin HL, Lin YH, Leung PO, Chuang YC, Lai CC. The impact of central line insertion bundle on central line-associated bloodstream infection. BMC Infect Dis. 2014;14:356.
25. Klintworth G, Stafford J, O'Connor M, Leong T, Hamley L, Watson K, et al. Beyond the intensive care unit bundle: implementation of a successful hospital-wide initiative to reduce central line-associated bloodstream infections. Am J Infect Control. 2014;42(6):685–7.
26. Shea G, Smith W, Koffarnus K, Knobloch MJ, Safdar N. Kamishibai cards to sustain evidence-based practices to reduce healthcare-associated infections. Am J Infect Control. 2019;47(4):358–65.
27. Linder LA, Gerdy C, Jo Y, Wilson A. Changes in central line-associated bloodstream infection rates among children with immune compromised conditions: an 11-year review. J Pediatr Oncol Nurs. 2018;35(6):382–91.
28. Chant C, Smith OM, Marshall JC, Friedrich JO. Relationship of catheter-associated urinary tract infection to mortality and length of stay in critically ill patients: a systematic review and meta-analysis of observational studies. Crit Care Med. 2011;39(5):1167–73.
29. Moghadamyeghaneh Z, Chen LJ, Alameddine M, Gupta AK, Burke GW, Ciancio G. Never events and hospital-acquired conditions after kidney transplant. Can Urol Assoc J. 2017;11(11):E431–E6.
30. Wu X, Dong Y, Liu Y, Li Y, Sun Y, Wang J, et al. The prevalence and predictive factors of urinary tract infection in patients undergoing renal transplantation: a meta-analysis. Am J Infect Control. 2016;44(11):1261–8.
31. Lee JR, Bang H, Dadhania D, Hartono C, Aull MJ, Satlin M, et al. Independent risk factors for urinary tract infection and for subsequent bacteremia or acute cellular rejection: a single-center report of 1166 kidney allograft recipients. Transplantation. 2013;96(8):732–8.
32. Dantas SR, Kuboyama RH, Mazzali M, Moretti ML. Nosocomial infections in renal transplant patients: risk factors and treatment implications associated with urinary tract and surgical site infections. J Hosp Infect. 2006;63(2):117–23.
33. Lo E, Nicolle LE, Coffin SE, Gould C, Maragakis LL, Meddings J, et al. Strategies to prevent catheter-associated urinary tract infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol. 2014;35(5):464–79.
34. Fakih MG, Dueweke C, Meisner S, Berriel-Cass D, Savoy-Moore R, Brach N, et al. Effect of nurse-led multidisciplinary rounds on reducing the unnecessary use of urinary catheterization in hospitalized patients. Infect Control Hosp Epidemiol. 2008;29(9):815–9.
35. Meddings J, Rogers MA, Macy M, Saint S. Systematic review and meta-analysis: reminder systems to reduce catheter-associated urinary tract infections and urinary catheter use in hospitalized patients. Clin Infect Dis. 2010;51(5):550–60.
36. Goldman JD, Julian K. Urinary tract infections in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13507.
37. Guler S, Cimen S, Hurton S, Molinari M. Risks and benefits of early catheter removal after renal transplantation. Transplant Proc. 2015;47(10):2855–9.
38. Dols JD, White SK, Timmons AL, Bush M, Tripp J, Childers AK, et al. A unique approach to dissemination of evidence-based protocols: a successful CAUTI reduction pilot. J Nurses Prof Dev. 2016;32(1):53–4.

39. Gould CV, Umscheid CA, Agarwal RK, Kuntz G, Pegues DA. Healthcare Infection Control Practices Advisory C. Guideline for prevention of catheter-associated urinary tract infections 2009. Infect Control Hosp Epidemiol. 2010;31(4):319–26.
40. Fasugba O, Cheng AC, Gregory V, Graves N, Koerner J, Collignon P, et al. Chlorhexidine for meatal cleaning in reducing catheter-associated urinary tract infections: a multicentre stepped-wedge randomised controlled trial. Lancet Infect Dis. 2019;19(6):611–9.
41. Willson M, Wilde M, Webb ML, Thompson D, Parker D, Harwood J, et al. Nursing interventions to reduce the risk of catheter-associated urinary tract infection: part 2: staff education, monitoring, and care techniques. J Wound Ostomy Cont Nurs. 2009;36(2):137–54.
42. Fakih MG, Krein SL, Edson B, Watson SR, Battles JB, Saint S. Engaging health care workers to prevent catheter-associated urinary tract infection and avert patient harm. Am J Infect Control. 2014;42(10 Suppl):S223–9.
43. Brown K, Valenta K, Fisman D, Simor A, Daneman N. Hospital ward antibiotic prescribing and the risks of *Clostridium difficile* infection. JAMA Intern Med. 2015;175(4):626–33.
44. Donnelly JP, Wang HE, Locke JE, Mannon RB, Safford MM, Baddley JW. Hospital-onset *Clostridium difficile* infection among solid organ transplant recipients. Am J Transplant. 2015;15(11):2970–7.
45. Ilett EE, Helleberg M, Reekie J, Murray DD, Wulff SM, Khurana MP, et al. Incidence rates and risk factors of *Clostridioides difficile* infection in solid organ and hematopoietic stem cell transplant recipients. Open Forum Infect Dis. 2019;6(4):ofz086.
46. Furuya-Kanamori L, Marquess J, Yakob L, Riley TV, Paterson DL, Foster NF, et al. Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications. BMC Infect Dis. 2015;15:516.
47. Revolinski SL, Munoz-Price LS. *Clostridium difficile* in immunocompromised hosts: a review of epidemiology, risk factors, treatment, and prevention. Clin Infect Dis. 2019;68(12):2144–53.
48. Jain T, Croswell C, Urday-Cornejo V, Awali R, Cutright J, Salimnia H, et al. *Clostridium difficile* colonization in hematopoietic stem cell transplant recipients: a prospective study of the epidemiology and outcomes involving toxigenic and nontoxigenic strains. Biol Blood Marrow Transplant. 2016;22(1):157–63.
49. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):987–94.
50. Anderson DJ, Moehring RW, Weber DJ, Lewis SS, Chen LF, Schwab JC, et al. Effectiveness of targeted enhanced terminal room disinfection on hospital-wide acquisition and infection with multidrug-resistant organisms and *Clostridium difficile*: a secondary analysis of a multicentre cluster randomised controlled trial with crossover design (BETR disinfection). Lancet Infect Dis. 2018;18(8):845–53.
51. Brite J, McMillen T, Robilotti E, Sun J, Chow HY, Stell F, et al. Effectiveness of ultraviolet disinfection in reducing hospital-acquired *Clostridium difficile* and vancomycin-resistant *Enterococcus* on a bone marrow transplant unit. Infect Control Hosp Epidemiol. 2018;39(11):1301–6.
52. Ray AJ, Deshpande A, Fertelli D, Sitzlar BM, Thota P, Sankar CT, et al. A multicenter randomized trial to determine the effect of an environmental disinfection intervention on the incidence of healthcare-associated *Clostridium difficile* infection. Infect Control Hosp Epidemiol. 2017;38(7):777–83.
53. Abbo LM, Grossi PA, Practice AICo. Surgical site infections: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13589.
54. Berrios-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, et al. Centers for disease control and prevention guideline for the prevention of surgical site infection, 2017. JAMA Surg. 2017;152(8):784–91.

55. Munoz-Price LS, Bowdle A, Johnston BL, Bearman G, Camins BC, Dellinger EP, et al. Infection prevention in the operating room anesthesia work area. Infect Control Hosp Epidemiol. 2019;40(1):1–17.
56. Schweizer ML, Chiang HY, Septimus E, Moody J, Braun B, Hafner J, et al. Association of a bundled intervention with surgical site infections among patients undergoing cardiac, hip, or knee surgery. JAMA. 2015;313(21):2162–71.
57. Clancy CJ, Bartsch SM, Nguyen MH, Stuckey DR, Shields RK, Lee BY. A computer simulation model of the cost-effectiveness of routine *Staphylococcus aureus* screening and decolonization among lung and heart-lung transplant recipients. Eur J Clin Microbiol Infect Dis. 2014;33(6):1053–61.
58. Paterson DL, Rihs JD, Squier C, Gayowski T, Sagnimeni A, Singh N. Lack of efficacy of mupirocin in the prevention of infections with *Staphylococcus aureus* in liver transplant recipients and candidates. Transplantation. 2003;75(2):194–8.
59. Anesi JA, Blumberg EA, Abbo LM. Perioperative antibiotic prophylaxis to prevent surgical site infections in solid organ transplantation. Transplantation. 2018;102(1):21–34.
60. Bratzler DW, Dellinger EP, Olsen KM, Perl TM, Auwaerter PG, Bolon MK, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. Am J Health Syst Pharm. 2013;70(3):195–283.
61. Fernandes Agreli H, Murphy M, Creedon S, Ni Bhuachalla C, O'Brien D, Gould D, et al. Patient involvement in the implementation of infection prevention and control guidelines and associated interventions: a scoping review. BMJ Open. 2019;9(3):e025824.
62. Tartari E, Weterings V, Gastmeier P, Rodriguez Bano J, Widmer A, Kluytmans J, et al. Patient engagement with surgical site infection prevention: an expert panel perspective. Antimicrob Resist Infect Control. 2017;6:45.
63. Beam E, Razonable RR. A survey of infection prevention and control practices among solid organ transplantation centers. Am J Infect Control. 2019;47(1):101–4.
64. Duffy J, Harris J, Gade L, Sehulster L, Newhouse E, O'Connell H, et al. Mucormycosis outbreak associated with hospital linens. Pediatr Infect Dis J. 2014;33(5):472–6.
65. Cheng VCC, Chen JHK, Wong SCY, Leung SSM, So SYC, Lung DC, et al. Hospital outbreak of pulmonary and cutaneous zygomycosis due to contaminated linen items from substandard laundry. Clin Infect Dis. 2016;62(6):714–21.
66. Sehulster L, Chinn RY, Cdc, Hicpac. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep. 2003;52(RR-10):1–42.
67. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis. 2011;52(4):427–31.
68. Gayet-Ageron A, Iten A, van Delden C, Farquet N, Masouridi-Levrat S, Von Dach E, et al. In-hospital transfer is a risk factor for invasive filamentous fungal infection among hospitalized patients with hematological malignancies: a matched case-control study. Infect Control Hosp Epidemiol. 2015;36(3):320–8.
69. Pokala HR, Leonard D, Cox J, Metcalf P, McClay J, Siegel J, et al. Association of hospital construction with the development of healthcare associated environmental mold infections (HAEMI) in pediatric patients with leukemia. Pediatr Blood Cancer. 2014;61(2):276–80.
70. Kanamori H, Rutala WA, Sickbert-Bennett EE, Weber DJ. Review of fungal outbreaks and infection prevention in healthcare settings during construction and renovation. Clin Infect Dis. 2015;61(3):433–44.
71. Ananda-Rajah MR, Bergmeir C, Petitjean F, Slavin MA, Thursky KA, Webb GI. Toward electronic surveillance of invasive mold diseases in hematology-oncology patients: an expert system combining natural language processing of chest computed tomography reports, microbiology, and antifungal drug data. JCO Clin Cancer Inform. 2017;1:1–10.

72. Kanamori H, Weber DJ, Rutala WA. Healthcare outbreaks associated with a water reservoir and infection prevention strategies. Clin Infect Dis. 2016;62(11):1423–35.
73. Falkinham JO 3rd. Common features of opportunistic premise plumbing pathogens. Int J Environ Res Public Health. 2015;12(5):4533–45.
74. Almeida D, Cristovam E, Caldeira D, Ferreira JJ, Marques T. Are there effective interventions to prevent hospital-acquired Legionnaires' disease or to reduce environmental reservoirs of Legionella in hospitals? A systematic review. Am J Infect Control. 2016;44(11): e183–e8.
75. Shih HY, Lin YE. Efficacy of copper-silver ionization in controlling biofilm- and plankton-associated waterborne pathogens. Appl Environ Microbiol. 2010;76(6):2032–5.
76. Duda S, Kandiah S, Stout JE, Baron JL, Yassin M, Fabrizio M, et al. Evaluation of a new monochloramine generation system for controlling Legionella in building hot water systems. Infect Control Hosp Epidemiol. 2014;35(11):1356–63.
77. Demirjian A, Lucas CE, Garrison LE, Kozak-Muiznieks NA, States S, Brown EW, et al. The importance of clinical surveillance in detecting Legionnaires' disease outbreaks: a large outbreak in a hospital with a Legionella disinfection system-Pennsylvania, 2011–2012. Clin Infect Dis. 2015;60(11):1596–602.
78. Baron JL, Vikram A, Duda S, Stout JE, Bibby K. Shift in the microbial ecology of a hospital hot water system following the introduction of an on-site monochloramine disinfection system. PLoS One. 2014;9(7):e102679.
79. Cristino S, Legnani PP, Leoni E. Plan for the control of Legionella infections in long-term care facilities: role of environmental monitoring. Int J Hyg Environ Health. 2012;215(3):279–85.
80. Dyck A, Exner M, Kramer A. Experimental based experiences with the introduction of a water safety plan for a multi-located university clinic and its efficacy according to WHO recommendations. BMC Public Health. 2007;7:34.
81. Oren I, Zuckerman T, Avivi I, Finkelstein R, Yigla M, Rowe JM. Nosocomial outbreak of *Legionella pneumophila* serogroup 3 pneumonia in a new bone marrow transplant unit: evaluation, treatment and control. Bone Marrow Transplant. 2002;30(3):175–9.
82. Knirsch CA, Jakob K, Schoonmaker D, Kiehlbauch JA, Wong SJ, Della-Latta P, et al. An outbreak of *Legionella micdadei* pneumonia in transplant patients: evaluation, molecular epidemiology, and control. Am J Med. 2000;108(4):290–5.
83. Palmore TN, Stock F, White M, Bordner M, Michelin A, Bennett JE, et al. A cluster of cases of nosocomial legionnaires disease linked to a contaminated hospital decorative water fountain. Infect Control Hosp Epidemiol. 2009;30(8):764–8.
84. Soda EA, Barskey AE, Shah PP, Schrag S, Whitney CG, Arduino MJ, et al. Vital signs: health care-associated Legionnaires' disease surveillance data from 20 states and a large metropolitan area-United States, 2015. Am J Transplant. 2017;17(8):2215–20.
85. Iroh Tam PY, Kline S, Wagner JE, Guspiel A, Streifel A, Ward G, et al. Rapidly growing mycobacteria among pediatric hematopoietic cell transplant patients traced to the hospital water supply. Pediatr Infect Dis J. 2014;33(10):1043–6.
86. Kline S, Cameron S, Streifel A, Yakrus MA, Kairis F, Peacock K, et al. An outbreak of bacteremias associated with *Mycobacterium mucogenicum* in a hospital water supply. Infect Control Hosp Epidemiol. 2004;25(12):1042–9.
87. Baker AW, Lewis SS, Alexander BD, Chen LF, Wallace RJ Jr, Brown-Elliott BA, et al. Two-phase hospital-associated outbreak of *Mycobacterium abscessus*: investigation and mitigation. Clin Infect Dis. 2017;64(7):902–11.
88. Allen KB, Yuh DD, Schwartz SB, Lange RA, Hopkins R, Bauer K, et al. Nontuberculous mycobacterium infections associated with heater-cooler devices. Ann Thorac Surg. 2017;104(4):1237–42.
89. Wong V, Levi K, Baddal B, Turton J, Boswell TC. Spread of *Pseudomonas fluorescens* due to contaminated drinking water in a bone marrow transplant unit. J Clin Microbiol. 2011;49(6):2093–6.

90. Lyytikainen O, Golovanova V, Kolho E, Ruutu P, Sivonen A, Tiittanen L, et al. Outbreak caused by tobramycin-resistant *Pseudomonas aeruginosa* in a bone marrow transplantation unit. Scand J Infect Dis. 2001;33(6):445–9.
91. Decraene V, Phan HTT, George R, Wyllie DH, Akinremi O, Aiken Z, et al. A large, refractory nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Escherichia coli* demonstrates carbapenemase gene outbreaks involving sink sites require novel approaches to infection control. Antimicrob Agents Chemother. 2018;62(12)
92. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, Palmore TN, et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. Sci Transl Med. 2012;4(148):148ra16.
93. Leitner E, Zarfel G, Luxner J, Herzog K, Pekard-Amenitsch S, Hoenigl M, et al. Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing *Klebsiella oxytoca* on a hematology ward. Antimicrob Agents Chemother. 2015;59(1):714–6.
94. Cadnum JL, Shaikh AA, Piedrahita CT, Sankar T, Jencson AL, Larkin EL, et al. Effectiveness of disinfectants against *Candida auris* and other Candida species. Infect Control Hosp Epidemiol. 2017;38(10):1240–3.
95. Nellore A, Huprikar S, Practice AICo. Vancomycin-resistant *Enterococcus* in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13549.
96. Marzec NS, Bessesen MT. Risk and outcomes of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia among patients admitted with and without MRSA nares colonization. Am J Infect Control. 2016;44(4):405–8.
97. Vigil DI, Harden WD, Hines AE, Hosokawa PW, Henderson WG, Bessesen MT. Risk of MRSA infection in patients with intermittent versus persistent MRSA Nares colonization. Infect Control Hosp Epidemiol. 2015;36(11):1292–7.
98. Lam JC, Gregson DB, Robinson S, Somayaji R, Conly JM, Parkins MD. Epidemiology and outcome determinants of *Staphylococcus aureus* bacteremia revisited: a population-based study. Infection. 2019;47:961–71.
99. Austin ED, Sullivan SS, Macesic N, Mehta M, Miko BA, Nematollahi S, et al. Reduced mortality of *Staphylococcus aureus* bacteremia in a retrospective cohort study of 2139 patients: 2007–2015. Clin Infect Dis. 2019.
100. Pereira MR, Rana MM, Practice AICo. Methicillin-resistant *Staphylococcus aureus* in solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13611.
101. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R, et al. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992–2003. Clin Infect Dis. 2006;42(3):389–91.
102. Gaynes R, Edwards JR, National Nosocomial Infections Surveillance S. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005;41(6):848–54.
103. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis. 2011;11(5):381–93.
104. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol. 2013;34(1):1–14.
105. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol. 2016;37(11):1288–301.
106. Kalpoe JS, Sonnenberg E, Factor SH, del Rio MJ, Schiano T, Patel G, et al. Mortality associated with carbapenem-resistant *Klebsiella pneumoniae* infections in liver transplant recipients. Liver Transpl. 2012;18(4):468–74.

107. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol. 2008;29(12):1099–106.
108. Lanini S, Costa AN, Puro V, Procaccio F, Grossi PA, Vespasiano F, et al. Incidence of carbapenem-resistant gram negatives in Italian transplant recipients: a nationwide surveillance study. PLoS One. 2015;10(4):e0123706.
109. Pouch SM, Patel G. Multidrug-resistant Gram-negative bacterial infections in solid organ transplant recipients-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13594.
110. Scheich S, Lindner S, Koenig R, Reinheimer C, Wichelhaus TA, Hogardt M, et al. Clinical impact of colonization with multidrug-resistant organisms on outcome after allogeneic stem cell transplantation in patients with acute myeloid leukemia. Cancer. 2018;124 (2):286–96.
111. Russell DL, Flood A, Zaroda TE, Acosta C, Riley MM, Busuttil RW, et al. Outcomes of colonization with MRSA and VRE among liver transplant candidates and recipients. Am J Transplant. 2008;8(8):1737–43.
112. Ziakas PD, Pliakos EE, Zervou FN, Knoll BM, Rice LB, Mylonakis E. MRSA and VRE colonization in solid organ transplantation: a meta-analysis of published studies. Am J Transplant. 2014;14(8):1887–94.
113. Macesic N, Gomez-Simmonds A, Sullivan SB, Giddins MJ, Ferguson SA, Korakavi G, et al. Genomic surveillance reveals diversity of multidrug-resistant organism colonization and infection: a prospective cohort study in liver transplant recipients. Clin Infect Dis. 2018;67(6):905–12.
114. Giannella M, Bartoletti M, Morelli MC, Tedeschi S, Cristini F, Tumietto F, et al. Risk factors for infection with carbapenem-resistant *Klebsiella pneumoniae* after liver transplantation: the importance of pre- and posttransplant colonization. Am J Transplant. 2015;15(6):1708–15.
115. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory C. 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am J Infect Control. 2007;35(10 Suppl 2):S65–164.
116. Centers for Disease C, Prevention. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. MMWR Morb Mortal Wkly Rep. 2009;58(10):256–60.
117. Carmeli Y, Akova M, Cornaglia G, Daikos GL, Garau J, Harbarth S, et al. Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control. Clin Microbiol Infect. 2010;16(2):102–11.
118. Tschudin-Sutter S, Lucet JC, Mutters NT, Tacconelli E, Zahar JR, Harbarth S. Contact precautions for preventing nosocomial transmission of extended-spectrum beta lactamase-producing *Escherichia coli*: a point/counterpoint review. Clin Infect Dis. 2017;65(2):342–7.
119. Huskins WC, Huckabee CM, O'Grady NP, Murray P, Kopetskie H, Zimmer L, et al. Intervention to reduce transmission of resistant bacteria in intensive care. N Engl J Med. 2011;364(15):1407–18.
120. Bearman G, Abbas S, Masroor N, Sanogo K, Vanhoozer G, Cooper K, et al. Impact of discontinuing contact precautions for methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*: an interrupted time series analysis. Infect Control Hosp Epidemiol. 2018;39(6):676–82.
121. Marra AR, Edmond MB, Schweizer ML, Ryan GW, Diekema DJ. Discontinuing contact precautions for multidrug-resistant organisms: a systematic literature review and meta-analysis. Am J Infect Control. 2018;46(3):333–40.
122. De Angelis G, Cataldo MA, De Waure C, Venturiello S, La Torre G, Cauda R, et al. Infection control and prevention measures to reduce the spread of vancomycin-resistant enterococci in hospitalized patients: a systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(5):1185–92.

123. Stelfox HT, Bates DW, Redelmeier DA. Safety of patients isolated for infection control. JAMA. 2003;290(14):1899–905.
124. Abbo L, Shukla BS, Giles A, Aragon L, Jimenez A, Camargo JF, et al. Linezolid- and vancomycin-resistant *Enterococcus faecium* in solid organ transplant recipients: infection control and antimicrobial stewardship using whole genome sequencing. Clin Infect Dis. 2019;69(2):259–65.
125. Ben-David D, Masarwa S, Fallach N, Temkin E, Solter E, Carmeli Y, et al. Success of a national intervention in controlling carbapenem-resistant *Enterobacteriaceae* in Israel's long-term care facilities. Clin Infect Dis. 2019;68(6):964–71.
126. van Loon K, Voor In 't Holt AF, Vos MC. A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant *Enterobacteriaceae*. Antimicrob Agents Chemother. 2018;62(12). pii: e01689-18. https://doi.org/10.1128/AAC.01689-18. Print 2018 Dec.
127. van Duin D, van Delden C, Practice ASTIDCo. Multidrug-resistant gram-negative bacteria infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):31–41.
128. Huang SS, Septimus E, Kleinman K, Moody J, Hickok J, Avery TR, et al. Targeted versus universal decolonization to prevent ICU infection. N Engl J Med. 2013;368(24):2255–65.
129. Singh N, Squier C, Wannstedt C, Keyes L, Wagener MM, Cacciarelli TV. Impact of an aggressive infection control strategy on endemic *Staphylococcus aureus* infection in liver transplant recipients. Infect Control Hosp Epidemiol. 2006;27(2):122–6.
130. Abbas S, Raybould JE, Sastry S, de la Cruz O. Respiratory viruses in transplant recipients: more than just a cold. Clinical syndromes and infection prevention principles. Int J Infect Dis. 2017;62:86–93.
131. Manuel O, Estabrook M, American Society of Transplantation Infectious Diseases Community of P. RNA respiratory viral infections in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13511.
132. Sokol KA, De la Vega-Diaz I, Edmondson-Martin K, Kim S, Tindle S, Wallach F, et al. Masks for prevention of respiratory viruses on the BMT unit: results of a quality initiative. Transpl Infect Dis. 2016;18(6):965–7.
133. Offeddu V, Yung CF, Low MSF, Tam CC. Effectiveness of masks and respirators against respiratory infections in healthcare workers: a systematic review and meta-analysis. Clin Infect Dis. 2017;65(11):1934–42.
134. Smith JD, MacDougall CC, Johnstone J, Copes RA, Schwartz B, Garber GE. Effectiveness of N95 respirators versus surgical masks in protecting health care workers from acute respiratory infection: a systematic review and meta-analysis. CMAJ. 2016;188(8):567–74.
135. Kumar D, Michaels MG, Morris MI, Green M, Avery RK, Liu C, et al. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. Lancet Infect Dis. 2010;10(8):521–6.
136. Reid G, Huprikar S, Patel G, Razonable RR, Mossad S, Levi M, et al. A multicenter evaluation of pandemic influenza A/H1N1 in hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2013;15(5):487–92.
137. Uyeki TM, Bernstein HH, Bradley JS, Englund JA, File TM, Fry AM, et al. Clinical practice guidelines by the infectious diseases Society of America: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenzaa. Clin Infect Dis. 2019;68(6):e1–e47.
138. Bosaeed M, Kumar D. Seasonal influenza vaccine in immunocompromised persons. Hum Vaccin Immunother. 2018;14(6):1311–22.
139. Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P, et al. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67(9):1322–9.

140. Danziger-Isakov L, Kumar D, Practice AICo. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13563.
141. Natori Y, Shiotsuka M, Slomovic J, Hoschler K, Ferreira V, Ashton P, et al. A double-blind, randomized trial of high-dose vs standard-dose influenza vaccine in adult solid-organ transplant recipients. Clin Infect Dis. 2018;66(11):1698–704.
142. Radonovich LJ Jr, Simberkoff MS, Bessesen MT, Brown AC, Cummings DAT, Gaydos CA, et al. N95 respirators vs medical masks for preventing influenza among health care personnel: a randomized clinical trial. JAMA. 2019;322(9):824–33.
143. Khawaja F, Chemaly RF. Respiratory syncytial virus in hematopoietic cell transplant recipients and patients with hematologic malignancies. Haematologica. 2019;104:1322–31.
144. Kelly SG, Metzger K, Bolon MK, Silkaitis C, Mielnicki M, Cullen J, et al. Respiratory syncytial virus outbreak on an adult stem cell transplant unit. Am J Infect Control. 2016;44(9):1022–6.
145. French CE, McKenzie BC, Coope C, Rajanaidu S, Paranthaman K, Pebody R, et al. Risk of nosocomial respiratory syncytial virus infection and effectiveness of control measures to prevent transmission events: a systematic review. Influenza Other Respir Viruses. 2016;10(4):268–90.
146. Michaels MG, Fonseca-Aten M, Green M, Charsha-May D, Friedman B, Seikaly M, et al. Respiratory syncytial virus prophylaxis: a survey of pediatric solid organ transplant centers. Pediatr Transplant. 2009;13(4):451–6.
147. Danziger-Isakov LA, Arslan D, Sweet S, Benden C, Goldfarb S, Wong J. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the International Pediatric Lung Transplant Collaborative. Pediatr Transplant. 2012;16(6):638–44.
148. Teusink-Cross A, Davies SM, Danziger-Isakov L, El-Bietar J, Grimley MS. Restrictive palivizumab use does not lead to increased morbidity and mortality in pediatric hematopoietic stem cell transplantation patients. Biol Blood Marrow Transplant. 2016;22(10):1904–6.
149. American Academy of Pediatrics Committee on Infectious Diseases, American Academy of Pediatrics Bronchiolitis Guidelines Committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. Pediatrics. 2014;134(2):415–20.
150. Science M, Akseer N, Asner S, Allen U. Risk stratification of immunocompromised children, including pediatric transplant recipients at risk of severe respiratory syncytial virus disease. Pediatr Transplant. 2019;23(2):e13336.
151. Kassis C, Champlin RE, Hachem RY, Hosing C, Tarrand JJ, Perego CA, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. Biol Blood Marrow Transplant. 2010;16(9):1265–71.
152. Ison MG. Adenovirus infections in transplant recipients. Clin Infect Dis. 2006;43(3):331–9.
153. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. 2014;27(3):441–62.
154. Florescu DF, Schaenman JM, Practice ASTIDCo. Adenovirus in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13527.
155. Rutala WA, Peacock JE, Gergen MF, Sobsey MD, Weber DJ. Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities. Antimicrob Agents Chemother. 2006;50(4):1419–24.
156. Pergam SA, Limaye AP. Varicella zoster virus (VZV) in solid organ transplantation: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13622.
157. Cordonnier C, Einarsdottir S, Cesaro S, Di Blasi R, Mikulska M, Rieger C, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(6):e200–e12.

158. Chong PP, Avery RK. A comprehensive review of immunization practices in solid organ transplant and hematopoietic stem cell transplant recipients. Clin Ther. 2017;39(8):1581–98.
159. Advisory Committee on Immunization Practices, Centers for Disease Control and Prevention (CDC). Immunization of health-care personnel: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2011;60(RR-7):1–45.
160. Marin M, Guris D, Chaves SS, Schmid S, Seward JF, Advisory Committee on Immunization Practices CfDC, et al. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2007;56(RR-4):1–40.
161. Paules CI, Marston HD, Fauci AS. Measles in 2019 – going backward. N Engl J Med. 2019;380(23):2185–7.
162. Patel M, Lee AD, Redd SB, Clemmons NS, McNall RJ, Cohn AC, et al. Increase in measles cases – United States, January 1-April 26, 2019. MMWR Morb Mortal Wkly Rep. 2019;68(17):402–4.
163. McLean HQ, Fiebelkorn AP, Temte JL, Wallace GS, Centers for Disease C, Prevention. Prevention of measles, rubella, congenital rubella syndrome, and mumps, 2013: summary recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2013;62(RR-04):1–34.
164. Han G, Batra N, Vallejo A, Schechter R, Zipprich J, Harriman K. Notes from the field: measles outbreak in an era of stricter immunization requirements – California, March 2018. MMWR Morb Mortal Wkly Rep. 2019;68(8):201–2.
165. Fiebelkorn AP, Seward JF, Orenstein WA. A global perspective of vaccination of healthcare personnel against measles: systematic review. Vaccine. 2014;32(38):4823–39.
166. Basu S, Giri P, Adisesh A, Mc NR. Healthcare workers and measles-mumps-rubella (MMR) status: how worried should we be about further outbreaks? Epidemiol Infect. 2014;142(8):1688–94.

Antimicrobial Stewardship for Transplant Candidates and Recipients

7

Catherine Liu and Erica J. Stohs

Contents

Introduction 132
Implementing Antimicrobial Stewardship in Transplant Patients 133
Formulary Management 135
Pre-prescription Authorization and Post-prescriptive Review 136
Antimicrobial Agent Selection 137
Antibiotic Allergy Assessment 137
Dose Optimization and Drug Interactions 139
Intravenous-to-Oral Conversion 139
Clinical Pathways and Guideline Development 140
Diagnostic Test Stewardship 140
Syndrome- and Drug-Specific Targets 141
Antibiotic Stewardship 141
Antiviral Stewardship 144
Antifungal Stewardship 146
Antimicrobial Stewardship Program Metrics 147
Conclusion 147
Key Points 148
References 149

C. Liu (✉)
Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Division of Allergy and Infectious Diseases, University of Washington, Seattle, WA, USA
e-mail: catherine.liu@fredhutch.org

E. J. Stohs
Division of Infectious Diseases, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA
e-mail: erica.stohs@unmc.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_6

Introduction

Antimicrobial stewardship has been recognized as a key strategy to combat the growing threat of antibiotic resistance. Antimicrobial stewardship is defined as coordinated interventions designed to improve and measure the appropriate use of antimicrobial agents by promoting the optimal antimicrobial drug regimen including dosing, duration of therapy, and route of administration [1]. The primary goal of antimicrobial stewardship is to optimize patient outcomes while minimizing the development of resistance, toxicity, and other untoward consequences such as *Clostridioides difficile* [2]. It has emerged as a national priority in the USA with regulatory mandates requiring all acute care hospitals and ambulatory care centers to establish antimicrobial stewardship programs (ASP). The Centers for Disease Control and Prevention has developed core elements of a successful antibiotic stewardship program (Table 1) which complement guidance published by the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America on implementation of an antimicrobial stewardship program [3].

Antimicrobial stewardship is highly relevant for transplant recipients where infectious complications remain a leading cause of morbidity and mortality following solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT). Transplant candidates and recipients represent a population at high risk for antimicrobial resistance due to frequent and prolonged exposure to antibiotics for prevention and treatment of infections. They are at increased risk for drug toxicity due to polypharmacy and complex drug interactions often in the setting of underlying hepatic or renal dysfunction. Additionally, they require use of high-cost antimicrobials and contribute substantially to the overall antimicrobial budget [4]. Transplant recipients have a higher incidence of *C. difficile* infection when compared to the general population; *C. difficile* infection is associated with significant morbidity including increased risk of graft rejection in certain populations (e.g., kidney

Table 1 Summary of CDC core elements of hospital antibiotic stewardship program

Leadership Commitment: Dedicating necessary human, financial, and information technology resources
Accountability: Appointing a single leader responsible for program outcomes. Experience with successful programs show that a physician leader is effective
Drug Expertise: Appointing a single pharmacist leader responsible for working to improve antibiotic use
Action: Implementing at least one recommended action, such as systemic evaluation of ongoing treatment need after a set period of initial treatment (i.e., "antibiotic time out" after 48 h)
Tracking: Monitoring antibiotic prescribing and resistance patterns
Reporting: Regular reporting of information on antibiotic use and resistance to doctors, nurses, and relevant staff
Education: Educating clinicians about resistance and optimal prescribing

Source: Centers for Disease Control and Prevention, https://www.cdc.gov/antibiotic-use/healthcare/implementation/core-elements.html. Accessed March 29, 2019

transplant recipients) [5]. Furthermore, emerging data regarding the impact of antibiotic use on the microbiome, graft versus host disease (GVHD) [6], and relapse risk [7] suggest that antimicrobial stewardship efforts may have broader impact on important clinical outcomes.

It is important to consider antimicrobial stewardship among both transplant candidates and recipients as antimicrobial use prior to transplant may have a significant impact on selection of resistance and thus capacity for downstream use of certain antibiotics. Furthermore, antibiotic use prior to HSCT may impact intestinal microbiota composition at the time of transplant. Weber and colleagues found that compared to posttransplant, early exposure to antibiotics (day −7 to 0) was associated with greater disruption of commensal microbiota and was an independent risk factor for transplant related mortality (TRM) including GVHD-related TRM [8]. Additionally, decreases in gut microbiota diversity prior to HSCT has been associated with increased risk of bloodstream infection posttransplant [9].

Diagnostic and therapeutic uncertainties were identified as the major challenges to antimicrobial stewardship in transplant patients in a survey of adult and pediatric infectious disease physicians and pharmacists at US SOT and HSCT centers [10]. Timely accurate diagnosis and management of infections in transplant patients can be difficult due to delayed test turnaround time as well as underlying comorbidities which may limit ability to perform invasive diagnostic procedures such as biopsy or other surgical interventions required to achieve source control. Difficulty establishing a diagnosis coupled with lack of outcomes data to guide duration of therapy among transplant patients often leads to prolonged antibiotic use in this immunocompromised population. Additionally, concern over the potential for antibiotic-resistant organisms may lead to escalation of therapy thereby contributing further to the cycle of antibiotic resistance. Finally, prescriber opposition and differences in risk perception or motivation between the ASP team and primary team have been identified as important barriers to stewardship in transplant populations [10].

This chapter reviews practical considerations when implementing antimicrobial stewardship programs targeting transplant recipients and candidates and describes opportunities for optimizing antimicrobial use.

Implementing Antimicrobial Stewardship in Transplant Patients

A multidisciplinary approach is important for successful implementation of an ASP among transplant patients. These patients are often managed by a team approach including physicians, mid-level providers, nurses, and pharmacists; hence, it is critical to engage all of these individuals as key stakeholders when developing stewardship interventions. See Fig. 1.

An infectious disease physician and infectious disease-trained pharmacist generally serve as the backbone of the ASP team. The number of ASP team members needed varies based on hospital size. For example, a staffing calculator by the Veterans Health Administration determined that 1.0 full-time equivalent clinical

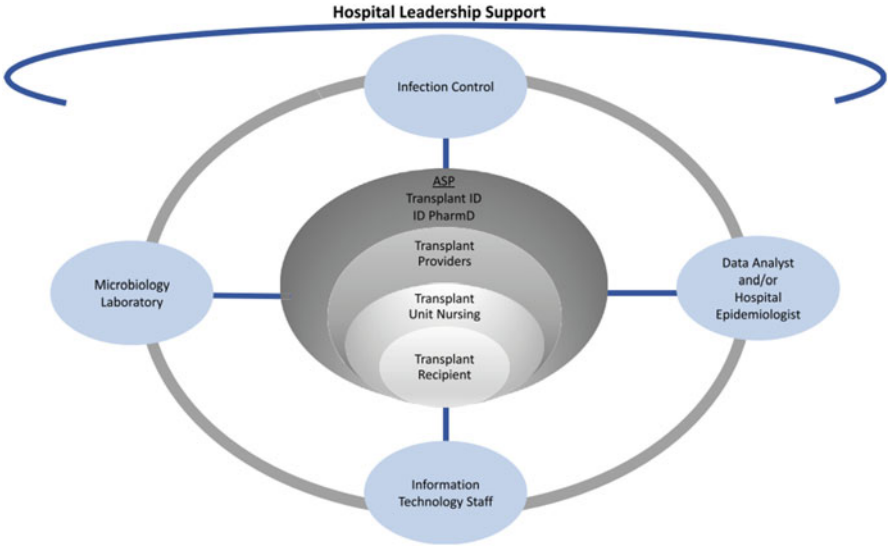


Fig. 1 Multidisciplinary approach to antimicrobial stewardship in transplantation [35]. (Permission for use of this table granted from Elsevier Inc.)

pharmacist per 100 occupied beds was needed to perform antimicrobial stewardship in a general hospital population [11]. Familiarity with the complexities of infections and novel therapeutics in immunocompromised patient population is key as these patients utilize a significant proportion of antimicrobials. Involving transplant and oncology infectious disease colleagues in ASP decisions encourages adherence to guidelines and recommendations [12]. Understanding providers' perceptions and behaviors regarding antimicrobial prescribing patterns particularly in the face of diagnostic uncertainty and complex clinical situations provides insight into ASP approaches to this population. Additionally, close partnership with the infection prevention team is important to develop strategies to prevent transmission of multidrug-resistant organisms.

Microbiology laboratory representatives are also instrumental for ASPs when integrating diagnostic modalities with appropriate antimicrobials and clinical management decisions. For instance, increasingly available rapid molecular diagnostic techniques for positive blood cultures and respiratory and gastrointestinal pathogens have had implications for the care of transplant patients but often require ASP guidance in conjunction with microbiology to optimize their benefit. Likewise, assistance from information technology personnel can be key to interface with providers via order sets, electronic decision support tools, and guidance documents.

Nursing staff may provide valuable contributions to ASP activities. A 2017 White Paper by Centers for Disease Control and Prevention and American Nursing Association outlined opportunities and emphasized the value of including and educating registered nurses in antimicrobial stewardship, particularly because they serve on the frontline of patient care [13]. Additionally, nurses are often already

engaged in infection prevention efforts which complement antimicrobial stewardship activities (e.g., *C. difficile*).

Administrative support is instrumental to maintaining a successful ASP. As accreditation standards for antimicrobial stewardship evolve, hospital administrators have a vested interest in establishing and maintaining antimicrobial stewardship efforts. ASP policies, reporting parameters, and funding require regular engagement and information exchange with members of hospital administration and various medical center committees.

There are a broad range of activities that ASP programs can initiate or expand when implementing antimicrobial strategies in SOT and HSCT recipients (Table 2).

Formulary Management

The ASP team plays a key role in making formulary decisions about antimicrobial agents through the hospital's pharmacy and therapeutics committee. Formulary choices should take into consideration not only therapeutic efficacy, adverse effects, and cost but also local infectious disease epidemiology and resistance patterns in

Table 2 Antimicrobial stewardship strategies and interventions in SOT and HSCT recipients

Antimicrobial stewardship intervention	Examples
Formulary management	Participation in Pharmacy &Therapeutics Committee Formulary review Management of drug shortages
Pre-prescription authorization	High-cost drugs used by transplant recipients New antimicrobial agents
Post-prescriptive review (prospective audit and feedback)	Targeted antibiotics (e.g., carbapenems, antipseudomonal agents, double-anaerobic coverage) Handshake stewardship
Antimicrobial agent selection	Transplant-specific antibiogram Individualized antibiotic plan (e.g., based on history of or colonization with MDRO) Precision medicine using advanced molecular diagnostics to characterize resistance genes
Antibiotic allergy assessment	Beta-lactam allergy testing Pre-transplant antibiotic allergy evaluation
Dose optimization	PK/PD-based dosing and monitoring (e.g., vancomycin, aminoglycosides, extended infusion β-lactams, voriconazole, posaconazole)
Intravenous-to-oral conversion	Antibiotics, antivirals (e.g., IV ganciclovir to oral valganciclovir) and antifungals (e.g., echinocandin to oral triazole)
Clinical pathways and guideline development	Febrile neutropenia, antifungal prophylaxis and treatment, CMV prophylaxis and treatment, posttransplant vaccination
Diagnostic test stewardship	*C. difficile* testing, surveillance blood and urine cultures

the context of the different populations served [12]. For transplant patients, it is important to review formularies with particular attention to new antibiotics targeting multidrug-resistant organisms (MDROs), as well as antifungals and antivirals that may be used for prophylaxis and treatment in the peri-transplant period. As part of the formulary review process, ASPs should consider what additional processes are needed to ensure the safe prescribing, distribution, and use of the drug. This may include pre-authorization or restriction, guideline development, medication use evaluation (MUE) plans, and educational efforts [14]. Additionally, it will be important for ASPs to coordinate with the microbiology laboratory regarding availability of antimicrobial drug susceptibility tests for new agents.

Recent challenges faced by ASPs that have direct impact on the management of infections among transplant patients include antimicrobial drug shortages [15] and the dramatic rise in costs of certain medications (e.g., aerosolized ribavirin and pyrimethamine) [16, 17]. The ASP can play a key role in managing these scenarios through development of drug use guidelines during drug shortages and whenever a prescriber orders selected high-cost drugs. Such guidelines may incorporate consideration of therapeutic alternatives and often require direct ASP communication with providers.

Pre-prescription Authorization and Post-prescriptive Review

One core strategy to reduce use and cost of certain antimicrobials is pre-prescription authorization, known simply as pre-authorization [2]. Typically an ASP pharmacist or infectious disease physician must provide approval for use of an antimicrobial prior to release [18]. Pre-prescription authorization may include high-cost drugs used in transplant patients, such as intravenous posaconazole, inhaled ribavirin, and newer antimicrobial agents targeting resistant pathogens. This strategy has been shown to reduce costs in transplant units [19]. A carbapenem restriction policy in one center resulted in a significant decrease in meropenem use for hematology-oncology and bone marrow transplant units [20]. Efforts to promote appropriate use of carbapenems may prevent emergence of carbapenem resistance.

Post-prescriptive review, also known as prospective audit and feedback (PAF), is another foundational antimicrobial stewardship intervention that engages the provider after an antibiotic is prescribed [3]. PAF involves review of clinical and laboratory results to guide further therapy and allows opportunities to address de-escalation and duration. One study investigating post-prescriptive reviews in hematopoietic cell transplant patients reported trends in reduced third- and fourth-generation cephalosporin prescribing and increased compliance with ASP recommendations [21].

In a survey of US adult and pediatric HSCT and SOT centers, pre-authorization and post-prescriptive review were among the six most common antimicrobial stewardship interventions [10]. Both strategies have been evaluated in centers that include oncology and/or transplant patients with a resultant decrease in antimicrobial utilization and costs without resulting in harm [18, 22].

Handshake stewardship is an emerging ASP strategy that builds upon PAF and refers to active engagement with frontline providers including review of all

antimicrobials and a rounding-based, in-person approach to feedback by a pharmacist-physician team [23]. This approach may be particularly useful for ASPs when establishing credibility and rapport with transplant teams. Participation in in-person rounds increases visibility and familiarity with the transplant services and provides a unique opportunity for bidirectional exchange of information and education [24]. However, this approach is labor- and time-intensive, and not all hospitals may have the resources to support this. Establishing regular meetings to increase face time with providers may be more feasible if resources are limited; in one study of pediatric hematology-oncology and HSCT patients, this strategy in combination with others was found to be effective in reducing antimicrobial use and cost without adverse outcomes [22].

Antimicrobial Agent Selection

Transplant patients present unique considerations when it comes to empiric antimicrobial selection, including previous antibiotic exposure, history of resistant organisms, organ dysfunction, and medication interactions [18]. A transplant-specific antibiogram can be useful in guiding empiric antimicrobial selection. One study observed significant differences in susceptibilities in blood and urine isolates of *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* from SOT recipients as compared to the hospital-wide antibiogram [25]. In another study, a disease-specific antibiogram for urinary tract infections in kidney transplant recipients found that *E. coli* urinary isolates were significantly more resistant to multiple antibiotic drug classes compared to the institutional antibiogram [26]. One strategy proposed by some HSCT centers is the development of individualized antibiotic plans for future inevitable episodes of febrile neutropenia, based on results of screening cultures to identify colonization with MDROs [18]. Pre-lung transplant patients represent another group in which an individualized antibiotic plan at the time of transplant may be developed based on prior bronchoalveolar lavage cultures and past history of *Aspergillus* or mycobacterial colonization, with additional modifications based on available donor cultures at time of transplant. A "precision medicine" approach to antibiotic selection using advanced molecular diagnostic techniques has also been increasingly recognized as an important strategy for the management of transplant patients with MDROs. This approach may be helpful in guiding use of novel antibiotic combinations [27].

A conceptual framework developed by the Agency for Healthcare Research and Quality (AHRQ) Safety Program for Improving Antibiotic Use that is readily adaptable to the transplant patients is the 4 moments of antibiotic decision-making [28] (Table 3).

Antibiotic Allergy Assessment

Antibiotic allergy assessment has been increasingly recognized as an important stewardship intervention that may help to guide antibiotic selection and use of preferred agents. Penicillin allergies are most commonly reported in up to 10–15% of the general

Table 3 Incorporating the 4 moments of antibiotic decision-making into daily practice [28]

Question[a]	Patient scenario	ASP strategy
1. Does this patient have an infection that requires antibiotics?	Renal transplant patient with asymptomatic bacteriuria	Clinical pathways and guideline development Post-prescriptive review
2. Have I ordered appropriate cultures before starting antibiotics? What empiric antibiotic should I initiate?	HSCT patient with febrile neutropenia	Clinical pathways and guideline development Dose optimization Transplant-specific antibiogram
3. Cultures have returned. Can I change from IV to oral therapy?	Liver transplant patient with fluconazole-sensitive candidemia	IV-to-PO conversion
4. What duration of antibiotic therapy is needed for this patient's diagnosis?	HSCT patient with surveillance blood culture demonstrating growth of coagulase negative *Staphylococcus*	Diagnostic test stewardship Post-prescriptive review

ASP antimicrobial stewardship program, *HSCT* hematopoietic stem cell transplant, *IV* intravenous, *PO* oral

[a]Questions posed by Tamma and colleagues [28]; patient scenarios and ASP strategies are original

population [3, 29]. When compared to nonallergic patients, those with a penicillin allergy label have increased exposure to alternative and less optimal antibiotic therapy, increased risk for antibiotic resistance, and higher healthcare costs [29].

There are limited data on the prevalence of antibiotic allergies among transplant patients. In a study of 4671 patients with hematologic malignancies of which approximately 20% underwent HSCT, 35% reported antibiotic allergy with 14% having a beta-lactam allergy [30]. In an Australian liver transplant cohort of 313 patients, 16% reported at least one antibiotic allergy [31]. Similar to the general population, antibiotic allergy labels in immunocompromised populations largely consisted of beta-lactams, particularly penicillin [30–32]. In patients with hematologic malignancies, antibiotic allergy labels have been associated with use of more antibiotic classes [30], increased antibiotic duration [30, 32], longer lengths of stay [30], higher readmission rates [30, 32], higher rates of *C. difficile* [30], and reduced concordance with prescribing guidelines [32]. Integrating an antibiotic allergy testing program at one large center successfully led to the prescription of narrower-spectrum beta-lactams, more guideline appropriate antibiotic use, and use of fewer restricted antibiotics. Forty-eight percent of these patients were immunocompromised with up to 8% developing febrile neutropenia during their clinical course [33].

Some centers may consider incorporating antibiotic allergy assessment as part of the routine pre-transplant evaluation to optimize posttransplant antibiotic use, though further data are needed to assess the impact of such programs.

Dose Optimization and Drug Interactions

Dose optimization using pharmacokinetic (PK) and pharmacodynamic (PD) principles is a key function of an antimicrobial stewardship program managing transplant patients [18]. Critical illness, impaired renal function, differences in PK/PD among selected patient populations (e.g., cystic fibrosis), and frequent drug-drug interactions underscore the importance of a stewardship team with experience in the management of complex transplant scenarios. PK monitoring and adjustment programs for aminoglycosides and vancomycin are encouraged and can decrease adverse effects and costs [3]. Extended infusion compared to standard dosing for broad-spectrum β-lactams has been associated with decreased costs though there are limited data in general and transplant populations to assess the impact on clinical outcomes [3]. In one single-center, nonblinded, randomized clinical trial of febrile neutropenia, extended infusion β-lactams was found to be associated with improved treatment outcomes when compared to bolus infusion with the greatest benefit observed among those patients with clinically documented infections and, in particular, pneumonia [34]. The importance of antiviral PK and PD principles has been recognized in the management of CMV infections with the goal of reducing toxicities, decreasing the emergence of resistance and improving treatment response [35]. Therapeutic drug monitoring of antifungal agents including voriconazole and posaconazole has been associated with a reduced risk of adverse drug events and improved outcomes [36].

Use of older antimicrobial agents, such as polymyxins, in the face of MDROs often requires expert infectious disease pharmacy consultation to optimize dosing. Additionally, ASP pharmacists play a critical role in reviewing complex medication lists for drug-drug interactions and assessing the need for dose modification or use of an alternative agent. One of the most common examples is the use of calcineurin inhibitors and triazoles. As additional therapies emerge for the management of transplant patients, it is important that the ASP team recognizes the potential for new drug-drug interactions and monitor accordingly.

Intravenous-to-Oral Conversion

Timely intravenous-to-oral (IV-to-PO) agent conversion is recommended as an ASP intervention to reduce costs, hospital length of stay, outpatient parenteral antimicrobials, and need for intravenous catheters [3]. Approximately half of surveyed transplant center ASPs reported IV-to-PO conversion as a stewardship strategy [10]. Although the presence of mucositis among HSCT recipients may

preclude oral therapy, IV-to-PO conversion may be considered among transplant recipients for whom oral absorption is not a concern. A recent study found no difference in 30-day mortality among patients (approximately 10% SOT and 6% HSCT) who received early oral step-down versus continued parenteral therapy for the treatment of *Enterobacteriaceae bacteremia* with adequate source control [37]; larger studies in transplant recipients are needed to validate the safety of this approach. Antifungals are an important target in transplant patients as echinocandins can be transitioned to oral fluconazole for susceptible *Candida* blood isolates and IV-to-PO triazoles utilized in management of invasive fungal infections [18, 35]. Oral valganciclovir has been shown to be noninferior to IV ganciclovir for treatment of CMV disease among SOT patients who are able to absorb oral medication and do not have severe gastrointestinal CMV disease [38].

Clinical Pathways and Guideline Development

Development of clinical pathways and guidelines is another important ASP strategy and was described as an intervention performed by over 70% of HSCT and SOT centers surveyed [10]. There are many opportunities for guideline development including antifungal prophylaxis and treatment of invasive fungal infections, management of CMV infections including antiviral prophylaxis, as well as posttransplant vaccination. Implementation of guidelines for management of febrile neutropenia has been associated with reduction in empiric vancomycin [39, 40] and carbapenem use [41] (see section on "Febrile Neutropenia"). A clinical pathway for febrile neutropenia among pediatric oncology patients presenting to the emergency room was shown to improve time to antibiotics and reduce septic shock, ICU admissions, and mortality [42].

Engagement of appropriate stakeholders including both the transplant (e.g., HSCT or SOT) and transplant infectious disease (ID) consult teams with opportunities for feedback and education is key prior to implementation of clinical pathways. Guidelines can be disseminated in a variety of ways including presentations, standardized order sets, pocket cards, ASP website, and computerized decision support. In addition to guideline implementation and dissemination, it is important to assess adherence to treatment guidelines and to monitor intended outcomes [3].

Diagnostic Test Stewardship

Diagnostic test stewardship aims to optimize and reduce inappropriate use of diagnostic tests and thus unnecessary antibiotic use. It involves efforts to modify the process of ordering, performing, and reporting of diagnostic tests [43]. As part of the American Board of Internal Medicine (ABIM) Choosing Wisely initiative, the Society for Healthcare Epidemiology of America (SHEA) identified orders for urine cultures, blood cultures, and stool tests for *C. difficile* in the absence of signs and symptoms of infection as high-priority targets for provider education to reduce

overdiagnosis and overtreatment [44]. This is highly relevant to transplant patients where diagnostic tests are frequently performed due to concern for possible infectious complications among a vulnerable population.

While several studies have demonstrated the effectiveness of computerized clinical decision support (CCDS) and other tools in reducing *C. difficile* tests and rates in general hospitalized patient populations [45, 46], there are limited published data in transplant populations. A bundled intervention including CCDS-based interventions and educational efforts led to a significant reduction in *C. difficile* tests with a trend toward fewer *C. difficile* infection (CDI) events among SOT recipients [45]. Implementation of *C. difficile* diagnostic stewardship strategies among HSCT candidates and recipients poses some unique challenges. HSCT patients represent a high-risk population for CDI due to frequent healthcare and antibiotic exposure along with impaired host immunity [47], but high prevalence of diarrhea due to chemotherapy and transplant-related complications such as graft versus host disease (GVHD) may lead to high rates of testing and thus overdiagnosis of CDI in a population with a high baseline prevalence of colonization [48]. Further research is needed to refine criteria for *C. difficile* testing among HSCT patients.

Surveillance blood cultures are performed at some transplant centers for early detection of bloodstream infections among allogeneic HSCT recipients receiving high-dose glucocorticoids, with neutropenia, or central venous catheters [49]. Among centers that perform surveillance blood cultures, there is variability in the frequency (e.g., daily, weekly) and conditions in which surveillance cultures are performed; however, in general, studies have suggested that surveillance blood cultures have limited value and infrequently detect clinically significant pathogens while leading to excess antibiotic use [49].

Another area of opportunity for improved diagnostic stewardship is limiting screening for and treatment of asymptomatic bacteriuria and is discussed in detail in Section Vb.

While overuse of diagnostic tests when not indicated may lead to unnecessary antibiotic use, appropriate use of diagnostic studies may be helpful in limiting antibiotic use. For example, respiratory viral PCR testing was associated with decreased antibiotic use for upper respiratory infections at an ambulatory cancer center [50].

Syndrome- and Drug-Specific Targets

ASP programs can also consider a variety of infectious disease syndrome and/or drug targets when implementing stewardship interventions.

Antibiotic Stewardship

Febrile Neutropenia

The 2016 Infectious Diseases Society of America (IDSA) Guidelines on Implementing an Antibiotic Stewardship Program recommend development of

institution-specific guidelines for fever and neutropenia (F&N) in hematology-oncology patients which can reduce unnecessary antibiotic use without adverse outcomes [3]. Several studies suggest that implementation of pathways for F&N management is associated with improved clinical outcomes [42, 51].

There are several aspects to the management of F&N that represent potential targets for antimicrobial stewardship: (1) addition of vancomycin to initial antibiotic therapy or for persistent febrile neutropenia and (2) duration of empiric antibiotic therapy and de-escalation. Routine addition of gram-positive coverage to an anti-pseudomonal beta-lactam for initial empiric therapy of F&N has not been associated with improved outcomes [52, 53]. Guidelines from IDSA, NCCN, and ECIL indicate that vancomycin should only be used in selected scenarios when managing a patient with F&N (Table 4). Despite these recommendations, vancomycin is often given along with an antipseudomonal beta-lactam as part of empiric therapy for febrile neutropenia. In a retrospective study of 128 patients with F&N most of whom had an underlying hematologic malignancy, 62% had an inappropriate indication for vancomycin use [54]. Another scenario in which vancomycin is commonly used is as an add-on to empiric antipseudomonal beta-lactam monotherapy following several days of persistent fever. In a randomized clinical trial, the addition of vancomycin did not have any impact on time to defervescence among neutropenic patients with persistent fever 48–60 h after the initiation of empiric piperacillin-tazobactam monotherapy [55]. Implementation of a clinical pathway including specific guidance on vancomycin use was associated with a significant reduction in inappropriate use from 35.9% to 11.4% pre- and post-pathway implementation, respectively ($p = 0.001$) [39].

Table 4 Indications for empiric vancomycin use among patients with febrile neutropenia

Indications	2011 IDSA Guidelines	2011 ECIL	2019 NCCN Guidelines
Clinically apparent, serious IV catheter-related infection (e.g., chills or rigors with infusion through catheter and cellulitis around the catheter entry/exit site)	X	X	X
Blood cultures positive for gram-positive bacteria before final identification and susceptibility testing	X		X
Known colonization with MRSA or penicillin-/cephalosporin-resistant pneumococci	X	X	X
Clinical instability (e.g., hypotension, shock) pending culture results	X	X	X
Soft tissue infection	X	X	X
Pneumonia documented radiographically	X	X	
Severe mucositis, if fluoroquinolone prophylaxis and *ceftazidime* used as empiric therapy	X		

IDSA Infectious Diseases Society of America, *ECIL* European Conference on Infections in Leukaemia, *NCCN* National Comprehensive Cancer Network, *IV* intravenous, *MRSA* methicillin-resistant *Staphylococcus aureus*

There is some variation among national and international guidelines regarding the duration of empiric antibiotic therapy in F&N. Clinical practice guidelines by the IDSA [56] and National Comprehensive Cancer Network (NCCN) [57] recommend continuing empiric broad-spectrum antibiotic therapy until absolute neutrophil count (ANC) recovery (ANC $\geq$500 cells/ mcL). An alternative approach is suggested in patients in whom an appropriate treatment course has been completed with resolution of all signs and symptoms of infection; in these cases, oral fluoroquinolone prophylaxis may be considered until ANC recovery. The ECIL guidelines indicate that empiric antibiotics may be discontinued after $\geq$72 h of intravenous antibiotics in patients who have been hemodynamically stable since presentation and afebrile for $\geq$48 h or more, regardless of ANC or expected duration of neutropenia [58]. Recent studies have suggested that earlier discontinuation or de-escalation of antibiotic therapy may be a reasonable approach [59]. A randomized clinical trial of high-risk patients with hematologic malignancies and febrile neutropenia found that discontinuation of empiric antibiotic therapy after 72 h of apyrexia and clinical recovery resulted in less antibiotic exposure compared to an ANC recovery directed approach and was safe [60].

Asymptomatic Bacteriuria in Organ Transplant Recipients

The 2019 IDSA Clinical Practice Guidelines for Asymptomatic Bacteriuria and 2019 American Society of Transplantation Infectious Disease Community of Practice Guidelines for Urinary Tract Infections in Solid Organ Transplant Recipients recommend against treatment of asymptomatic bacteriuria (ASB) after 1 or 2 months post-kidney transplant, respectively [61, 62]. This includes ASB with multidrug-resistant organisms.

Two prospective randomized comparative trials suggest no benefit of treatment versus no treatment of ASB in renal transplant recipients. Origüen et al. analyzed 112 patients with bacteriuria identified $\geq$ 2 months posttransplant who were randomized to treatment versus no treatment of ASB episodes identified by screening urine cultures performed at clinic visits over a 2-year follow-up period [63]. The primary outcome, acute pyelonephritis, occurred with the same frequency in each group. Secondary outcomes, long-term graft function, all-cause mortality, cumulative incidence of UTI, acute graft rejection, *C. diff* infection, and colonization or infection with MDROs were also the same. Among patients who received treatment of ASB, microbiologic cure was achieved in only 51%, while 33% of those untreated cleared bacteriuria spontaneously [63]. Another prospective quasi-randomized trial assigned 88 recipients of kidney transplants with ASB identified by monthly screening urine cultures over a 1-year period to antibiotic treatment versus observation alone. There were no difference in outcomes: bacteriuric episodes, symptomatic UTIs, and graft function [64].

The threat of inducing drug-resistant organisms by treating ASB outweighs the theoretical benefit of treatment [61, 62]. American Society of Transplantation Guidelines for UTI in Solid Organ Transplant highlight retrospective studies suggesting increased development of drug resistance as a consequence of treating ASB or UTI. Studies of trimethoprim-sulfamethoxazole used for both *Pneumocystis*

carinii and UTI suppression in renal transplant are also linked to the emergence of UTIs from ESBL and/or carbapenemase-producing *Enterobacteriaceae* [62]. In one study, treatment of asymptomatic *E. coli* or *E. faecalis* bacteriuria led to selection of resistant organisms in 78% of treated cases with no difference in progression to UTI between treated and untreated groups [65].

There is insufficient evidence to inform recommendations for ASB screening and treatment within 1–2 months following transplant [61, 62]. Some have justified ASB screening and treatment during the early posttransplant period due to concerns about progression to pyelonephritis, sepsis, and early graft dysfunction and rejection [61, 62]. Balancing these potential outcomes with the risk of inducing drug-resistant organisms requires careful consideration.

Distinguishing asymptomatic bacteriuria (ASB) from urinary tract infection (UTI) is a recognized challenge in renal transplant patients. Indwelling ureteral stents, surgical manipulation of the urinary tract, and induction immunosuppression are UTI risk factors unique to renal transplant recipients [61, 62]. See Table 5 for definitions of ASB, simple cystitis, complicated UTI, and pyelonephritis in renal transplant recipients.

In recipients of nonrenal organ transplants, there is no evidence to support screening for or treating ASB [61, 62]. Much of the data comes from the Spanish Network for the Study of Infections in Transplantation (RESITRA) registry of 2,405 organ transplant recipients citing low incidence of cystitis and UTI-derived bacteremia in nonrenal organ transplant recipients. In the registry, incidence of cystitis per 100 recipient years was 3.09 for liver, 2.41 for heart, and 1.36 for lung transplant recipients compared with 13.84 for renal transplant; UTI was the source of bacteremia in 3% of liver, 3% of heart, and 0% of lung transplant recipients compared with 39% of renal transplant patients [66].

Antiviral Stewardship

Aerosolized Ribavirin for Management of RSV

Aerosolized ribavirin has long been considered the standard of care for management of RSV upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI) [67]. However, aerosolized ribavirin is expensive, cumbersome to administer, and associated with adverse effects such as bronchospasm and teratogenicity. In recent years, the cost of aerosolized ribavirin has increased to nearly $30,000 per day [16]. For all of these reasons, aerosolized ribavirin represents an important opportunity for antiviral stewardship and development of institutional guidelines regarding use. Recent studies suggest that oral ribavirin may be a safe and effective alternative to aerosolized ribavirin for treatment of RSV infection. In a retrospective study of 124 HSCT recipients with RSV infection, there was no difference in rates of progression to LRTI and mortality among patients receiving oral compared to aerosolized ribavirin [68]. In one study that evaluated 21 lung transplant patients who received either aerosolized or oral ribavirin for RSV infection, no significant differences in 6-month outcomes were noted between the two groups [69].

Table 5 Classification of asymptomatic bacteriuria in urinary tract infection (UTI) in renal transplant recipients

Classification	Description	Laboratory definitions
Asymptomatic bacteriuria	No urinary of systemic symptoms of infection	$>10^5$ CFU/mL uropathogen[a,b]
Acute simple cystitis	Dysuria, urinary urgency/frequency, or suprapubic pain but no systemic symptoms and no ureteral stent/nephrostomy tube/chronic urinary catheter	>10 WBC/mm^3 [c] $>10^3$ CFU/mL uropathogen[b]
Acute pyelonephritis/complicated UTI	Fever, chills, malaise, hemodynamic instability, or leukocytosis (without other apparent etiology); flank/allograft pain; or bacteremia with same organisms as in urine Dysuria, urgency, frequency, suprapubic pain may or may not be present	>10 WBC/mm^3 [c] $>10^4$ CFU/mL uropathogen[b]
Recurrent UTI	>/= 3 UTIs in prior 12-month period	As above

Adapted from Table 1 from AST ID COP UTI Guidelines [62]. Permission for use of this table granted from John Wiley & Sons, Inc.

CFU colony forming units, *mL* milliliter, *WBC* white blood cell, *mm* millimeter

[a]While routine treatment of asymptomatic bacteriuria is not recommended, if considering treatment (e.g., in the immediate posttransplant period), a repeat urine culture is recommended to assess persistence of the same uropathogen. Spontaneous resolution is common

[b]*Staphylococcus epidermidis* (except if ureteral stent present), lactobacillus, and *Gardnerella* sp. are unlikely to be uropathogens. Regarding CFU/mL, while most patients will UTI will have $>10^5$ CFU/mL of a uropathogen in a midstream urine sample, some patients with pyelonephritis may have only 10^4–10^5 CFU/mL of a uropathogen, and some patients with cystitis may have even fewer CFU/mL (most data on cystitis with low CFU/mL is only for *E. coli*). Not all labs report $<10^4$ CFU/mL

[c]While not an absolute criteria (depending on the performance characteristics of the urinalysis or presence of neutropenia), <10 WBC/mm^3 should prompt consideration of a diagnosis other than UTI

Cytomegalovirus (CMV)

With the threat of CMV resistance and the introduction of new antivirals, CMV management is a target of antimicrobial stewardship interventions. Locally developed guidelines for CMV prophylaxis and treatment, respectively, are utilized by 82% and 70% of 71 US transplant centers surveyed regarding stewardship strategies [10]. Data on the effectiveness of such strategies are limited, but multidisciplinary collaboration is encouraged to optimize management [70].

A pharmacist-driven antimicrobial stewardship intervention in the outpatient setting was reported at one transplant center performing SOT [70]. In this quasi-experiment, CMV DNA surveillance results were conducted by a pharmacist and recommendations for drug therapy provided at the time of result notification based on institution, organ-specific protocols and compared to the previous standard of care. Time-to-CMV eradication was decreased (25.5 vs. 29 days; $p = 0.003$); there were also non-statistically significant decreases in CMV-related admissions, CMV disease, and episodes of CMV resistance at 1 year [70].

ASP programs may also consider evaluating the impact of new antivirals (e.g., letermovir use for CMV prophylaxis) on subsequent use of existing agents such as ganciclovir and foscarnet.

Antifungal Stewardship

Antifungal stewardship is important among SOT and HSCT recipients, who disproportionately experience a large burden of fungal infections and excess use of antifungal drugs compared to other hospitalized patients [4, 71, 72]. Invasive *Candida* and *Aspergillus* infections represent the most common invasive fungal diseases (IFD) in SOT and HSCT recipients [73]. Given their morbidity and mortality in these populations, antifungal prophylaxis is routine in high-risk HSCT [72] and SOT recipients, particularly in the first 100 days posttransplant [74]. The high cost of IFD prevention [4], management [75], and emerging antifungal resistance [72, 75] are rationales for establishing antifungal stewardship strategies using national antimicrobial stewardship guidelines [3]. Antifungal overuse, potential toxicity with prolonged use, attention to drug-drug interactions, and need for drug level monitoring are additional reasons to establish antifungal stewardship programs focused on transplant patients.

Opportunities exist to optimize antifungal use. Prolonged antifungal therapy and prophylaxis account for 42–66% of antifungal overuse [75]. Delays in IFD recognition and poor sensitivity of fungal diagnostics contribute to this [72, 75]. Given the poor outcomes associated with delays in appropriate therapy for IFD, many clinicians choose to start empiric antifungal treatment [71, 72]. Empiric antifungal therapy accounts for up to two thirds of inpatient antifungal prescribing; de-escalation of empiric therapy remains one of the most challenging aspects of antifungal stewardship [71].

Post-prescriptive review and feedback is a recommended antifungal stewardship intervention [71] which has been reported to reduce antifungal consumption and cost [75]. Intravenous-to-oral conversion for azole-sensitive candidemia is recommended by national antimicrobial stewardship guidelines [3]. Often, post-prescriptive review and feedback is combined with other interventions, such as guideline implementation, prescriber education, therapeutic drug monitoring, and incorporation of fungal diagnostics to optimize antifungal use [75]. High-risk hematologic malignancy, HSCT, and SOT (particularly lung transplant) patients are recommended target populations [71].

National ASP guidelines suggest that non-culture-based fungal markers may help to optimize antifungal use [3]. Incorporating fungal markers such as galactomannan, (1,3)-β-D-glucan, or fungal PCR (single or multi-pathogen) into IFD diagnostic and therapeutic algorithms for high-risk patients may assist in decreasing duration of empiric antifungal therapy in high-risk hematologic malignancy and allogeneic HSCT patients [3, 71, 72]. In SOT recipients, there is limited utility for serum fungal biomarkers due to reduced sensitivity; bronchoalveolar aspergillus galactomannan is

the preferred biomarker for diagnosis of invasive pulmonary aspergillosis in SOT patients [76].

Transplant recipients can benefit from attention to therapeutic drug monitoring and drug-drug interactions (DDIs) to avoid toxicities. Patient comorbidities and the complex pharmacokinetics of concurrent immunosuppressive drug regimens put transplant recipients at increased risk. DDIs with antifungal therapy are common, especially in SOT and HSCT patients treated with triazoles, with the potential for associated adverse drug events [75]. Therapeutic drug monitoring of voriconazole can improve efficacy, safety, and compliance in patients with IFD [71, 75]. IDSA Guidelines for management of aspergillosis recommend therapeutic drug monitoring of voriconazole, itraconazole, and posaconazole; currently there is insufficient data to support isavuconazole level monitoring [77]. Cost, accessibility, and turnaround time of fungal diagnostics and antifungal therapeutic drug monitoring are implementation factors to consider at each institution [75].

Antimicrobial Stewardship Program Metrics

Previously described antimicrobial use, process, and outcomes measures [3] should be applied to assess the impact of an antimicrobial stewardship program on transplant populations (Table 6). Ideally, metrics should be captured in a way that enables tracking and feedback by a service line and/or unit. Additionally, the impact of interventions to improve antibiotic use should be assessed. Limitations may include identifying appropriate benchmarks for transplant programs for metrics such as days of therapy (DOT). Assessment of process measures such as guideline adherence and intervention acceptance rates requires robust data capture and analytic tools but may be possible with enhanced electronic medical record (EMR) programs. It is important to partner with transplant services to identify clinically meaningful quality and outcome metrics although feasibility of data capture and presence of confounders when interpreting outcomes such as readmissions and mortality may pose challenges [76].

Conclusion

Transplant candidates and recipients benefit greatly from antimicrobial stewardship because they are at high risk for complications from antimicrobial-resistant organisms and drug toxicities. Implementation of antimicrobial stewardship strategies requires a multidisciplinary approach and should target infectious syndromes and specific antimicrobials. Metrics need to be carefully chosen based on a stewardship intervention goal, such as antimicrobial use, guideline, or order utilization, or a selected patient outcome.

Table 6 Antimicrobial stewardship metrics for transplant programs [3]

Antimicrobial metrics	Considerations
Process metrics	
Antibiotic utilization	
Days of therapy (DOT)	Preferred method for measuring antibiotic utilization, used by the CDC National Healthcare Safety Network (NHSN) for antimicrobial use reporting
Defined daily dose	Alternative method for sites that cannot obtain patient-level antibiotic use data
Adherence to facility-specific antibiotic treatment guidelines	Requires robust system for data capture
ASP intervention acceptance rate	Requires robust system for data capture
Outcome metrics	
Clostridioides difficile infection (CDI)	Required reporting of hospital-onset CDI (HO-CDI) to NHSN; partner with infection prevention team to track transplant unit HO-CDI rates. Limitations: HO-CDI rates influenced by multiple factors including infection control and diagnostic testing practices
Antibiotic resistance	Resistance trends can be tracked through transplant-specific antibiograms
Antimicrobial costs	Use prescription and administration rather than purchasing data and report as cost per patient day
Adverse drug events/toxicities	Limited by feasibility of event capture
Readmission related to infections	Limited by feasibility of event capture

Key Points

- Antimicrobial stewardship is an essential component of transplant programs to improve patient outcomes and reduce the threat of antimicrobial resistance and the risks of drug toxicity.
- A multidisciplinary approach including physicians, mid-level providers, nurses, and pharmacists that leverages the existing team-based structure of transplant programs is important for successful implementation of antimicrobial stewardship strategies.
- Antimicrobial stewardship strategies and interventions applicable to SOT and HSCT recipients include formulary management, pre-prescription authorization, post-prescriptive review, antimicrobial agent selection, antibiotic allergy assessment, dose optimization, IV-to-oral conversion, clinical pathways and guideline development, and diagnostic test stewardship.
- A variety of opportunities exist in antibiotic, antiviral, and antifungal stewardship targeting specific drugs commonly used among transplant patients and infectious disease syndromes frequently encountered by transplant patients.
- Antimicrobial stewardship programs should partner with key stakeholders in transplant services to capture and report relevant process and outcome metrics and to identify opportunities for improvement.

References

1. Fishman N. Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). Infect Control Hosp Epidemiol. 2012;33(4):322–7.
2. Dellit TH, Owens RC, Mcgowan JE, Gerding DN, Weinstein RA, Burke JP, et al. Guidelines for developing an institutional program to enhance antimicrobial stewardship. Clin Infect Dis. 2007;44(2):159–77.
3. Barlam TF, Fishman NO, Hamilton CW, Jenkins TC, Lipsett PA, Malani PN, et al. Implementing an antibiotic stewardship program: guidelines by the infectious diseases society of America and the society for healthcare epidemiology of America. Clin Infect Dis. 2016;62(10):e51–77.
4. Dela-Pena J, Kerstenetzky L, Schulz L, Kendall R, Lepak A, Fox B. Top 1% of inpatients administered antimicrobial agents comprising 50% of expenditures: a descriptive study and opportunities for stewardship intervention. Infect Control Hosp Epidemiol. 2017;38(3):259–65.
5. Revolinski SL, Munoz-Price LS. *Clostridium difficile* in immunocompromised hosts: a review of epidemiology, risk factors, treatment, and prevention. Clin Infect Dis. 2019;68(12):2144–53.
6. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med. 2016;8(339):339ra71.
7. Bergeron A, Chevret S, Granata A, Chevallier P, Vincent L, Huynh A, et al. Effect of azithromycin on airflow decline–free survival after allogeneic hematopoietic stem cell transplant. J Am Med Assoc. 2017;318(6):557–66.
8. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota disruption induced by early use of broad-spectrum antibiotics is an independent risk factor of outcome after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2017;23(5):845–52.
9. Montassier E, Al-Ghalith GA, Ward T, Corvec S, Gastinne T, Potel G, et al. Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. Genome Med. 2016;8(1):49.
10. Seo SK, Lo K, Abbo LM. Current state of antimicrobial stewardship at solid organ and hematopoietic cell transplant centers in the United States. Infect Control Hosp Epidemiol. 2016;37(10):1195–200.
11. Echevarria K, Groppi J, Kelly AA, Morreale AP, Neuhauser MM, Roselle GA. Development and application of an objective staffing calculator for antimicrobial stewardship programs in the Veterans Health Administration. Am J Heal Pharm. 2017;74(21):1785–90.
12. Abbo LM, Ariza-Heredia EJ. Antimicrobial stewardship in immunocompromised hosts. Infect Dis Clin N Am. 2014;28(2):263–79.
13. American Nurses Association, Centers for Disease Control and Prevention. Redefining the antibiotic stewardship team: recommendations from the American Nurses Association/Centers for Disease Control and Prevention Workgroup on the role of registered nurses in hospital antibiotic stewardship practices. 2017.
14. Tyler LS, Cole SW, May JR, Miliares M, Valentino MA, Vermeulen LC, et al. ASHP guidelines on the pharmacy and therapeutics committee and the formulary system. Am J Health Syst Pharm. 2008;65:1272–83.
15. Quadri F, Mazer-Amirshahi M, Fox ER, Hawley KL, Pines JM, Zocchi MS, et al. Antibacterial drug shortages from 2001 to 2013: implications for clinical practice. Clin Infect Dis. 2015;60(12):1737–42.
16. Chemaly RF, Aitken SL, Wolfe CR, Jain R, Boeckh MJ. Aerosolized ribavirin: the most expensive drug for pneumonia. Transpl Infect Dis. 2016;18(4):634–6.
17. McCarthy M. Drug's 5000% price rise puts spotlight on soaring US drug costs. BMJ. 2015;351:h5114.
18. Robilotti E, Holubar M, Seo SK, Deresinski S. Feasibility and applicability of antimicrobial stewardship in immunocompromised patients. Curr Opin Infect Dis. 2017;30(4):346–53.

19. Standiford HC, Chan S, Tripoli M, Weekes E, Forrest GN. Antimicrobial stewardship at a large tertiary care academic medical center: cost analysis before, during, and after a 7-year program. Infect Control Hosp Epidemiol. 2012;33(4):338–45.
20. Kirk A, Pierce J, Doll M, Lee K, Pakyz A, Kim J, et al. Effect of carbapenem restriction on prescribing trends for immunocompromised wards at an academic medical center. Am J Infect Control. 2019;47(8):1035–7.
21. Yeo CL, Chan DSG, Earnest A, Wu TS, Yeoh SF, Lim R, et al. Prospective audit and feedback on antibiotic prescription in an adult hematology-oncology unit in Singapore. Eur J Clin Microbiol Infect Dis. 2012;31(4):583–90.
22. Horikoshi Y, Kaneko T, Morikawa Y, Isogai M, Suwa J, Higuchi H, et al. The north wind and the sun: pediatric antimicrobial stewardship program combining restrictive and persuasive approaches in hematology-oncology ward and hematopoietic stem cell transplant unit. Pediatr Infect Dis J. 2018;37(2):164–8.
23. Hurst AL, Child J, Pearce K, Palmer C, Todd JK, Parker SK. Handshake stewardship: a highly effective rounding-based antimicrobial optimization service. Pediatr Infect Dis J. 2016;35(10):1104–10.
24. Messacar K, Campbell K, Pearce K, Pyle L, Hurst AL, Child J, et al. A handshake from antimicrobial stewardship opens doors for infectious disease consultations. Clin Infect Dis. 2017;64(10):1449–52.
25. Rosa R, Simkins J, Camargo JF, Martinez O, Abbo LM. Solid organ transplant antibiograms: an opportunity for antimicrobial stewardship. Diagn Microbiol Infect Dis. 2016;86(4):460–3.
26. Korayem GB, Zangeneh TT, Matthias KR. Recurrence of urinary tract infections and development of urinary-specific antibiogram for kidney transplant recipients. J Glob Antimicrob Resist. 2018;12:119–23.
27. Rosa R, Rudin SD, Rojas LJ, Perez-Cardona A, Aragon L, Nicolau DP, et al. Application of "precision medicine" through the molecular characterization of extensively drug-resistant *Klebsiella pneumoniae* in a multivisceral transplant patient. Clin Infect Dis. 2017;65(4):701–2.
28. Tamma PD, Miller MA, Cosgrove SE. Rethinking how antibiotics are prescribed. JAMA. 2019;321(2):139–40.
29. Shenoy ES, Macy E, Rowe T, Blumenthal KG. Evaluation and management of penicillin allergy: a review. JAMA. 2019.
30. Huang KHG, Cluzet V, Hamilton K, Fadugba O. The impact of reported beta-lactam allergy in hospitalized patients with hematologic malignancies requiring antibiotics. Clin Infect Dis. 2018;67(1):27–33.
31. Khumra S, Chan J, Urbancic K, Worland T, Angus P, Jones R, et al. Antibiotic allergy labels in a liver transplant recipient study. Antimicrob Agents Chemother. 2017;61(5):10–2.
32. Trubiano JA, Leung VK, Chu MY, Worth LJ, Slavin MA, Thursky KA. The impact of antimicrobial allergy labels on antimicrobial usage in cancer patients. Antimicrob Resist Infect Control. 2015;4(1):1–4.
33. Trubiano JA, Thursky KA, Stewardson AJ, Urbancic K, Worth LJ, Jackson C, et al. Impact of an integrated antibiotic allergy testing program on antimicrobial stewardship: a multicenter evaluation. Clin Infect Dis. 2017;65(1):166–74.
34. Ram R, Halavy Y, Amit O, Paran Y, Katchman E, Yachini B, et al. Extended vs bolus infusion of broad-spectrum β-lactams for febrile neutropenia: an unblinded, randomized trial. Clin Infect Dis. 2018;67(8):1153–60.
35. Hand J. Strategies for antimicrobial stewardship in solid organ transplant recipients. Infect Dis Clin N Am. 2018;32(3):535–50.
36. Ashbee HR, Barnes RA, Johnson EM, Richardson MD, Gorton R, Hope WW. Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the british society for medical mycology. J Antimicrob Chemother. 2014;69(5):1162–76.
37. Tamma PD, Conley AT, Cosgrove SE, Harris AD, Lautenbach E, Amoah J, et al. Association of 30-day mortality with oral step-down vs continued intravenous therapy in patients hospitalized with Enterobacteriaceae Bacteremia. JAMA Intern Med. 2019;179(3):316–23.

38. Åsberg A, Humar A, Jardine AG, Rollag H, Pescovitz MD, Mouas H, et al. Long-term outcomes of CMV disease treatment with valganciclovir versus IV ganciclovir in solid organ transplant recipients. Am J Transplant. 2009;9(5):1205–13.
39. Vicente M, Al-Nahedh M, Parsad S, Knoebel RW, Pisano J, Pettit NN. Impact of a clinical pathway on appropriate empiric vancomycin use in cancer patients with febrile neutropenia. J Oncol Pharm Pract. 2017;23(8):575–81.
40. Nucci M, Landau M, Silveira F, Spector N, Pulcheri W. Application of the IDSA guidelines for the use of antimicrobial agents in neutropenic patients: impact on reducing the use of glycopeptides. Infect Control Hosp Epidemiol. 2001;22(10):651–3.
41. la Martire G, Robinson C, Oubaya N, Lepeule R, Beckerich F, Leclerc M, et al. De-escalation and discontinuation strategies in high-risk neutropenic patients: an interrupted time series analyses of antimicrobial consumption and impact on outcome. Eur J Clin Microbiol Infect Dis. 2018;37(10):1931–40.
42. Pakakasama S, Surayuthpreecha K, Pandee U, Anurathapan U, Maleewan V, Udomsubpayakul U, et al. Clinical practice guidelines for children with cancer presenting with fever to the emergency room. Pediatr Int. 2011;53(6):902–5.
43. Morgan DJ, Malani P, Diekema DJ. Diagnostic stewardship – leveraging the laboratory to improve antimicrobial use. J Am Med Assoc. 2017;318(7):607–8.
44. Morgan DJ, Croft LD, Deloney V, Popovich KJ, Crnich C, Srinivasan A, et al. Choosing Wisely in healthcare epidemiology and antimicrobial stewardship. Infect Control Hosp Epidemiol. 2016;37(7):755–60.
45. Madden GR, Sifri CD. Reduced *Clostridioides difficile* tests among solid organ transplant recipients through a diagnostic stewardship bundled intervention. Ann Transplant. 2019;24:304–11.
46. Mizusawa M, Small BA, Hsu Y-J, Sharara SL, Advic E, Kauffman C, et al. Prescriber behavior in *Clostridium difficile* testing: a 3-hospital diagnostic stewardship intervention. Clin Infect Dis. 2019;69(11):2019–2021. https://doi.org/10.1093/cid/ciz295
47. Alonso CD, Marr KA. *Clostridium difficile* infection among hematopoietic stem cell transplant recipients: beyond colitis. Curr Opin Infect Dis. 2013;26(4):326–31.
48. Kamboj M, Brite J, Aslam A, Kennington J, Babady NE, Calfee D, et al. Artificial differences in clostridium difficile infection rates associated with disparity in testing. Emerg Infect Dis. 2018;24(3):584–7.
49. Stohs E, Chow VA, Liu C, Bourassa L, Miles-Jay A, Knight J, et al. Limited utility of outpatient surveillance blood cultures in hematopoietic cell transplant recipients on high-dose steroids for treatment of acute graft-versus-host-disease. Biol Blood Marrow Transplant. 2019;25(6):1247–52.
50. Krantz EM, Zier J, Stohs E, Ogimi C, Sweet A, Marquis S, et al. Antibiotic prescribing and respiratory viral testing for acute upper respiratory infections among adult patients at an Ambulatory Cancer Center. Clin Infect Dis 2019;1–8.
51. Rosa RG, Goldani LZ, dos Santos RP. Association between adherence to an antimicrobial stewardship program and mortality among hospitalised cancer patients with febrile neutropaenia: a prospective cohort study. BMC Infect Dis. 2014;14:286.
52. Beyar-Katz O, Dickstein Y, Borok S, Vidal L, Leibovici L, Paul M. Empirical antibiotics targeting gram-positive bacteria for the treatment of febrile neutropenic patients with cancer. Cochrane Database Syst Rev. 2017;6:CD003914. https://doi.org/10.1002/14651858.CD003914.pub4.
53. Vardakas KZ, Samonis G, Chrysanthopoulou SA, Bliziotis IA, Falagas ME. Role of glycopeptides as part of initial empirical treatment of febrile neutropenic patients: a meta-analysis of randomised controlled trials. Lancet Infect Dis. 2005;5(7):431–9.
54. Libuit J, Whitman A, Wolfe R, Washington CS. Empiric vancomycin use in febrile neutropenic oncology patients. Open Forum Infect Dis. 2014;1(Suppl 1):1–8.
55. Cometta A, Kern WV, De Bock R, Paesmans M, Vandenbergh M, Crokaert F, et al. Vancomycin versus placebo for treating persistent fever in patients with neutropenic cancer receiving piperacillin-tazobactam monotherapy. Clin Infect Dis. 2003;37(3):382–9.

56. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients. IDSA Guidel. 2010;52(4):e56–93.
57. Baden LR, Swaminathan S, Angarone M, Blouin G, Camins BC, Casper C, et al. Prevention and treatment of cancer-related infections, version 2.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2016;14(7):882–913.
58. Averbuch D, Orasch C, Cordonnier C, Livermore DM, Mikulska M, Viscoli C, et al. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia. Haematologica. 2013;98(12):1826–35.
59. Pillinger KE, Bouchard J, Withers ST, Mediwala K, Mcgee EU, Gibson GM, et al. Inpatient antibiotic stewardship interventions in the adult oncology and hematopoietic stem cell transplant population: a review of the literature. Ann Pharmacother. 2019;1060028019890886. https://doi.org/10.1177/1060028019890886. [Epub ahead of print]
60. Aguilar-Guisado M, Espigado I, Martín-Peña A, Gudiol C, Royo-Cebrecos C, Falantes J, et al. Optimisation of empirical antimicrobial therapy in patients with haematological malignancies and febrile neutropenia (how long study): an open-label, randomised, controlled phase 4 trial. Lancet Haematol. 2017;4(12):e573–83.
61. Nicolle LE, Gupta K, Bradley SF, Colgan R, DeMuri GP, Drekonja D, et al. Clinical practice guideline for the management of asymptomatic bacteriuria: 2019 update by the Infectious Diseases Society of America. Clin Infect Dis. 2019;68(10):1611–5.
62. Goldman JD, Julian K. Urinary tract infections in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13507.
63. Origüen J, López-Medrano F, Fernández-Ruiz M, Polanco N, Gutiérrez E, González E, et al. Should asymptomatic bacteriuria be systematically treated in kidney transplant recipients? Results from a randomized controlled trial. Am J Transplant. 2016;16(10):2943–53.
64. Moradi M, Abbasi M, Moradi A, Boskabadi A, Jalali A. Effect of antibiotic therapy on asymptomatic bacteriuria in kidney transplant recipients. Urol J. 2005;2(1):32–5.
65. El Amari EB, Hadaya K, Bühler L, Berney T, Rohner P, Martin P-Y, et al. Outcome of treated and untreated asymptomatic bacteriuria in renal transplant recipients. Nephrol Dial Transplant. 2011;26(12):4109–14.
66. Vidal E, Torre-Cisneros J, Blanes M, Montejo M, Cervera C, Aguado JM, et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. Transpl Infect Dis. 2012;14(6):595–603.
67. Hynicka LM, Ensor CR. Prophylaxis and treatment of respiratory syncytial virus in adult immunocompromised patients. Ann Pharmacother. 2012;46(4):558–66.
68. Foolad F, Aitken SL, Shigle TL, Prayag A, Ghantoji S, Ariza-Heredia E, et al. Oral versus aerosolized ribavirin for the treatment of respiratory syncytial virus infections in hematopoietic cell transplant recipients. Clin Infect Dis. 2019;68(10):1641–9.
69. Li L, Avery R, Budev M, Mossad S, Danziger-Isakov L. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. J Heart Lung Transplant. 2012;31(8):839–44.
70. Wang N, Athans V, Neuner E, Bollinger J, Spinner M, Brizendine K. A pharmacist-driven antimicrobial stewardship intervention targeting cytomegalovirus viremia in ambulatory solid organ transplant recipients. Transpl Infect Dis. 2018;20(6):1–8.
71. Ananda-Rajah MR, Slavin MA, Thursky KT. A case for antifungal stewardship. Curr Opin Infect Dis. 2012;25(1):107–15.
72. Hamdy RF, Zaoutis TE, Seo SK. Antifungal stewardship considerations for adults and pediatrics. Virulence. 2017;8(6):658–72.
73. Schwartz IS, Patterson TF. The emerging threat of antifungal resistance in transplant infectious diseases. Curr Infect Dis Rep. 2018;20(3):1–10.

74. Neofytos D, Fishman JA, Horn D, Anaissie E, Chang CH, Olyaei A, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2010;12(3):220–9.
75. Urbancic KF, Thursky K, Kong DCM, Johnson PDR, Slavin MA. Antifungal stewardship: developments in the field. Curr Opin Infect Dis. 2018;31(6):490–8.
76. Hand J, Patel G. Antimicrobial stewardship in transplant patients. Curr Opin Organ Transplant. 2019;24(4):497–503.
77. Patterson TF, Thompson GR, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Executive summary: practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the infectious diseases society of America. Clin Infect Dis. 2016;63(4):e1–60.

Antimicrobial Prophylaxis in the Age of Emerging Transplant Infections

8

Jonathan Hand

Contents

Introduction 155
Perioperative Antibacterial Prophylaxis in Solid Organ Transplantation 156
Antibiotic Prophylaxis After Transplantation 158
Antifungal Prophylaxis After Transplantation 159
Mycobacterial Prophylaxis After Transplantation 162
Influenza Prophylaxis After Transplantation 162
Herpes Simplex Virus and Varicella Zoster Virus Prophylaxis After Transplantation 163
Cytomegalovirus Prophylaxis After Transplantation 163
BK Polyomavirus Prophylaxis After Transplantation 165
Parasitic Prophylaxis in Transplantation 165
Conclusion 167
Key Points 167
References 168

Introduction

Emerging multidrug-resistant bacterial, viral, and fungal infections are a common cause of morbidity and mortality after solid organ transplantation (SOT) and hematopoietic cell transplantation (HCT) worldwide. With limited antimicrobial options to combat these pathogens, prevention of infection is critical for optimal patient and graft outcomes. Antimicrobial prophylaxis (AP) is routinely used to prevent infections in SOT and HCT recipients. In the current era of emerging resistant infections the efficacy of prophylactic regimens may be diminished in these patients and thoughtful, personalized approaches are warranted. Conceptually, AP used to prevent infection should aim to be efficacious while minimizing toxicities, cost, and

J. Hand (✉)
Division of Infectious Diseases, Ochsner Health, University of Queensland School of Medicine, Ochsner Clinical School, New Orleans, LA, USA
e-mail: jonathan.hand@ochsner.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_7

Table 1 Risk factors for early posttransplant infections

Intraoperative infection risk factors	Donor and recipient pretransplant risk factors	Early posttransplant infection risk factors
Prolonged surgery or substantial transfusion requirement Choice of surgical technique (roux-en-Y biliary anastomosis for liver transplantation) Technical problems affecting the transplant's functional integrity and vascular supply (lung: Bronchial anastomotic leaks; liver: Hepatic artery thrombosis; pancreas: Duodenal leaks; kidney: Vesicoureteral reflux etc.)	Chronic malnutrition Colonization: MDR-bacteria or yeasts (ESBL, CRAB, MRSA, CRE, drug-resistant *Candida*) Filamentous fungi (*Aspergillus*, Mucor) Endemic fungi (*Histoplasma, Coccidioides*) Latent infections: *Mycobacterium tuberculosis, M. avium or M. abcessus* Viruses (CMV, VZV, HSV, EBV, HIV, HHV8) *Toxoplasma gondii, Strongyloides stercoralis, Trypanosoma cruzii*	Prolonged mechanical ventilation Protracted use of indwelling devices (central line catheters, abdominal or chest drains, ureteral catheters) Persistent hematomas or leaks (biliary, urinary, bronchial) Prolonged renal replacement therapies (hemodialysis) Repeated open surgery Prolonged exposure to broad-spectrum antibiotics Nosocomial exposure (respiratory viruses, filamentous fungi, drug resistance bacteria)

antimicrobial resistance using the lowest effective dose for the shortest acceptable duration. In the following sections, antimicrobial prophylactic strategies will be reviewed in the context of emerging antimicrobial resistance.

There are many factors that impact a patient's individual risk of infection, and thereby alter the indications of a prophylactic strategy. Some of these reasons are summarized in Table 1.

Perioperative Antibacterial Prophylaxis in Solid Organ Transplantation

Incidence of surgical site infections (SSI) range widely in SOT recipients, from 3 to 53%, and vary by the organ transplanted. By nature of organ-specific surgical differences, causative pathogens also vary, although reporting bias often leads to more frequent descriptions of infections with multidrug-resistant organisms (MDROs) [1]. Greater antimicrobial use prior to SOT has been associated with MDRO infection post-transplant [2]. Surgical antibacterial prophylaxis (SAP), used to prevent SSI, is standard of care for solid organ transplant operations. During the operative procedure, antibacterials minimize the burden of bacteria in the surgical site environment to decrease infections and promote healing [1]. World Health Organization guidelines suggest SAP should be administered within 120 min before the incision and re-dosed throughout based on the agent's half-life and procedure duration [3]. There is limited and low quality evidence to inform the

Table 2 Suggested peri-transplant antibacterial prophylaxis for prevention of early posttransplant infections

Transplant	Antibiotics for 24–72 h posttransplant[a] (duration as short as possible)
Kidney	Cefuroxime or cefazolin
Liver	Cefuroxime, ampicillin-sulbactam or piperacillin-tazobactam
Pancreas	Piperacillin-tazobactam, or ampicillin-sulbactam for 5–7 days
Heart	Cefuroxime or cefazolin
Lung	Cefuroxime or cefepime Tailor to recipient/donor bronchial cultures after transplant
Intestinal	Piperacillin-tazobactam or cefepime + metronidazole, variable duration

[a]Should be tailored to reflect local epidemiology and antibiogram, as well as culture results from recipient before and after transplant

recommendation for optimal pre-incision SAP timing. Historical studies and guidance have, somewhat arbitrarily, recommended dosing 30–60 min prior to incision. However, a recent systemic review and meta-analysis found no significant difference between the 120–60 min dosing when compared to the 60–0 dosing [4]. The shortest effective duration should be used to minimize toxicities of antibacterials. For an example of a solid-organ transplant SAP regimen, refer to Table 2. Though national and international guidelines recommend stopping SAP at closure of surgical incision, transplant-specific evidence and guidance is limited leading to significant variation in duration across centers. A recent single-center, randomized controlled pilot study found no significant differences in liver transplant SSI rates when SAP limited to intra-operative dosing was compared to SAP extended to 72 h [5]. SAP agent choice also varies between institutions and should be based on local epidemiology while considering patient-specific microbiological factors such as recipient and donor colonization. As MDROs are more frequently implicated in SSIs, antibacterial agents targeting these pathogens may be more frequently used. MDRO colonization has been associated with post-transplant infection though differences in site of colonization and organ transplanted are important [1]. For example, lung transplants are more significantly impacted by changes in respiratory flora, renal transplant by urogenital organisms, and liver transplant by biliary pathogens. Evidence to assess the impact of altering SAP to account for MDROs with which recipients are colonized is lacking. However, attempts to target colonizing MDROs with SAP are common practice in many centers. Adjunctive strategies to eradicate MDRO colonization and restore gut microbial diversity through gut decontamination and fecal microbiota transplantation are being explored in certain centers through research protocols, but at this time they are of uncertain benefit and their utility in routine, transplant care has not yet been established [6]. Transmission of MDROs from donors to recipients has been described and antimicrobials targeted to these organisms have prevented recipient infection [7]. In these situations, communication between organ procurement organizations and accepting transplant centers is critical to establish the optimal perioperative treatment regimen. Transplanting organs from donors with MDR Gram-negative organisms in special circumstances may become more feasible as new agents targeting MDR Gram-negative

organisms are developed. Current guidelines recommend 7–14 days of antimicrobial therapy for recipients of organs from infected donor [7].

Antibiotic Prophylaxis After Transplantation

In the post-transplant setting, trimethoprim-sulfamethoxazole (TMP-SMX) is the most commonly used prophylactic antibacterial agent. It is the first-line option for *Pneumocystis jiroveci* prevention and may also help to prevent other opportunistic infections with *Listeria, Toxoplasma gondii, Legionella, Nocardia,* and certain gastrointestinal pathogens. After SOT and allogeneic (allo-) HCT TMP-SMX is continued for at least 6 months and may be extended in patients requiring longer or intensified immunosuppression (e.g., treatment for graft vs. host disease (GVHD)) or immunomodulatory co-infections such as CMV and EBV. Given the importance of TMP-SMX prophylaxis, patients with reported sulfa allergies and other intolerances should be evaluated for a rechallenge after transplant, or pretransplant, if time permits, through an infectious disease, or allergy consultation. In a recent single-center, retrospective study a rechallenge protocol was safe and successful [8]. Resistance mutations in *P. jiroveci* dihydropteroate synthase (DHPS), an enzyme involved in folate metabolism, have been described and result from long-term TMP-SMX exposure in HIV settings. However, these mutations seem to have limited clinical importance in relation to treatment failure and transplant-specific data are inadequate [9]. TMP-SMX resistant *Nocardia* isolates have been described in both SOT and HCT though reported rates vary widely by geography and species [10]. Though *Nocardia* infections may occur while patients are receiving TMP-SMX prophylaxis, other clinical factors contributing to net-state of immunosuppression likely play a role. In kidney transplant (KT) recipients TMP-SMX also reduces the occurrence of urinary tract infections (UTI) though emerging resistance in certain institutions and regions may limit its efficacy [11]. Additional antibacterials added during ureteral stent removal after KT have not been found to be efficacious and should be avoided [12]. Other antibacterials such as fluoroquinolones have been used as UTI primary prophylaxis in KT recipients though with rising rates of fluoroquinolone resistance and a growing list of associated toxicities this drug class is not recommended for prevention [11].

After HCT, antibacterial regimes depend on agent toxicities, and begin around the time of administration of the conditioning regimen, after stem cell infusion, or at the time of engraftment. Antibacterial prophylaxis is not typically given to autologous HCT recipients as these patients engraft more quickly and have less-severe mucositis. Fluoroquinolones are most commonly used to prevent neutropenic fever in high-risk patients given their activity against Gram-negative bacilli such as *Pseudomonas aeruginosa* and Enterobacteriaceae as well as viridans group streptococcus. In randomized trials, antibacterial prophylaxis has been associated with less Gram-negative infections with no significant reduction in mortality. Meta-analyses have reported mixed effects on all-cause mortality [13]. The increasingly worrisome side effect profile of the fluoroquinolones and associated consequences of their use

including emerging MDR infectious complications, *Clostridioides difficile* infection, and gastrointestinal dysbiosis demand a close evaluation of these drugs for routine prophylaxis. Additionally, worldwide rates of fluoroquinolone resistance continue to rise and may limit efficacy of prophylaxis particularly when local *Escherichia coli* resistance rates are greater than 20% [14–16]. Antibacterial prophylaxis is typically continued until engraftment. Patients with chronic graft-versus-host disease actively receiving immunosuppression remain at risk for encapsulated bacteria. Antibacterial prophylaxis against *Streptococcus pneumoniae* is recommended and may extend into the post-engraftment period [16]. In regions with growing rates of penicillin and fluoroquinolone resistant *S. pneumoniae*, alternative drug choices must be considered. Further studies are needed to better define personalized risk and evaluate other antibacterial classes to employ as prophylactic agents in required settings. Recently, cefpodoxime was evaluated as an alternative to fluoroquinolone prophylaxis and was associated with similar rates of neutropenic fever and AP failure [17].

Antifungal Prophylaxis After Transplantation

In non-lung SOT, *Candida* is the most common fungal organism responsible for invasive infection [18]. Infections with azole-resistant, non-*albicans Candida* species, particularly *C. glabrata,* are an emerging threat. Many countries have also witnessed a concerning rise in drug-resistant *C. auris* infections transmitted in hospital settings. Though universal prophylaxis for *Candida* is common after small-bowel transplantation, a targeted strategy is recommended for other abdominal and thoracic organ recipients [19]. For a suggested antifungal prophylaxis pathway, refer to Table 3. Echinocandins, azoles, and formulations of amphotericin B have been studied and have demonstrated successful reductions in invasive *Candida* infections. Though fluconazole is commonly recommended as a first-line prophylactic agent, hospital epidemiology, patient risk factors, and colonization should be used to create a personalized strategy, employing alternative broad-spectrum agents as needed to prevent post-operative infections with resistant species. Isavuconazole, a newer, broad-spectrum, triazole approved for the treatment of invasive aspergillosis (IA) and mucormycosis, is a promising prophylactic agent for emerging fungal infections. However, limited data exists for prophylactic use and its role for *Candida* prophylaxis after SOT is unclear. Though isavuconazole was inferior to caspofungin when used as treatment for invasive candidiasis [20], a recent single-center, retrospective study found no increase in *Candida* or other yeast infections using isavuconazole prophylaxis in lung transplant recipients [21].

Similarly, *Candida* prophylaxis during the pre-engraftment period after myeloablative, allogeneic HCT reduces *Candida* infections, and universal prophylaxis is recommended [22]. Though fluconazole is the currently recommended prophylactic agent of choice, other broader spectrum tri-azoles, echinocandins, and polyenes have been studied [23]. Breakthrough infections with azole-resistant *Candida* species have been described, and in combination with the risk of invasive molds

Table 3 Fungal infection prevention peri-transplant

Transplant	Fungus	Risk factors	Prophylaxis	Suggested duration[a]
Kidney	*Pneumocystis* *Candida*	All patients Candiduria	TMP-SMX[a] Fluconazole	6 months 7–14 days
Pancreas/ intestine	*Pneumocystis* *Candida*	All patients All patients	TMP-SMX Fluconazole, echinocandin	6 months 2–4 weeks
Liver	*Pneumocystis* *Candida*	High meld score (>30), ATG, CMV disease, second transplant Based on combinations of risk factors: Protracted antibiotics >5 days, yeast colonization >3 body sites, ICU >5 days, posttransplant hemodialysis, retransplantation or need for second surgery, choledocojejunostomy, high transfusion requirement, and pancreatitis	TMP-SMX Echinocandin or fluconazole	6 months 2 weeks
Lung	*Pneumocystis* *Candida* *Aspergillus*	All patients None All patients or according to risk factors: Proven colonization, high-doses steroids, retransplantation, acute rejection, CMV infection	TMP-SMX Aerosolized amphotericin B, voriconazole	Lifelong 4–6 weeks, lifelong
Heart	*Pneumocystis* *Candida*	All patients None	TMP-SMX	12 months versus lifelong (depending on toxoplasma status)

[a]TMP-SMX is preferred agent. Atovaquone is preferred alternative for patients who are also at risk of toxoplasmosis. Dapsone and Pentamidine are alternative options

may necessitate echinocandin or extended spectrum azole prophylactic strategies in many centers.

Aspergillus fumigatus is the most commonly identified etiology of IA after SOT. Though uncommonly reported in the United States, other countries are observing increasing rates of azole-resistant *A. fumigatus* which have been associated with high mortality [24]. While risk of IA after SOT varies by transplanted organ, prophylactic strategies after liver and lung transplantation have been most studied. Universal prophylaxis with a mold-active agent is not recommended after liver transplantation

and an approach targeted to risk factors for IA is favored [25]. In certain studies, triazoles and echinocandins have successfully prevented IA but breakthrough *Aspergillus* infections have been occasionally reported [25]. Though many centers employ universal prophylactic strategies after lung transplantation, preemptive therapy has also been shown to be successful and is an acceptable approach [25]. Triazoles such as itraconazole and voriconazole are common prophylactic agents used after lung transplantation and have demonstrated reductions in IA. A recent single-center evaluation of experience with aerosolized amphotericin B lipid complex with and without micafungin during transplant hospitalization found the prevalence rate of invasive candidiasis and non-*Candida* invasive fungal infections was 11.4% and 8.8%, respectively [26]. Although isavuconazole primary prophylaxis has not been well studied nor should be considered standard of care, a recent single center report found it safe and effective for the prevention of mold infections in lung transplant recipients and found no increase in candida or other yeast infections [21].

Aspergillus accounts for a majority of invasive mold infections after HCT. While fluconazole is recommended as first-line prophylaxis, its lack of *Aspergillus* activity has led some centers to employ mold-active azoles for primary prophylaxis, particularly for patients transplanted for acute myelogenous leukemia or patients who received haploidentical, or umbilical cord blood grafts. Voriconazole and posaconazole are most commonly used and micafungin and itraconazole may be considered as alternatives. Isavuconazole has been studied as treatment for invasive molds in HCT recipients. Though it is a promising agent to prevent emerging fungal infections after HCT, studies for this use are limited. A recent single-center, retrospective study evaluating isavuconazole primary prophylaxis for HCT recipients and patients with hematologic malignancies observed an increased rate of breakthrough invasive mold infections [27]. Conversely, in a similar, single-center, open-label trial of isavuconazole primary prophylaxis after HCT, 3.1% of patients experienced breakthrough candidemia and no patients developed mold infections [28]. Finally for patients with GVHD, posaconazole has been shown to prevent IA and reduce fungal related deaths when used as prophylaxis [16]. In patients with prior invasive fungal infections, secondary prophylaxis should be strongly considered particularly during periods of prolonged neutropenia or intensified immunosuppression. Uncommon fungal infections may also emerge while on triazole prophylaxis after HCT, leaving limited options for treatment or secondary prophylaxis [29].

Coccidiomycoses after SOT and HCT can cause significant morbidity and mortality. SOT recipients transplanted in *Coccidioides*-endemic regions should receive primary azole prophylaxis post-transplant. SOT deceased donors, especially pulmonary, who spent significant time living in the southeast of the United States, should also trigger posttransplant prophylaxis in their recipients. Living donors, where transplant timing is more flexible, should be clinically and serologically screened for *Coccidioides* risk, and prophylaxis strategy determined accordingly. Similarly, transplant candidates should be assessed for prior travel to or residence in endemic regions and screened with chest imaging and serologic testing to help guide posttransplant prophylaxis strategies. Additionally, given the significant risk of coccidiomycoses relapse following primary infection in SOT, lifelong azole secondary prophylaxis

should be considered [30]. Though fluconazole is most commonly used, other triazoles are active against *Coccidiodes* and have been used successfully for treatment [31]. However, limited prophylaxis data has been reported for alternative azoles.

Primary prophylaxis for histoplasmosis and blastomycosis is not recommended. In patients who have recently recovered from histoplasmosis or blastomycosis, secondary prophylaxis with antigen monitoring may be considered during periods of intense immunosuppression [30]. For example, even lung recipients can be managed by following serial urinary histoplasma antigens rather than embarking on protracted antifungal prophylaxis. Recommendations for prophylaxis of endemic mycoses after HCT are lacking. Similar nuanced strategies using epidemiologic risk and serologic screening should be considered. Further, while azole prophylaxis is more common after HCT, personalized, secondary prophylaxis should be considered during periods of prolonged neutropenia and during GVHD therapy.

Mycobacterial Prophylaxis After Transplantation

Drug-resistant *Mycobacterium tuberculosis* (TB) has been increasingly described throughout the world and has been reported in transplant recipients [32]. Transplant recipients who have been in contact with persons known to be infected with drug-resistant TB should receive prophylaxis. Though fluoroquinolones are not recommended as first line agents for latent tuberculosis infection (LTBI) due to limited efficacy data and their many toxicities, they have been used for prevention of MDR TB and may be considered if concern for resistance exists [33]. Optimally, LTBI therapy should be initiated and completed before SOT or HCT. If prophylaxis is used in the posttransplant setting, careful attention is needed to avoid drug-drug interactions of rifamycins with HCT conditioning regimens and GVHD prophylaxis as well as SOT maintenance immunosuppression. Other emerging, difficult-to-treat, non-tuberculous mycobacterial (NTM) infections often complicate pre- and post-lung transplant care. Specifically, pretransplant identification of *Mycobacterium abscessus* presents a significant challenge to lung transplant candidacy. Decisions to move forward with lung transplantation in such patients infected or colonized with this organism, though not absolutely contraindicated, should be diligently individualized. A recent single center experience reported optimal outcomes when nuanced, tolerable, and effective antimicrobials were used to treat and clear AFB smears and cultures pretransplant and extended into the posttransplant setting [34, 35].

Influenza Prophylaxis After Transplantation

Data does support post-exposure prophylaxis against influenza in high-risk patients [36]. Historically, neuraminidase inhibitor resistance rates for influenza have varied seasonally but have been relatively low in pandemic and post-pandemic virus. Antiviral resistance is more common in HCT and SOT recipients and is associated with prolonged viral shedding in the setting of immunosuppression and oseltamivir exposure [37]. Nosocomial clusters of resistant influenza A and B have been

described. Transplant recipients who are exposed to close household contacts or involved in a nosocomial outbreak should receive post-exposure antiviral prophylaxis. If concern for antiviral resistance exists in seasonal virus or in a healthcare setting, post-exposure prophylaxis with an oseltamivir alternative should be considered. The H275Y mutation, most frequently responsible for oseltamivir resistance, does not reduce zanamivir activity. Inhaled zanamivir should be used as first-line post-exposure prophylaxis if exposure to oseltamivir-resistant virus is suspected. Baloxavir marboxil, a novel inhibitor of cap-dependent endonuclease, may also have a role in post-exposure prophylaxis though no data for this indication exists and studies of this agent in SOT and HCT are lacking. Pre-exposure prophylaxis in high-risk patients, for example, lung transplant recipients in the winter, has not been studied in formal settings, and is generally not recommended.

Herpes Simplex Virus and Varicella Zoster Virus Prophylaxis After Transplantation

Since the advent of HCT and SOT herpesvirus infections have been a common cause of post-transplant infectious complications. For suggested prophylactic details of antiviral prophylaxis, in SOT, refer to Table 4. However, significant advances in antiviral therapy and testing have drastically decreased their associated morbidity and mortality. Acyclovir (ACV) and valacyclovir (vACV) are active against herpes simplex virus (HSV) type-1 and 2 and may be used for HSV prophylaxis. These agents also have minor activity against cytomegalovirus (CMV) though are not recommended first line for prophylaxis and should not be used for treatment. Historically acyclovir and valacyclovir resistant HSV has been reported in 2.5% of SOT recipients [38]. Rates are higher in HCT recipients ranging from 4% to over 30% [39]. ACV and vACV prophylaxis are recommended and routinely used in the first 30 days following allo-HCT and may be extended if reactivations are recurrent or during periods of intensified immunosuppression. Additionally, given the activity against VZV, antiviral prophylaxis can be given routinely for 1 year to VZV seropositive recipients. HSV resistance to ACV is most frequently mediated by mutations in the *viral thymidine kinase* gene conferring cross-resistance to other oral herpes-active agents. Treatment of drug-resistant HSV typically includes alternative agents such as intravenous foscarnet or topical cidofovir for cutaneous recurrences. Given these agents have substantial toxicities they are not recommended for prophylactic use [16]. Brincidofovir has in vitro activity against HSV though there is no data to support its use for posttransplant prophylaxis. Maribavir and Letermovir, CMV-active agents, do not have activity against other herpesviruses such as HSV and VZV.

Cytomegalovirus Prophylaxis After Transplantation

Cytomegalovirus (CMV) remains a common infectious complication after SOT and allo-HCT. Risk is largely dictated by donor and recipient serostatus and remains an independent risk factor for negative outcomes. The highest risk serostatus pairing in

Table 4 Human herpesvirus prophylaxis after kidney, pancreas, liver, or heart transplant

Induction agent	Donor CMV	Recipient CMV	Prophylaxis
Basiliximab or steroids	**Positive**	**Negative**	**Valganciclovir 3-6 m**
	Positive	**Positive**	
	Negative	**Positive**	
	Negative	**Negative**	Acyclovir, famciclovir, or valacyclovir 3–6 m
Anti-thymocyte globulin or alemtuzumab induction	**Positive**	**Negative**	**Valganciclovir 6 m**
	Positive	**Positive**	
	Negative	**Positive**	
	Negative	**Negative**	Acyclovir, famciclovir, or valacyclovir 6 m

Comments on viral prophylaxis:

•Dosages of all antiviral agents need to be adjusted for renal function. The eGFR or creatinine clearance should be used (not simply the serum creatinine).

•For prophylaxis, the first doses may be intravenous ganciclovir or oral valganciclovir, converting intravenous to oral once patient tolerating oral medications. Data supports the safety and efficacy of either administration for treatment (not prophylaxis, although should be similar) [48, 49].

•"Mini-dosing" not recommended as it increases the risk of resistant/refractory CMV infections.

•Lung transplant prophylaxis would often be similar, with longer courses of prophylaxis more common.

•For centers wishing to take a mixed approach, preemptive monitoring can be done more readily with R+ recipients using Basiliximab or steroid induction.

•Monitoring while on prophylaxis only done if clinically indicated by symptoms; consider weekly monitoring after prophylaxis completed for 8–12 weeks in higher risk patients, those with deep lymphopenia, and those on more potent immunosuppression [50].

SOT and HCT are different: seropositive solid organ donor grafts transplanted into CMV seronegative recipients pose the greatest risk; conversely seronegative HCT donor grafts given to CMV seropositive recipients are more concerning in stem cell transplantation. The incidence of ganciclovir (GCV)-resistant CMV after SOT and HCT is 0%–3% and 0%–4%, respectively, and has been reported up to 14.5% in haploidentical-HCT recipients [40, 41]. Risk factors for resistant CMV infection include prolonged anti-CMV drug exposure with inadequate dosing, high-degree immunosuppression, and donor/recipient serostatus mismatch. Though preemptive therapy and antiviral prophylaxis prevention strategies have been studied, prophylaxis with valganciclovir (vGCV) is most commonly employed after SOT while preemptive therapy is favored after HCT given concerns for vGCV/GCV-induced marrow toxicity (Table 4). Recently, other antiviral agents have been studied for CMV prevention. Letermovir, a novel inhibitor of the viral terminase complex (encoded by *UL56*) has recently been approved by the United States Food and Drug Administration for CMV prophylaxis in the first 100 days after allo-HCT for seropositive recipients. In a multicenter phase III trial, patients who received letermovir prophylaxis through week 14 after HCT developed less CMV infection and had lower all-cause mortality at week 24 [42]. Though letermovir appears to have less myelosuppressive effects, it has a low barrier to resistance which may limit

its use to prophylaxis. A phase III trial evaluating letermovir prophylaxis for CMV prevention after kidney transplantation is ongoing. Letermovir has a distinctly different viral target than DNA polymerase inhibitors GCV and vGCV making it an attractive option for CMV secondary prophylaxis in patients with GCV/vGCV resistant virus. Published experience with letermovir secondary prophylaxis in SOT and HCT is limited to case reports at this time, and cannot be routinely recommended. Investigational agents, brincidofovir, a lipid conjugate of cidofovir, and maribavir, a DNA synthesis inhibitor, may also retain activity against resistant CMV. Maribavir has not demonstrated benefit when used for primary CMV prophylaxis after liver transplantation and after engraftment in HCT [43]. Neither drug is currently available outside trials or compassionate access pathways. Similarly, a recent placebo-controlled phase III trial of brincidofovir primary prophylaxis found similar rates of CMV when compared to placebo with more serious adverse events noted in the patients given brincidfovir [43]. No data exists for secondary prophylaxis using these agents. Though antiviral prophylaxis is favored at many solid organ transplant centers a recent randomized controlled trial found incidence of CMV disease was significantly lower at 1 year in patients undergoing preemptive therapy when compared to antiviral prophylaxis. Patients who received antiviral prophylaxis were also found to have less CMV-specific neutralizing antibody and T-cell responses compared to those patients who received preemptive therapy [44]. The exact role of CMV-specific immune monitoring strategies used to dictate preemptive therapy or prophylaxis has not yet been established and more studies are needed.

BK Polyomavirus Prophylaxis After Transplantation

BK polyomavirus reactivation leading to viruria and DNAemia is common after transplant. Though patients may be asymptomatic, BK polyomavirus may cause polyomavirus-associated nephropathy after kidney transplantation and hemorrhagic cystitis after allo-HCT. Other organ recipients are rarely clinically impacted. The key element of treatment and prevention for BK is reduction of immunosuppression. Fluoroquinolones can inhibit BK polyomavirus replication in vitro and have been studied to prevent BK replication and subsequent disease. Randomized trials have found no benefit of fluoroquinolones used for prophylaxis or treatment of BK. The most recently published trial found ciprofloxacin was not only ineffective at preventing BK DNAemia but also associated with an increased risk of fluoroquinolone-resistant infections [45]. Brincidofovir has also been evaluated in complex BK, but generally not accepted as a viable option at this time.

Parasitic Prophylaxis in Transplantation

Globally, changing environmental and patient demographic variables will likely impact the epidemiology of infections seen in the posttransplant setting. *Strongyloides stercoralils,* a parasite endemic to the tropics and subtropics, has

Table 5 Suggested duration of toxoplasmosis prophylaxis after heart transplant (based on Serologic Combinations)

Serologic combination	Risk group	Treatment and Dosing	Duration of therapy
Donor seropositive/ recipient seronegative	Highest risk	TMP-SMX DS[a] (some centers use SS) every day (if DS dose reduce to Bactrim SS q day if GFR < 30) $\times$ 12 months then TMP-SMX SS every day (no need for dose reduction with renal insufficiency even ESRD/dialysis).[b]	Lifetime, if possible
Recipient seropositive (donor either serostatus)	Moderate risk	TMP-SMX SS[a] every day (no need for dose reduction with renal insufficiency even ESRD/dialysis)	Can stop at 1 year after transplant, or when on low-dose immunosuppression whichever is *later/longer* Restart if intensification of immunosuppression for same period as after transplant.
Donor seronegative/ recipient seronegative	Lowest risk	Same as for recipient seropositive for Pneumocystis and other prevention, although not needed for toxoplasmosis	

[a]Trimethoprim-sulfamethoxazole (TMP-SMX) SS (single strength) is sulfamethoxazole 400 mg and trimethoprim 80 mg, while TMP-SMX DS (double strength) is 200% that dose
[b]If true TMP-SMX allergy documented, second-line prophylaxis would be dapsone 100 mg a day or atovaquone 1500 mg a day. With dapsone, methemoglobinemia or breakthrough toxoplasmosis infection could occur, and G6PD should be checked prior to starting treatment (to avoid hemolysis if G6PD deficient)

also been described in the southeastern United States. De novo infection, reactivation of chronic, latent infection, and donor-derived infection have all been described in SOT and HCT recipients and can be associated with significant morbidity and mortality [46]. Though transplant center and organ procurement organization practice is variable, screening deceased and living donors as well as transplant candidates with epidemiological risk factors is recommended [46]. Because cases have been seen with recipients of all organs, prophylaxis is recommended for recipients of all organs coming from seropositive donors (refer to Table 5).

Oral ivermectin is the treatment of choice for seropositive candidates, ideally before transplant. Additionally, treating recipients of donors who have tested positive for *Strongyloides* with oral ivermectin can minimize the risk of donor-derived infection [46].

Infections caused by *Trypanosoma cruzi*, the protozoan parasite causing Chagas disease, most commonly occurs in Latin America and has been reported in the United States in areas with large Latin American communities [47]. *T. cruzi* is primarily transmitted by the reduviid bug resulting in de novo infection though may be transmitted through transplantation or present as reactivation in transplant recipients in the setting of immunosuppression. Prevention relies on screening transplant donors and candidates. In the posttransplant setting, preemptive parasitological and PCR testing are recommended to allow for treatment in patients who develop evidence of *T. cruzi* reactivation or transmission [47]. Though benznidazole

and nifurtimox, antitrypanosomal agents used to treat T. *cruzi,* have been used for prophylaxis, their associated toxicities make preemptive therapy the strategy of choice. In the United States, posttransplant prospective monitoring strategies should be coordinated with the Centers for Disease Control and Prevention, and when early preclinical parasitemia is detected, therapy can be started, rather than using any drug prophylactically.

The parasite *Toxoplasma gondii* can also be a significant clinical issue post-transplant; it most commonly presents in mismatched heart transplants, where transfer within the donor cardiac muscle is possible. Approximately 10% of the North American population is seropositive to *T. gondii*, leaving a substantial portion of the community at risk. Other solid organ transplants and HCT recipients appear to be at lower, but not zero risk. Trimethoprim-sulfamethoxazole (TMP-SMX) is the preferred agent for high-risk prophylaxis in heart transplants. For patients who are sulfa intolerant atovaquone 1500 mg daily can be used. Both these agents are effective for joint prophylaxis against *Pneumocystis jiroveci*, unlike dapsone or pentamidine which have no meaningful protection against toxoplasmosis (Table 5).

Conclusion

Infections complicate the care of patients after transplant, and antimicrobial prophylaxis has long been a cornerstone of infection prevention in this setting. However, as emerging, multidrug-resistant infections become more common, routine prophylactic regimens may have less efficacy. Thoughtful antimicrobial prophylaxis strategies are needed to protect patients from these pathogens as well as antimicrobial toxicities. Future research should focus on personalized approaches to prophylaxis to optimize targeted antimicrobials and durations, while evaluating the role of newer antimicrobials for prophylaxis.

Key Points

- Antimicrobial agents commonly used post-transplant may have limited efficacy in the era of emerging, multidrug-resistant infections.
- Antimicrobial prophylaxis should aim to be efficacious while minimizing toxicities, cost, and antimicrobial resistance using the lowest effective dose for the shortest acceptable duration.
- Intraoperative, donor/recipient, and early post-transplant factors should be used to guide personalized prophylaxis strategies.
- Surgical antimicrobial prophylaxis to prevent surgical site infection should consider prior recipient MDRO colonization and may need to be extended post-transplant if targeting donor organisms.
- Trimethoprim-sulfamethoxazole remains the linchpin of prophylaxis after SOT and HCT preventing a broad range of opportunistic infections.

- With rising rates of fluoroquinolone resistance and the worrisome side effect profile of this drug class, their use should be minimized when possible.
- Studies evaluating alternative approaches to prevent infection after cancer chemotherapy-related neutropenia are needed.
- More data are needed to understand the prophylactic role of newer antiviral and antifungal agents in the setting of emerging resistance.

References

1. Abbo LM, Grossi PA, Practice AICo. Surgical site infections: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13589.
2. Reid GE, Grim SA, Sankary H, Benedetti E, Oberholzer J, Clark NM. Early intra-abdominal infections associated with orthotopic liver transplantation. Transplantation. 2009;87(11):1706–11.
3. Allegranzi B, Zayed B, Bischoff P, et al. New WHO recommendations on intraoperative and postoperative measures for surgical site infection prevention: an evidence-based global perspective. Lancet Infect Dis. 2016;16(12):e288–303.
4. de Jonge SW, Gans SL, Atema JJ, Solomkin JS, Dellinger PE, Boermeester MA. Timing of preoperative antibiotic prophylaxis in 54,552 patients and the risk of surgical site infection: a systematic review and meta-analysis. Medicine (Baltimore). 2017;96(29):e6903.
5. Berry PS, Rosenberger LH, Guidry CA, Agarwal A, Pelletier S, Sawyer RG. Intraoperative versus extended antibiotic prophylaxis in liver transplant surgery: a randomized, controlled pilot trial. Liver Transpl. 2019;25:1043–53.
6. Tacconelli E, Mazzaferri F, de Smet AM, et al. ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant Gram-negative bacteria carriers. Clin Microbiol Infect. 2019;25:807–17.
7. Wolfe CR, Ison MG, Practice ASTIDCo. Donor-derived infections: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13547.
8. Urbancic KF, Ierino F, Phillips E, Mount PF, Mahony A, Trubiano JA. Taking the challenge: a protocolized approach to optimize Pneumocystis pneumonia prophylaxis in renal transplant recipients. Am J Transplant. 2018;18(2):462–6.
9. Navin TR, Beard CB, Huang L, et al. Effect of mutations in Pneumocystis carinii dihydropteroate synthase gene on outcome of P carinii pneumonia in patients with HIV-1: a prospective study. Lancet. 2001;358(9281):545–9.
10. Restrepo A, Clark NM, Infectious Diseases Community of Practice of the American Society of T. Nocardia infections in solid organ transplantation: guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation. Clin Transpl. 2019;33(9):e13509. https://doi.org/10.1111/ctr.13509. Epub 2019 Mar 19.
11. Goldman JD, Julian K. Urinary tract infections in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13507.
12. Lee JH, Muthukumar T, Kim J, et al. Antibiotic prophylaxis for ureteral stent removal after kidney transplantation. Clin Transpl. 2019;33(3):e13491.
13. Mikulska M, Averbuch D, Tissot F, et al. Fluoroquinolone prophylaxis in haematological cancer patients with neutropenia: ECIL critical appraisal of previous guidelines. J Infect. 2018;76(1):20–37.
14. Kern WV, Weber S, Dettenkofer M, et al. Impact of fluoroquinolone prophylaxis during neutropenia on bloodstream infection: data from a surveillance program in 8755 patients

receiving high-dose chemotherapy for haematologic malignancies between 2009 and 2014. J Infect. 2018;77(1):68–74.
15. Satlin MJ, Chavda KD, Baker TM, et al. Colonization with levofloxacin-resistant extended-spectrum beta-lactamase-producing Enterobacteriaceae and risk of bacteremia in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2018;67(11):1720–8.
16. Ullmann AJ, Schmidt-Hieber M, Bertz H, et al. Infectious diseases in allogeneic haematopoietic stem cell transplantation: prevention and prophylaxis strategy guidelines 2016. Ann Hematol. 2016;95(9):1435–55.
17. Doan VP, Yeh JC, Gulbis AM, Aitken SL, Ariza-Heredia E, Ahmed S. Levofloxacin versus cefpodoxime for antibacterial prophylaxis in allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2019;25:1637–41.
18. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.
19. Aslam S, Rotstein C, Practice ASTIDCo. Candida infections in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13623.
20. Kullberg BJ, Viscoli C, Pappas PG, et al. Isavuconazole versus caspofungin in the treatment of candidemia and other invasive Candida infections: the ACTIVE trial. Clin Infect Dis. 2019; 68(12):1981–9.
21. Samanta P, Clancy CJ, Marini RV, et al. Isavuconazole is as effective as and better tolerated than voriconazole for antifungal prophylaxis in lung transplant recipients. Clin Infect Dis. 2020; ciaa652. https://doi.org/10.1093/cid/ciaa652. Online ahead of print.
22. Taplitz RA, Kennedy EB, Bow EJ, et al. Antimicrobial prophylaxis for adult patients with cancer-related immunosuppression: ASCO and IDSA clinical practice guideline update. J Clin Oncol. 2018;36(30):3043–54.
23. Baden LR, Swaminathan S, Angarone M, et al. Prevention and treatment of cancer-related infections, version 2.2016, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2016;14(7):882–913.
24. Schwartz IS, Patterson TF. The emerging threat of antifungal resistance in transplant infectious diseases. Curr Infect Dis Rep. 2018;20(3):2.
25. Husain S, Camargo JF. Invasive aspergillosis in solid-organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13544.
26. Baker AW, Maziarz EK, Arnold CJ, et al. Invasive fungal infection after lung transplantation: epidemiology in the setting of antifungal prophylaxis. Clin Infect Dis. 2020;70(1):30–9.
27. Fontana L, Perlin DS, Zhao Y, et al. Isavuconazole prophylaxis in patients with hematologic malignancies and hematopoietic cell transplant recipients. Clin Infect Dis. 2020;70 (5):723–30.
28. Stern A, Su Y, Lee YJ, et al. A single-center, open-label trial of isavuconazole prophylaxis against invasive fungal infection in patients undergoing allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2020;26(6):1195–202.
29. Corzo-Leon DE, Satlin MJ, Soave R, et al. Epidemiology and outcomes of invasive fungal infections in allogeneic haematopoietic stem cell transplant recipients in the era of antifungal prophylaxis: a single-centre study with focus on emerging pathogens. Mycoses. 2015; 58(6):325–36.
30. Miller R, Assi M, Practice ASTIDCo. Endemic fungal infections in solid organ transplant recipients-guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13553.
31. Thompson GR 3rd, Rendon A, Ribeiro Dos Santos R, et al. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. Clin Infect Dis. 2016;63(3):356–62.
32. Subramanian AK, Theodoropoulos NM, Infectious Diseases Community of Practice of the American Society of T. Mycobacterium tuberculosis infections in solid organ transplantation: guidelines from the infectious diseases community of practice of the American Society of Transplantation. Clin Transpl. 2019;33:e13513.

33. Subramanian AK. Tuberculosis in solid organ transplant candidates and recipients: current and future challenges. Curr Opin Infect Dis. 2014;27(4):316–21.
34. Longworth SA, Daly JS, Practice ASTIDCo. Management of infections due to non-tuberculous mycobacteria in transplant recipients – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13588.
35. Perez AA, Singer JP, Schwartz BS, et al. Management and clinical outcomes after lung transplantation in patients with pre-transplant Mycobacterium abscessus infection: a single center experience. Transpl Infect Dis. 2019;21:e13084.
36. Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenzaa. Clin Infect Dis. 2019;68(6):895–902.
37. Manuel O, Estabrook M, American Society of Transplantation Infectious Diseases Community of P. RNA respiratory viral infections in solid organ transplant recipients: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13511.
38. Lee DH, Zuckerman RA, Practice ASTIDCo. Herpes simplex virus infections in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13526.
39. Ariza-Heredia EJ, Chemaly RF, Shahani LR, Jang Y, Champlin RE, Mulanovich VE. Delay of alternative antiviral therapy and poor outcomes of acyclovir-resistant herpes simplex virus infections in recipients of allogeneic stem cell transplant – a retrospective study. Transpl Int. 2018;31(6):639–48.
40. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13512.
41. Shmueli E, Or R, Shapira MY, et al. High rate of cytomegalovirus drug resistance among patients receiving preemptive antiviral treatment after haploidentical stem cell transplantation. J Infect Dis. 2014;209(4):557–61.
42. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med. 2017;377(25):2433–44.
43. Kotton CN. Updates on antiviral drugs for cytomegalovirus prevention and treatment. Curr Opin Organ Transplant. 2019;24(4):469–75.
44. Limaye AP, Green ML, Edmison BC, et al. Prospective assessment of cytomegalovirus immunity in high-risk donor-seropositive/recipient-seronegative liver transplant recipients receiving either preemptive therapy or antiviral prophylaxis. J Infect Dis. 2019;220:752–60.
45. Patel SJ, Knight RJ, Kuten SA, et al. Ciprofloxacin for BK viremia prophylaxis in kidney transplant recipients: results of a prospective, double-blind, randomized, placebo-controlled trial. Am J Transplant. 2019;19(6):1831–7.
46. La Hoz RM, Morris MI, Practice ASTIDCo. Intestinal parasites including Cryptosporidium, Cyclospora, Giardia, and microsporidia, *Entamoeba histolytica*, Strongyloides, schistosomiasis, and Echinococcus: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13618.
47. La Hoz RM, Morris MI, Infectious Diseases Community of Practice of the American Society of T. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, Babesia, Acanthamoeba, Balamuthia, and Naegleria in solid organ transplant recipients- guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13546.
48. Asberg A, Humar A, Rollag H, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant. 2007;7(9):2106–13.
49. Len O, Gavalda J, Aguado JM, et al. Valganciclovir as treatment for cytomegalovirus disease in solid organ transplant recipients. Clin Infect Dis. 2008;46(1):20–7.
50. Kotton CN, Kumar D, Caliendo AM, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018; 102(6):900–31.

9 Pretransplant Screening for Infection Exposure: Approach to the Patients with Epidemiologic and Geographic Risk Factors

Deepa Nanayakkara and Joanna Schaenman

Contents

Introduction 172
Bacterial Infections 172
Bacterial Pathogens of Special Interest 176
Mycobacterial Infections 177
Tuberculosis 177
Nontuberculous Mycobacteria 178
M. abscessus 178
Non-*M. abscessus* Non-*M. tuberculosis* Mycobacterial Infections 178
Fungi 179
Endemic Fungal Infections 179
Aspergillus and Other Molds 183
Parasitic Infections 184
Chagas Disease 184
Schistosomiasis 186
Strongyloidiasis 186
Leishmaniasis 186
Malaria 186
Toxoplasmosis 187
Viral Infections in the Transplant Candidate 187
Blood-borne Pathogens 187
Endemic Human Herpes Viruses 188
Viral Infections with Geographic Associations 189
Human Herpesvirus-8 (HHV-8) 189
HTLV-1 189

D. Nanayakkara
Division of Infectious Diseases, City of Hope, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
e-mail: dnanayakkara@coh.org

J. Schaenman (✉)
Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
e-mail: JSchaenman@mednet.ucla.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_9

Flaviviruses (Arboviruses) 190
West Nile Virus (WNV) 190
Zika, Chikungunya, and Dengue Viruses 191
Conclusion 191
KeyPoints 191
References 192

Introduction

Hematopoietic stem cell and solid organ transplantation is increasing throughout the world. With this increasing incidence of transplantation, the age and medical complexity of transplant candidates continue to increase. With this increased medical complexity comes increased social complexity, with immigration and global travel potentially increasing risk of acquiring infectious diseases. Transplant candidates or recipients may reside in or have migrated between geographic regions where different organisms are endemic. Further, the geographic distribution of pathogens can change over time. Pretransplant screening can identify infections that may disqualify the recipient as well as treat active or latent infections prior to undergoing transplantation (Tables 1 and 2). Also, recognizing potential risk factors can guide use of prophylactic treatment in the recipient, decreasing the risk of infection.

Approaches to pretransplant screening of infectious diseases as well as management of positive screening studies vary across transplant centers. A thorough medical history, including place of residence, travel history, occupation, exposure to animals and pets, hobbies, sexual practices, recreational drug use, and prior infections should be obtained from the transplant candidates [1]. The pretransplant period is an optimal time for providers to counsel candidates on vaccines and exposure risks associated with travel, outdoor hobbies, pets, and consumption of unpasteurized and raw foods. Moreover, it is best to initiate or complete treatment for latent infections prior to undergoing transplantation and starting immunosuppression.

Bacterial Infections

Pretransplant infection can influence the risk for post-transplant bacterial infection, especially for patients admitted to the hospital or with history of frequent hospitalizations while awaiting transplantation. It is therefore crucial to review this history including review of positive culture results to estimate post-transplant infection risk and target perioperative prophylaxis and empiric postoperative therapy. Each transplant type demonstrates a specific class of infectious syndromes that are common. For kidney transplant candidates with end-stage renal disease, history of urinary tract infection, or peritonitis associated with peritoneal dialysis, especially with resistant organisms, may be associated with post-transplant infection, including bacteremia [2]. Patients with anatomical issues including need for self-catheterization, bladder augmentation, or urostomy are also at increased risk for urinary tract infection after

Table 1 Pathogens that may infect transplant candidates and recipients with associated risk factors and management approaches

Pathogen	Risk factor	Approach
Bacterial infections		
Gram-positive and gram-negative infections	Indwelling devices, alterations in anatomy	Tailoring of antibiotic prophylaxis
Tuberculosis	Geographic exposure, incarceration	Prophylaxis with INH or rifampin
Nontuberculous mycobacterium	Cystic fibrosis, bronchiectasis	Pretransplant treatment if evidence of active infection, post-transplant treatment
Fungal infections		
Histoplasmosis	Geographic exposure: Mississippi and Ohio River valleys, Asia, and South America	Pretransplant treatment if evidence of active infection
Blastomycosis	Geographic exposure: Canada, US states bordering the Mississippi and Ohio Rivers	Pretransplant treatment if evidence of active infection
Coccidioidomycosis	Geographic exposure: Southwestern US, Mexico	Pretransplant treatment if evidence of active infection, post-transplant prophylaxis
Paracoccidioidomycosis	Geographic exposure: Latin America	Pretransplant treatment if evidence of active infection
Cryptococcosis	Bird exposure, immunosuppression, cirrhosis	Pretransplant treatment, consideration of post-transplant prophylaxis
Aspergillus and other molds	Hematologic malignancy, cystic fibrosis, immunosuppression, environmental exposure	Pretransplant treatment if evidence of active infection, post-transplant prophylaxis
Viral infections		
HIV	Sex, IVDU	Antiviral treatment
Hepatitis B	Sex, IVDU, geographic exposure	Antiviral treatment
Hepatitis C	Sex, IVDU, hemodialysis	Antiviral treatment
HHV-8	Geographic exposure (Africa, Brazil, Mediterranean, Caribbean), MSM	Post-transplant monitoring
West Nile virus	Mosquito exposure	Delay of transplantation until clinical syndrome resolved
Flavivirus (DENV, CHIKV, and ZIKV)	Geographic exposure, mosquito exposure	Delay of transplantation until clinical syndrome resolved
Parasites		
Chagas disease	Geographic exposure (Latin America)	Pretransplant treatment if evidence of active infection, post-transplant monitoring
Schistosomiasis	Geographic exposure (South America, Africa, and Asia)	Pretransplant treatment if evidence of active infection
Strongyloides	Geographic exposure (worldwide)	Pretransplant treatment

(continued)

Table 1 (continued)

Pathogen	Risk factor	Approach
Malaria	Geographic exposure (sub-Saharan Africa, Asia, and Latin America)	Pretransplant treatment if evidence of active infection, post-transplant monitoring
Leishmaniasis	Geographic exposure (Latin America, the Mediterranean, the Middle East, India)	Pretransplant treatment if evidence of active infection, post-transplant monitoring
Toxoplasmosis	Geographic exposure (worldwide)	Post-transplant monitoring

transplantation [3–5]. Liver and multivisceral transplant candidates may have peritonitis and other abdominal infection before transplant as well as associated bacteremias in the setting of prolonged need for intravascular catheterization which may potentially complicate the post-transplant course, especially when surgical complications occur [6–8]. For lung transplant candidates, a common issue is the presence of chronic colonization and recurrent infection with bacteria, especially for patients with cystic fibrosis. These may include *Staphylococcus aureus*, multi-drug resistant (MDR) *Pseudomonas*, *Acinetobacter*, or *Alcaligenes*, as well as mycobacterial infections (see below). These pathogens are frequently carried in the sinus cavities as well, and may subsequently infect the newly transplanted lung or lungs [9]. *Stenotrophomonas* infection was not associated with increased post-transplant mortality in a time-varying analysis in a cohort of patients with cystic fibrosis some of whom underwent lung transplantation; it is possible that negative relationships reported with *Stenotrophomonas* infection are due to association with severity of disease and history of extensive antibiotic use rather than direct pathogenic effect [10]. Other studies have also demonstrated no association with adverse outcomes in patients infected with *Stenotrophomonas* or *Achromobacter* [11].

For heart transplant candidates, a common pretransplant infection complicating transplantation is bacteremia after ventricular assist device (VAD) implantation, which can negatively impact patient survival [12]. However, there are data that VAD explantation can be curative of infection without significant impact on post-transplant course [13, 14], and this approach is suggested in both AST and ISHLT guidelines, albeit with expert infectious diseases input to guide management [15, 16]. Debridement before or during transplantation is useful to prevent infection recurrence and to obtain material for cultures that may guide treatment [15]. Bone involvement at the sternum, however, may not be cured by simple explantation and should prompt a strategy for debridement to ensure control of infection after immunosuppression starts. Review of history of previous infections including urinary tract infection, pneumonia, or blood stream infection is especially important for patients undergoing repeat transplantation or with prolonged hospitalization while awaiting transplantation. Dental evaluation with intervention for potentially problematic areas is ideal if it is medically safe to pursue in transplant candidates. Dental evaluation is

Table 2 Risk factors for post-transplant infection

Risk type	Risk details	Potential pathogens	Approach
Pretransplant immunosuppression	Receipt of prednisone, MMF	Bacteria, fungi	Review of infection history, treatment if active infection
Cystic fibrosis	Bronchiectatic lung leading to pulmonary colonization	MDR organisms, MRSA, NTM, aspergillus	Design of perioperative prophylaxis, treatment if active infection
Sexual history	Multiple sexual partners	STDs including syphilis, HIV, hepatitis	Vaccination, repeat screening while awaiting transplantation
Recreational drug use	IVDU, cannabis smoking or vaping	Intravascular infection, aspergillus and other molds	Review of infection history, treatment if active infection
Pets	Birds (parrots or pigeons), cats, reptiles	Cryptococcus, bartonella, Q fever, salmonella, campylobacter, toxoplasma	Review of infection history, treatment if active infection
Hobbies	Camping, spelunking, motorbiking, fresh water exposure	Cryptococcus, cocccidioides, Histoplasma, mold infection	Screening and prophylaxis if indicated
Food exposure	Unpasteurized dairy products or juices, raw foods	Mycobacterial infection, toxoplasma, norovirus	Review of infection history, treatment if active infection
Occupations			
Construction, landscaping, farming	Exposure to dust and soil	Coccidioides, aspergillus	Screening by serology or antigen testing
Work with animals	Potential for animal-borne infections	Cryptococcus, bartonella, Q fever, salmonella, campylobacter, toxoplasma	Screening if evidence of active infection
Health care worker	Risk for exposure to patients	TB	Screening and prophylaxis if indicated
Geographic exposures			
Mediterranean		HHV-8, HTLV	Screening
Caribbean		HHV-8, HTLV, strongyloides, TB	Screening
Asia		HTLV, TB, strongyloides, schistosoma, malaria	Screening

(continued)

Table 2 (continued)

Risk type	Risk details	Potential pathogens	Approach
Middle East		TB, strongyloides, schistosoma	Screening
Africa		TB, strongyloides, malaria	Screening
Latin America		TB, strongyloides, schistosoma, malaria	Screening
Southwest US/Mexico		Coccidioides	Screening

also important for hematopoietic stem cell transplant (HSCT) candidates where a common source of bacterial infection after immunosuppression is the mouth [17].

For HSCT candidates, history of previous myelosuppressive chemotherapy and accompanying hospitalization can lead to bacterial colonization with resistant organisms, which may cause recurrent infection [18].

MDR organisms are of special importance as the standard perioperative and post-transplant antibiotic regimens may not be sufficient. This is true for extended-spectrum beta-lactamase (ESBL) producing gram-negative infections, but is a particular problem with carbapenem-resistant *Enterobacteriaceae* (CRE). Epidemiologic risk factors for ESBL infection include previous exposure to broad-spectrum antibiotics, stay at a long-term care facility, or ICU stay [19, 20]. Risk factors for CRE are more geographic, with increased rates of colonization and infection in endemic areas, with studies performed in high prevalence areas such as New York City and Pittsburgh in the USA [21, 22]. History or positive testing demonstrating colonization with CRE increases risk for post-transplant infection up to fivefold [23, 24]. Studies of patients colonized with CRE have demonstrated that post-transplant infections are more common, with potential adverse impact on clinical outcomes [25]. However, interventions such as routine screening or attempts at decolonization have not proven to be effective in improving clinical outcomes and are not recommended at this time [26, 27].

Bacterial Pathogens of Special Interest

Burkholderiacepacia complex colonization and infection may be seen in patients with cystic fibrosis, and is associated with death or need for transplantation [28]. Although sometimes viewed as a contraindication to transplantation, ISHLT guidelines state that if sufficiently treated before transplant and if there is reasonable expectation for post-transplant control of infection, patients would be acceptable if transplanted at an experienced center [29]. However, within the species and subspecies of *Burkholderia*, there is data demonstrating that *B. cenocepacia*, genomovar III, is associated with a high risk of poor outcomes including possible mediastinitis and chest wall infections after transplantation, with higher rates of post-transplant infection and death compared with *B. cepacia, multivorans, stabilis,* and other

non-*cenocepacia Burkholderia* species [30–33]. In a meta-analysis, pretransplant colonization with *B. cenocepacia* complex was one of the few variables associated with poor outcomes after transplantation, while *Pseudomonas aeruginosa* colonization was not [34]. In contrast to the non-*cenocepacia* strains described above, *B. gladioli* is a *Burkholderia* species that is rare, but also associated with poor outcomes after transplantation [32]. However, several single-center reports of lung transplant recipients with non-*cenocepacia Burkholderia* demonstrate excellent clinical outcomes [31, 35, 36]. This demonstrates that the speciation and complete identification of *Burkholderia* subspecies is crucial for transplant evaluation, with *B. cenocepacia* (genomovar III) as a relative contraindication though other subspecies of *Burkholderia* are less concerning. However, if new antimicrobial approaches for resistant species emerge, this approach may need to be reevaluated.

Another important bacterial infection with potential negative impact post-transplant is syphilis. For this reason, it is recommended by the AST and UNOS/OPTN that all transplant candidates undergo screening with RPR testing, with treponemal-specific testing for confirmation when positive [1]. Transplant candidates with evidence of positive testing for syphilis should undergo further evaluation for neurologic involvement if indicated. For those with late latent disease without evidence of neurosyphilis, three once weekly treatments of 2.4 million units of benzathine penicillin should be administered prior to transplantation to prevent disease progression when immunosuppression is introduced.

Mycobacterial Infections

Tuberculosis

All transplant candidates should be screened for active and latent tuberculosis (TB). A careful history of prior exposure to tuberculosis should include details about prior tuberculin skin test (TST) and interferon gamma release assay (IGRA) testing results, exposure to individuals with active TB in the household or workplace including healthcare workers, prior residence or travel to areas highly endemic for TB, and history of homelessness or incarceration [37]. Any history of active or latent TB and details regarding length and type of treatment should be obtained and documented.

A TST can be used for screening with a test considered positive if there is $\geq$5 mm of induration at 48–72 h [38]. Experts recommend a second skin test 2 weeks later in patients with negative reactions if feasible, as the TST can convert from being falsely negative to positive due to a "boosting effect" in some with remote *Mycobacterium tuberculosis* (MTB) exposure [37, 39]. A history of Bacillus Calmette–Guerin (BCG) vaccination should not influence the screening for latent TB in transplant candidates. However, interferon gamma release assay (IGRA) testing is preferred to TST in this case as IGRA results will not be impacted by prior receipt of BCG. Furthermore, IGRA testing is reported to be more sensitive than TST in detecting LTBI in transplant candidates with advanced liver disease or end-stage renal disease

[39, 40]. A current negative screening test in patients awaiting organ transplantation does not refute a prior positive test. If TST and IGRA testing is negative or indeterminate, it is recommended that all transplant candidates also undergo screening with a chest X-ray. A chest computed tomography (CT) scan may be warranted in those with epidemiologic risk for latent or asymptomatic active TB to evaluate for granulomatous disease prior to immunosuppression start [41]. This recommendation applies to both solid organ transplant (SOT) and HSCT candidates.

Nontuberculous Mycobacteria

Evidence of colonization or infection with nontuberculous mycobacteria (NTM) can pose a special challenge prior to transplantation, especially for lung transplant candidates. These organisms are found in a wide range of environmental sources including soil, water, and plants, and may cause both colonization and invasive disease, complicating evaluation of positive respiratory cultures in transplant candidates [42–44]. Positive cultures pretransplant are associated with post-transplant positive cultures or clinical disease, even after bilateral lung transplant, suggesting that intraoperative contamination or the presence of lymphatic reservoirs are sources of reinfection [45, 46].

M. abscessus

Although a range of NTM have been associated with pre- and post-transplant infection, it is *M. abscessus* that is associated with the greatest incidence of invasive disease after transplantation including wound infection and pulmonary disease, and therefore isolation of resistant isolates has been considered to be a relative contraindication to transplantation, especially in lung transplant candidates with cystic fibrosis [29, 46, 47]. In a survey of practices in lung transplant centers worldwide, 76% considered *M. abscessus* infection to be a relative contraindication and 19% reported considering it to be in itself a contraindication to transplantation [48]. Reviews from experienced transplant centers demonstrate rates of patient and graft survival post-transplant that are comparable to patients without *M. abscessus* infection, albeit in carefully selected transplant candidates [49–52]. Diagnostic testing is important, including extensive antibiotic susceptibility testing, determination of inducible macrolide resistance, and identification to the subspecies level [46]. Experts generally recommend that patients be treated until asymptomatic and until smear negative and ideally culture negative pretransplant, and for a minimum of 6 weeks after transplantation [46].

Non-*M. abscessus* Non-*M. tuberculosis* Mycobacterial Infections

M. avium complex, *M. kansaii*, and *M. fortuitum*, and other NTM can also cause pretransplant infection that may cause post-transplant disease, especially in lung transplant candidates with cystic fibrosis or bronchiectasis. In a multicenter study of patients with cystic fibrosis, the prevalence of NTM infection was 13%, with

M. avium complex as the most prevalent species (72%) [53]. Many authors suggest that the prevalence of NTM infections are increasing over time [45, 54], which has been suggested as possibly due to global warming, although increased reporting and improved recovery methods are other possible explanations [43]. Leukopenia, immunosuppression, cystic fibrosis, and bronchiectasis are risk factors for development of infection [45, 47], so positive cultures from transplant candidates with these characteristics should be evaluated carefully for possible evidence of invasive disease, especially when accompanied by clinical symptoms of radiographic changes consistent with invasive disease. Geographic patterns of disease have also been identified, with *M. simiae* and *M. abscessus* more common than *M. avium* complex in Israel and presumably elsewhere in the Middle East [55], while in the UK, *M. abscessus* was the most common followed by *M. avium* complex [56], while inTaiwan, Korea, and Japan, *M. avium* complex is the most common isolate [57–60]. In terms of individual behavior, one case-control multicenter study in the USA identified residing in a single family house, the rusty or unclear appearance of tap water, and swimming in indoor pools to be associated with risk for NTM in patients with cystic fibrosis [61, 62]. In addition, prevalence of NTM infection has been shown to vary geographically within the USA, with increased rate of isolation in the southeastern USA and Hawaii, with a significant association with increased vapor pressure, a measure of moisture content of the air [61]. Some experts have recommended increasing the temperature of hot water heaters in homes and avoiding indoor swimming pools to decrease NTM risk.

Fungi

Endemic Fungal Infections

In North America, endemic fungal infections include coccidioidomycosis, blastomycosis, and histoplasmosis, while paracoccidioidomycosis is seen in South America. Endemic mycosis can present atypically and cause disseminated and severe disease in transplant recipients as compared to immunocompetent individuals, which stresses the importance of pretransplant screening [63]. Undergoing transplantation without adequate control of fungal infections increases morbidity and mortality post-transplant. The latest American Society of Transplantation (AST) guidelines recommend that a pretransplant candidate with evidence of invasive fungal infection should be treated until there is clinical, radiographic, and microbiologic resolution [1]. Endemic mycoses maybe difficult to diagnose as infection may be dormant. Therefore, obtaining a thorough history about travel, occupational exposures, pets, and outdoor hobbies/activities is important during the pretransplant screening process.

Histoplasmosis

The incidence of histoplasmosis post-SOT and HSCT is uncommon ($\leq$1%) but infection can cause severe, often disseminated, life-threatening disease [64]. In

transplant recipients, histoplasmosis may result from reactivation of latent infection. There are two subspecies that cause illness in humans – *Histoplasma capsulatum,* which has a global distribution, and *H. capsulatum duboisii*, which is found in Africa [65]. In the USA, histoplasmosis is highly endemic in the Mississippi and Ohio River valleys, namely the "Histo Belt." More than half of the population residing in the Histo Belt are exposed to *H. capsulatum*. *Histoplasma* infection in North America can also be found in patients originally from Asia and South America [66]. Routine pretransplant screening of transplant candidates is currently not recommended even in endemic areas, because of the low incidence of post-transplant histoplasmosis [63]. Incidental findings of calcified pulmonary, hilar, and splenic granuloma in radiographs can be consistent with prior *H. capsulatum* infection but do not warrant post-transplant prophylaxis in patients who are otherwise asymptomatic.

Blastomycosis

The incidence of blastomycosis in transplant recipients is rare in comparison to histoplasmosis and coccidioidomycosis [64, 67]. In North America, blastomycosis is endemic in the states bordering the Mississippi and Ohio Rivers, the Midwestern states, and extending into Kentucky, Arkansas, Tennessee, Louisiana, and the Canadian provinces that border the Great Lakes. It has also been reported in New York and Canada along the St. Lawrence River [68]. The distribution of *Blastomyces* may extend beyond the previously described geographic areas, as reporting of cases is voluntary in most states and is only required in six states. Most patients diagnosed with blastomycosis have a history of exposure to soil, wooded areas, lakes, or rivers, however, the exact natural habitat of *Blastomyces* has been difficult to identify. Routine pretransplant screening for blastomycosis is currently not recommended but should be considered in transplant candidates from endemic areas with lung and skin lesions of unknown etiology [69].

Paracoccidioidomycosis

Paracoccidioidomycosis is endemic to Latin America and typically affects men who work in agriculture [70]. Approximately 80% of cases of paracoccidioidomycosis have been identified in Brazil, particularly in the states of Sao Paulo, Parana, Rio Grande do Sul, Goias, Rio De Janeiro, and Rondonia [71]. Cases have also been identified in Venezuela, Ecuador, Columbia, and Argentina. Routine pretransplant screening is not recommended except in those from endemic areas with pulmonary findings and skin lesions of unclear etiology.

Coccidioidomycosis

Coccidioides spp. exhibit specific areas of geographic endemicity, with *Coccidioides immitis* found in California, especially the California Central Valley or San Joaquin Valley extending to southern California and Mexico, while *Coccidioides posadasii* is present in Arizona, New Mexico, Texas, Mexico, and South America, although the

clinical disease caused by these two organisms is similar [72, 73]. Recent reports of *C. immitis* in Northern California, Washington, and Utah suggest the possibility of spread of the current endemic region [74, 75], possibly related to global warming. Spores are stable in the environment for long periods of time, and when inhaled transform into spherules which replicate and then can disseminate throughout the body [76]. Risk factors for coccidioidomycosis infection include Filipino or African American ancestry, exposure in endemic areas via occupations such as landscaping, fieldwork, or construction, and history of immunocompromise including pregnancy and diabetes mellitus, and there are multiple reports suggesting that this incidence is increasing in both California and Arizona [76–78]. An important aspect of pretransplant evaluation is that infection may be completely asymptomatic, and that although pulmonary manifestations are the most common, the disease can also cause extrapulmonary manifestations including meningitis, osteomyelitis or joint infection, and cutaneous disease [79].

Patients with history of symptomatic infection will often recall subacute presentation with fatigue and cough followed by fever and shortness of breath, leading to hospitalization and improvement in symptoms with initiation of antifungal therapy. Although eosinophilia may be seen during acute presentations of infection, it is generally not seen after treatment of disease and is not useful for patient screening. Pretransplant screening for patients from endemic areas or with history of travel to or through endemic areas should include *Coccidioides* IgM and IgG antibody testing, typically using EIA testing [63]. Positive EIA test results should be investigated further with immunodiffusion (ID) and complement fixation (CF) testing which have higher specificity to confirm the diagnosis [80]. CF is also useful as a surrogate for disease activity, with higher titers associated with increased risk for disseminated disease. The role of *Coccidioides* antigen testing in screening of asymptomatic patients is unknown, although this test has demonstrated excellent sensitivity and specificity in evaluation of patients with CNS disease [81].

Pretransplant evaluation of candidates generally falls into one of three groups: Patients with personal history of coccidioidomycosis, patients with no symptoms or known history who present with positive screening tests, and patients living in an endemic area. For patients with a history of infection, outside records should be obtained as possible to determine the anatomic sites involved as well as the severity of the disease. Length and type of treatment should be documented as possible. Based on the site of disease involvement, radiographic testing should be performed to confirm resolution of disease and establish a baseline prior to transplantation [77]. For example, a patient with a history of pulmonary disease should have repeat noncontrast chest CT performed to evaluate for evidence of active infection. The presence of thick- or thin-walled cavitary lesions are concerning for active infection, or possibly progression with start of immunosuppression [82]. Calcified nodules, in contrast, especially if demonstrated to be stable over time, are less likely to progress. For patients with large lung lesions or other indicators of active disease, it may be reasonable to await transplantation after the start of azole therapy and demonstrated radiographic response.

Patients with positive laboratory testing in the absence of symptoms are often difficult to interpret. Positive EIA testing even in the absence of confirmatory testing by ID or CF should be evaluated carefully, especially if the patient is from an endemic area or has history of possible occupational exposure. Rates of IgM or IgG EIA positivity in asymptomatic patients from endemic areas can range from 1% to 4% [83]. Additional radiographic testing should be performed, starting with noncontrast chest CT to evaluate for presence of large nodules or cavitary lesions with potential for progression, as described above. PET scan should be considered for infection localization in patients with high pretest probability by laboratory testing but negative chest CT given the possibility of bone or joint disease [79]. As above, the decision should then be made as to whether to begin antifungal therapy and possibly delay transplantation if feasible, or to start antifungal prophylaxis at the time of transplantation.

The third group of potential transplant candidates needing evaluation are patients with no history of coccidioidomycosis, and negative serology testing, but with plans for ongoing residence in an endemic area. Blair et al. have recommended 12 months of therapy in this setting for kidney or liver transplant recipients in Arizona, while others have recommended longer courses, especially for lung transplant recipients [77, 84, 85]. The length of optimal prophylaxis therapy is unknown, but the length of prophylaxis should depend on risk for reactivation of coccidioidomycosis, with consideration for indefinite therapy in some cases [63].

Cryptococcosis

Although cryptococcal disease is generally thought of as primarily a post-transplant complication, pretransplant infection can also pose problems, especially in liver transplant candidates with cirrhosis. In a multicenter study conducted through the AST Infectious Diseases Community of Practice, 112 patients with cirrhosis and end-stage liver disease with cryptococcosis awaiting liver transplantation were identified, primarily diagnosed by evidence of cryptococcal fungemia on blood culture rather than cryptococcal serum antigen testing; CNS, pulmonary, and peritoneal disease were described [86]. The 90-day mortality rate was 57%, with over half of deaths attributed to cryptococcal disease. The eight patients who survived to transplantation were not delisted due to infection; all patients survived post-transplant with prolonged antifungal therapy. Therefore, although the high mortality rate should be taken into account in evaluation of pretransplant patients with cryptococcosis, especially disseminated disease, this diagnosis should not be viewed as an absolute contraindication to transplantation with effective antifungal therapy and continuation after transplantation for up to 12 months [86, 87]. The mechanism of vulnerability to infection and specifically peritonitis in patients with cirrhosis may be related to complement deficiency and macrophage impairment in cirrhotic ascites [88], in addition to the general immune dysfunction observed in patients with cirrhosis. Risk factors for fungal peritonitis include severity of liver disease and prolonged hospitalization [89]. In a case control study in Taiwan, HIV, chronic liver disease, and receipt of inhibitors of cell-mediated immunity were all found to significantly increase the risk of cryptococcal infection; however, diabetes mellitus

was not found to be a risk factor [90]. Reported close contact with pigeons did demonstrate an association in the single variable but not the multivariate analysis, possibly because of the low number of cases reporting this exposure.

While *Cryptococcus neoformans* is known to be associated with bird droppings but has no specific geographic association, *Cryptococcus gattii* is endemic to eucalyptus growing and other environmental regions in Australia, parts of Africa, the Mediterranean, India, Southeast Asia, Mexico, and Brazil, and has now extended to British Columbia and the US Pacific Northwest [91, 92]. Outdoor activities that may disturb the soil or involve logging or cutting trees may lead to dispersion of *C. gattii* in the air, increasing risk for fungal exposure [93]. However, given the difficulty of establishing clear risk factors for cryptococcal disease in asymptomatic transplant candidates, routine screening for all patients by cryptococcal serum antigen is not recommended [94]. Optimal management of asymptomatic patients with an isolated serum test positive for *Cryptococcus* is unknown, but antifungal treatment or prophylaxis should be considered [93]. Patients with a history of cryptococcal infection should receive at least several months of antifungal therapy and have no ongoing signs of symptoms of cryptococcal disease, ideally with negative culture results prior to transplant. There is no data supporting ongoing monitoring or surveillance with cryptococcal serum antigen testing after clinical resolution of disease [94]. Identification and adequate treatment is important as there is evidence of reactivation of latent infection after transplantation in seropositive patients, presumably due to release of dormant organisms present within a granuloma, generally with earlier post-transplant presentation of disease [95, 96].

Aspergillus and Other Molds

Although unusual as a pretransplant infection, transplant candidates may demonstrate positive cultures, especially respiratory cultures, for *Aspergillus*. There is some evidence from animal models and clinical experience suggesting that *Aspergillus* and other molds including agents of mucormycosis may reactivate after a period of dormancy without clinical symptoms [96]. Pretransplant colonization with *Aspergillus* has long been described as a risk factor for post-lung transplant invasive pulmonary aspergillosis as well as tracheobronchitis [97, 98]. This is a special problem in patients with cystic fibrosis who are often colonized with fungi pretransplantation at an incidence reported as ranging between 8% and 59%, with the predominant type identified as *Aspergillus* sp., although *Scedosporium* sp. and dematiaceous molds have also been described [97, 99]. Other than cystic fibrosis, lung transplant candidates receiving immunosuppression and patients with extensive environmental exposure are also at risk. For transplant programs performing targeted rather than universal prophylaxis after lung transplantation, the isolation of fungi from respiratory cultures prior to transplantation is important data used to direct azole start at the time of transplantation [100]. However, there is not clear evidence supporting antifungal therapy prior to lung transplantation in patients with positive respiratory cultures without evidence of invasive disease. The literature evaluating

impact of positive pulmonary cultures in patients with cystic fibrosis is inconsistent with some studies showing association with decline in pulmonary function and other studies showing no such association [101]. Therefore, many experts recommend only treating patients with cystic fibrosis who meet a certain criteria for symptomatic bronchitis, including failure of symptoms to respond to antibacterial therapy [102]. One mold with geographic association to exposure is *Penicillium marneffei*, which is endemic to Southeast Asia and Northeast Australia [103].

Isolation of mold in pretransplant candidates can guide post-transplant prophylaxis. HSCT candidates should have their medical records reviewed for a history of mold infection during previous chemotherapy, especially patients with a history of prolonged neutropenia. A history of invasive fungal infection significantly increases infection risk post-HSCT [104, 105]. Patients with a history of mold infection should receive secondary prophylaxis with a mold-active antifungal agent. Noncontrast chest CT should also be considered as a screen for previous fungal infection. A study of pretransplant evaluation found that 22% of HSCT candidates had abnormal findings on chest CT, which were primarily lung infiltrates or nodules [106]. Similarly, a history of deep-seated invasive *Candida* disease should also prompt consideration for extended antifungal prophylaxis.

Parasitic Infections

Parasitic infections often have strong geographic associations, emphasizing the importance of obtaining a complete history of birthplace, places of residence, and travel history (Fig. 1).

Chagas Disease

Chagas disease is endemic in Latin America, where 6–seven million people are infected [107]. Transplant candidates with chronic Chagas disease can reactivate the disease in the context of immunosuppression; the risk is highest in the first year after transplantation and subsequently with intensification of immunosuppression. Pretransplant screening in candidates who have resided in or traveled to endemic areas should include information about prolonged stays, residence in rural areas or thatched huts, or low socioeconomic status [103, 108].

Chronic Chagas disease is defined by having a positive epidemiology and serology, using at least two serological methods [107]. Conventional serological techniques are enzyme-linked immunosorbent assay, indirect immunofluorescence, and indirect hemagglutination. In case of inconclusive serology or discordant results, a third test is required. Inconclusive serologic results can be confirmed by polymerase chain reaction (PCR), which is able to detect low-level parasitemia. This screening recommendation applies to both SOT and HSCT candidates, who are also at risk for *T. cruzi* reactivation after immunosuppression. PCR should not be

Fig. 1 Geographic distribution of major parasitic infections seen in transplant recipients

used as a screening test in chronic patients, however, because its sensitivity ranges between 40% and 95% in the chronic phase due to intermittent parasitemia [107].

Schistosomiasis

Schistosomiasis should be considered in any transplant candidate who has resided or traveled to endemic areas with significant freshwater contact [107]. Geographic areas with schistosomiasis include South America, the Caribbean, Africa, the Middle East, and parts of Asia. Transplant candidates from endemic areas should be screened by serologic testing followed by stool examination for those with positive test results [109]. Patients with evidence of active infection can be treated with a single dose of praziquantel prior to transplantation. Serology assays offer high sensitivity for screening, however, antibodies remain detectable for a long period after infection and serology tests do not differentiate between current and past infections [107, 109].

Strongyloidiasis

Strongyloidiasis is an underdiagnosed disease and is endemic in Latin America, including Brazil, Peru, Venezuela, Argentina, and Ecuador, as well as Africa, Asia, Australia, and other areas with inadequate sanitary conditions [107, 108, 110]. Most infected patients are asymptomatic, however immunocompromised patients are at risk of life-threatening disseminated disease in the setting of immunosuppression. Patients who have emigrated from or lived in an endemic area should be screened by serologic testing. As the infection can last for decades, there is no time limit on how long it has been since exposure [107]. Screening in endemic areas should include evaluation for eosinophilia and at least 1 stool specimen for ova and parasites [103]. In nonendemic settings, serology can be used for screening [108]. Candidates testing positive should receive ivermectin prior to SOT or HSCT [107].

Leishmaniasis

Leishmaniasis is a disease transmitted through the bite of infected sandflies, and is predominantly found in tropical and Mediterranean regions. It is a rare disease among transplant recipients [111]. Manifestations may be cutaneous, mucocutaneous, or visceral. Diagnosis is via serology or parasite testing from affected lesions. Patients with active lesions should be treated, while those with a history of infection should be monitored closely for reactivation after initiation of immunosuppression [111, 112].

Malaria

Malaria should be considered in any transplant candidate who has resided or traveled to malaria-endemic areas, which include the tropical areas of sub-Saharan Africa,

Asia, Oceania, and Latin America [108]. Transplant candidates should be screened by epidemiologic history and laboratory testing and treated if there is evidence of active infection [112]. Due to reported cases of recrudescence after transplantation, infected recipients should be monitored clinically and via laboratory testing, ideally PCR, after transplantation [107].

Toxoplasmosis

Toxoplasmosis is a common parasitic infection worldwide, which can be acquired via ingestion of undercooked meat or unwashed vegetables that have been contaminated with oocysts or through contact with the feces of infected cats. Serologic screening for evidence of previous exposure should be performed in all transplant candidates, especially heart transplant and HSCT candidates [112]. Although reactivation of previous infection may occur after the initiation of immunosuppression, serologic testing is also important to identify seronegative patients who are at increased risk of donor-derived infection after receiving organs from seropositive donors, with the highest risk in heart transplant recipients since the parasite encysts in muscle including cardiac muscle [113]. Primary infection of seronegative individuals is another cause of post-transplant infection. Fortunately, the standard trimethoprim-sulfamethoxazole prophylaxis used for PCP prevention has been shown to be effective in preventing reactivation of toxoplasmosis, even in the highest risk situation, donor positive and recipient negative heart transplantation [114, 115]. Reactivation of toxoplasmosis has also been described after HSCT, so antibody testing is useful for patient risk stratification in HSCT candidates [116]. Seropositive HSCT recipients with seronegative stem cell donors are at increased risk.

Viral Infections in the Transplant Candidate

Blood-borne Pathogens

UNOS/OPTN guidelines recommend testing for HIV, hepatitis B (HBV), and hepatitis C (HCV) in all SOT candidates regardless of risk factors. Although specific epidemiologic and geographic evaluation is not necessary to screen for these relatively common infections, it is helpful to be aware of patient risk factors such as sexual activity and intravenous drug use that may increase the risk of infection. Similarly, HSCT candidates should also be screened for HIV, HBV, and HCV [117].

The approach to candidates with HIV is covered elsewhere in this volume, however, it is important to note that pretransplant evaluation of transplant candidates with HIV should include a review of antiretroviral therapy. Use of protease inhibitors and cobiscistat should be avoided whenever possible due to drug–drug interactions with calcineurin inhibitors and other medications. Kidney and liver transplantation in patients with HIV has become increasingly common and is associated with

excellent graft and patient outcomes [118–120]. HIV+ recipients have successfully undergone successful heart and lung transplantation without increased rates of infection, malignancy, or rejection [121–123].

Vaccination of patients who are nonimmune for hepatitis B is an important pretransplantation intervention. The new HBV vaccine Heplisav-B (HepB-CpG) has demonstrated improved vaccination response compared with Engerix [124]. Hepatitis B infection is more common in patients from endemic areas such as Africa, the Middle East, Asia, and Latin America. Patients with active or chronic HBV infection should be treated prior to transplantation according to AASLD guidelines [125].

The general practice for HCV+ abdominal organ transplant candidates has been to withhold treatment for HCV until after transplantation to facilitate receipt of an HCV positive organ; however, a recent retrospective study suggested that control of HCV viremia prior to kidney transplantation was associated with improved outcomes [126]. Candidates for thoracic organ transplantation are generally treated prior to transplantation to minimize adverse outcomes in the setting of generally higher levels of immunosuppression.

Similarly, HSCT candidates should be screened for HBV, HCV, and HIV. Those who test positive for HBV surface antigen or core antibody should receive third-generation antiviral prophylaxis after HSCT. As for SOT candidates, HSCT candidates with active disease should be treated prior to transplantation if possible, and should receive vaccination when feasible if HBV is naive to prevent HBV reactivation after immunosuppression or transmission of disease from donor products [127].

All transplant candidates may benefit from screening for hepatitis A IgG antibody. Patients who are IgG negative should be offered pretransplant vaccination if feasible prior to transplant. Hepatitis A/B combination vaccines can be used in patients who also require hepatitis B immunization.

Cases of hepatitis E virus (HEV) infection have been described in transplant recipients, either as donor-derived infection or as progression of latent infection present prior to transplantation. The presence of infection can be detected in asymptomatic patients using either HEV antigen or PCR testing [128]. Although routine screening is not currently recommended, it should be considered in transplant patients residing in or recently emigrated from the developing world [129].

Endemic Human Herpes Viruses

All HSCT candidates should be tested for herpes simplex virus (HSV) exposure prior to transplantation to identify patients at risk for HSV reactivation [17]. In addition, patient history should be reviewed for history of recurrent or resistant HSV infection in order to guide length and type of prophylaxis. Similarly, varicella zoster virus (VZV) IgG testing should be performed to determine if patients lack protective antibody and therefore need to take special precautions to avoid VZV exposure after transplant. Similarly, cytomegalovirus (CMV) and Epstein-Barr virus (EBV)

antibody status should be evaluated prior to HSCT or SOT to guide post-transplant prophylaxis.

Viral Infections with Geographic Associations

Human Herpesvirus-8 (HHV-8)

HHV-8 exhibits wide variation in incidence by geographic location, with the highest incidence in Africa and the Brazilian Amazon (>50%), followed by the Mediterranean, Eastern Europe, the Caribbean, and the Middle East (5–20% seroprevalence), followed by North American and Asia (<5%) [130]. Rates can also vary within different regions of a single country, with one study demonstrating a two- to threefold difference in incidence in liver transplant recipients depending on geographic region within Spain [131]. HHV-8 is associated with a variety of disease syndromes including Kaposi sarcoma, multicentric Castleman disease, and primary effusion lymphoma.

Increased incidence in men having sex with men (MSM) has been reported in both endemic and nonendemic countries, and it is thought to spread as a sexually transmitted disease given the association with increased rates of other sexually transmitted infections and high-risk sexual behavior as well as multiple partners [130, 132]. No association has been demonstrated with IV drug use, however. Studies of pretransplant rates in liver and kidney candidates in Italy demonstrated a prevalence of 16%, with an additional 11% of patients seroconverting after transplantation [133]. Although development of Kaposi sarcoma is classically associated with HIV, one study from France did not show a significant increase in incidence in liver and kidney transplant recipients with HIV compared with transplant patients without HIV, and disease presentation was not more severe in transplant recipients with HIV [134].

HHV-8 can reactivate with start of immunosuppression in patients previously infected, which has also been described as a donor-derived infection [135]. There are no clear guidelines regarding screening of transplant candidates, and recommendations are complicated by the lack of universal standards for serology or PCR-based laboratory diagnosis [135]. Given that proven prophylaxis regimens in SOT recipients have not been established, the recommendation for monitoring of positive patients after transplantation would primarily be clinical assessment; the role of screening PCR testing for surveillance has not been proven, in part given that an appropriate cutoff for clinically significant positive testing is unknown.

HTLV-1

HTLV-1 is a retrovirus which is also thought to be spread through sexual intercourse, although it can be transmitted from mother to child as well as by blood transfusion or IV drug use [136, 137]. Seroprevalence varies geographically, with highest

seroprevalence in Japan (up to 10%), especially southern Japan, the Caribbean (3–6%), and southern Africa (1–5%), as well as lower levels of incidence in the Middle East, Pacific Oceania, and South America [136, 138]. Pockets of increased incidence may also be seen in regions with concentrations of immigrants from endemic areas. Interestingly, in many countries, prevalence rates have been shown to be higher in hemodialysis patients or in patients receiving transfusions than in healthy controls [137]. Studies of transplant candidates in endemic areas such as Brazil and Japan have demonstrated incidence similar to the general population in that area [136]. The major disease manifestations are adult T cell leukemia or lymphoma and HTLV-1-associated myelopathy, and it does appear that immunosuppression after transplantation is associated with progression of HTLV-1-associated disease in infected transplant recipients, although the typical time to disease is not well described and the overall survival does not differ between infected and uninfected patients [139]. Although official screening recommendations are lacking, it would be reasonable to check HTLV-1 serology on transplant candidates from highly endemic regions, especially if they have been on hemodialysis. Confirmatory testing should be performed with Western blot or NAT testing, as false positive results can occur with ELISA [139]. The role of antiviral therapy to prevent primary infection or reactivation of disease after transplantation is unknown.

Flaviviruses (Arboviruses)

West Nile Virus (WNV)

WNV is a mosquito-borne flavivirus which can cause a wide range of clinical manifestations from mild disease to meningoencephalitis and death [140]. The geographic zone of involvement is large, including Asia, Africa, the Middle East, Europe, the Caribbean, North America, and Latin America, with seasonal activity in temperate zones when levels of infection in the bird reservoir are highest, typically peaking in July through November. Although OPTN/UNOS recommends screening for donors in endemic areas during the season where West Nile virus causes infection, no guidance exists for transplant candidates [1, 141]. Given that within endemic areas rates of seropositivity indicating previous exposure to infection are high, there is likely to be little benefit to testing in an asymptomatic transplant candidate. WNV is generally of more concern as a potential donor-derived infection in endemic areas, as even asymptomatic donors can transmit disease. NAT testing is more specific than serology testing, but may give false negative results depending on the stage of infection [141]. Therefore, only symptomatic patients should be tested as part of a more general diagnostic evaluation for causes of fever or meningoencephalitis; local disease activity and increased mosquito exposure increases risk of infection.

Zika, Chikungunya, and Dengue Viruses

Zika virus (ZIKV) is another viral infection transmitted by mosquitoes that has raised concerns regarding the possibility of donor-derived infection. Other methods of transmission are mother to child, sexual, and via blood transfusion [142]. Infection ranges in severity from mild or asymptomatic to meningoencephalitis or Guillain-Barre syndrome, and notable microcephaly in newborns. Chikungunya (CHIKV) is also a mosquito-borne virus, which has caused disease in Africa and Asia, and now has been found in Latin America [143]. Although able to cause meningoencephalititis and hepatitis, it typically presents as a nonspecific febrile illness with fever, chills, nausea, headache, rash, and sometimes with a chronic inflammatory arthritis. Given a shared mosquito vector and area of distribution, coninfection with dengue virus (DENV) has been reported to occur. Cases of CHIKV infection in transplant recipients has been reported, however the median time after transplantation was 7.5 years, suggesting acute infection rather than reactivation of preexisting disease [143].Transplant donors and candidates with epidemiologic risk factors for exposure and compatible symptoms should be screened for DENV, CHIKV, and ZIKV [142].

Conclusion

Each potential pathogen has its own specific risk factors for pretransplant exposure and post-transplant disease. These factors range from the association of cystic fibrosis and bronchiectasis with chronic bacterial infections such as *Burkholderia* and *Pseudomonas* to specific geographic regions associated with increased risk of endemic fungal infections. Tuberculosis infection is most common in selected countries in the developing world, and many parasitic and arbovirus infections are most common in tropical areas. While some viral infections have specific geographic associations, other viruses are more closely linked to behavior and potential blood-borne exposures, essential to include a careful medical, social, behavioral, and travel history in the evaluation of transplant candidates.

KeyPoints

- Pre-transplant screening is an important intervention to decrease infection risk after transplantation.
- In addition to the medical history, a patient's place of residence, travel history, occupation, exposure to animals and pets, hobbies, prior infections, and other aspects of the social issue are important to review to identify potential issues.
- Pre-transplant exposures that can pose dangers after start of immunosuppression include bacterial, mycobacterial, viral, fungal, and parasitic infections.

References

1. Malinis M, Boucher HW, Practice ASTIDCo. Screening of donor and candidate prior to solid organ transplantation-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13548.
2. Shao M, Wan Q, Xie W, Ye Q. Bloodstream infections among solid organ transplant recipients: epidemiology, microbiology, associated risk factors for morbility and mortality. Transplant Rev (Orlando). 2014;28:176–81.
3. Slagt IKB, Ijzermans JNM, Alamyar M, Verhagen PCMS, Weimar W, Roodnat JI, Terkivatan T. Long-term outcome of kidney transplantation in patients with a urinary conduit: a case–control study. Int Urol Nephrol. 2013;45:405–11.
4. Lopez Pereira P, Ortiz Rodriguez R, Fernandez Camblor C, Martinez Urrutia MJ, Lobato Romera R, Espinosa L, Jaureguizar ME. Renal transplant outcome in children with an augmented bladder. Front Pediatr. 2013;1:42.
5. Ariza-Heredia EJ, Beam EN, Lesnick TG, Kremers WK, Cosio FG, Razonable RR. Urinary tract infections in kidney transplant recipients: role of gender, urologic abnormalities, and antimicrobial prophylaxis. Ann Transplant. 2013;18:195–204.
6. Hara T, Soyama A, Takatsuki M, Hidaka M, Carpenter I, Kinoshita A, Adachi T, Kitasato A, Kuroki T, Eguchi S. The impact of treated bacterial infections within one month before living donor liver transplantation in adults. Ann Transplant. 2014;19:674–9.
7. Fagiuoli S, Colli A, Bruno R, Craxi A, Gaeta GB, Grossi P, Mondelli MU, Puoti M, Sagnelli E, Stefani S, et al. Management of infections pre- and post-liver transplantation: report of an AISF consensus conference. J Hepatol. 2014;60:1075–89.
8. Kim YJ, Yoon JH, Kim SI, Choi HJ, Choi JY, Yoon SK, You YK, Kim DG. Impact of pretransplant infections on clinical course in liver transplant recipients. Transplant Proc. 2018;50:1153–6.
9. Lease E, Zaas D. Complex bacterial infections pre- and Posttransplant. Semin Respir Crit Care Med. 2010;31:234–42.
10. Waters V, Atenafu EG, Lu A, Yau Y, Tullis E, Ratjen F. Chronic Stenotrophomonas maltophilia infection and mortality or lung transplantation in cystic fibrosis patients. J Cyst Fibros. 2013;12:482–6.
11. Lobo LJ, Tulu Z, Aris RM, Noone PG. Pan-resistant Achromobacter xylosoxidans and Stenotrophomonas maltophilia infection in cystic fibrosis does not reduce survival after lung transplantation. Transplantation. 2015;99:2196–202.
12. Aslam S, Xie R, Cowger J, Kirklin JK, Chu VH, Schueler S, de By T, Gould K, Morrissey O, Lund LH, et al. Bloodstream infections in mechanical circulatory support device recipients in the International Society of Heart and Lung Transplantation mechanically assisted circulation support registry: epidemiology, risk factors, and mortality. J Heart Lung Transplant. 2018;37:1013–20.
13. Rosenfeldt FL, Kwa LJ, Porapakkham P, Rajadurai S, Jones K, van de Merwe J, Billah B, Porapakkham P, Esmore DS, Halvorsen DS, et al. Bacteraemia in ventricular assist devices: a common complication that need not affect clinical outcomes. Heart Lung Circ. 2014;23:234–41.
14. Teoh TK, Hannan MM. Ventricular assist device-associated infection. Infect Dis Clin N Am. 2018;32:827–41.
15. Kusne S, Mooney M, Danziger-Isakov L, Kaan A, Lund LH, Lyster H, Wieselthaler G, Aslam S, Cagliostro B, Chen J, et al. An ISHLT consensus document for prevention and management strategies for mechanical circulatory support infection. J Heart Lung Transplant. 2017;36:1137–53.
16. Koval CE, Stosor V, Practice AICo. Ventricular assist device-related infections and solid organ transplantation-Guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13552.
17. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, Wingard JR, Young J-AH, Boeckh MA. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15:1143–238.

18. Sahin U, Toprak SK, Atilla PA, Atilla E, Demirer T. An overview of infectious complications after allogeneic hematopoietic stem cell transplantation. J Infect Chemother. 2016;22:505–14.
19. Linares L, Cervera C, Cofán F, Lizaso D, Marco F, Ricart MJ, Esforzado N, Oppenheimer F, Campistol JM, Moreno A. Risk factors for infection with extended-spectrum and AmpC β-lactamase-producing gram-negative rods in renal transplantation. Am J Transplant. 2008;8:1000–5.
20. Men TY, Wang JN, Li H, Gu Y, Xing TH, Peng ZH, Zhong L. Prevalence of multidrug-resistant gram-negative bacilli producing extended-spectrum beta-lactamases (ESBLs) and ESBL genes in solid organ transplant recipients. Transpl Infect Dis. 2013;15:14–21.
21. Satlin MJ, Jenkins SG, Walsh TJ. The global challenge of Carbapenem-resistant enterobacteriaceae in transplant recipients and patients with hematologic malignancies. Clin Infect Dis. 2014;58:1274–83.
22. Pouch SM, Satlin MJ. Carbapenem-resistant enterobacteriaceae in special populations: solid organ transplant recipients, stem cell transplant recipients, and patients with hematologic malignancies. Virulence. 2017;8:391–402.
23. Lubbert C, Becker-Rux D, Rodloff AC, Laudi S, Busch T, Bartels M, Kaisers UX. Colonization of liver transplant recipients with KPC-producing Klebsiella pneumoniae is associated with high infection rates and excess mortality: a case-control analysis. Infection. 2014;42:309–16.
24. Freire MP, Villela Soares Oshiro IC, Bonazzi PR, Pierrotti LC, de Oliveira LM, Machado AS, Van Der Heijdenn IM, Rossi F, Costa SF, Carneiro D'Albuquerque LA, et al. Surveillance culture for multidrug-resistant gram-negative bacteria: performance in liver transplant recipients. Am J Infect Control. 2017;45:e40–4.
25. Pouch SM, Kubin CJ, Satlin MJ, Tsapepas DS, Lee JR, Dube G, Pereira MR. Epidemiology and outcomes of carbapenem-resistant Klebsiella pneumoniae bacteriuria in kidney transplant recipients. Transp Infect Dis. 2015;17:800–9.
26. Tacconelli E, Mazzaferri F, de Smet AM, Bragantini D, Eggimann P, Huttner BD, Kuijper EJ, Lucet JC, Mutters NT, Sanguinetti M, et al. ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant gram-negative bacteria carriers. Clin Microbiol Infect. 2019;25:807–17.
27. Pouch SM, Patel G, Practice ASTIDCo. Multidrug-resistant gram-negative bacterial infections in solid organ transplant recipients – guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13594.
28. Nkam L, Lambert J, Latouche A, Bellis G, Burgel PR, Hocine MN. A 3-year prognostic score for adults with cystic fibrosis. J Cyst Fibros. 2017;16:702–8.
29. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, Lederer DJ, Mulligan MJ, Patterson GA, Singer LG, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the pulmonary transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2015;34:1–15.
30. Aris RM, Routh J, LiPuma J, Heath D, Gilligan PH. Lung transplantation for cystic fibrosis patients with Burkholderia cepacia complex. Am J Respir Crit Care Med. 2001;164:2102–6.
31. Alexander BD, Petzold EW, Reller LB, Palmer SM, Davis RD, Woods CW, Lipuma JJ. Survival after lung transplantation of cystic fibrosis patients infected with Burkholderia cepacia complex. Am J Transplant. 2008;8:1025–30.
32. Murray S, Charbeneau J, Marshall BC, LiPuma JJ. Impact of burkholderia infection on lung transplantation in cystic fibrosis. Am J Respir Crit Care Med. 2008;178:363–71.
33. Stephenson AL, Sykes J, Berthiaume Y, Singer LG, Aaron SD, Whitmore GA, Stanojevic S. Clinical and demographic factors associated with post-lung transplantation survival in individuals with cystic fibrosis. J Heart Lung Transplant. 2015;34:1139–45.
34. Koutsokera A, Varughese RA, Sykes J, Orchanian-Cheff A, Shah PS, Chaparro C, Tullis E, Singer LG, Stephenson AL. Pre-transplant factors associated with mortality after lung transplantation in cystic fibrosis: a systematic review and meta-analysis. J Cyst Fibros. 2019;18:407–15.

35. Boussaud V, Guillemain R, Grenet D, Coley N, Souilamas R, Bonnette P, Stern M. Clinical outcome following lung transplantation in patients with cystic fibrosis colonised with Burkholderia cepacia complex: results from two French centres. Thorax. 2008;63:732–7.
36. De Soyza A, Meachery G, Hester KL, Nicholson A, Parry G, Tocewicz K, Pillay T, Clark S, Lordan JL, Schueler S, et al. Lung transplantation for patients with cystic fibrosis and Burkholderia cepacia complex infection: a single-center experience. J Heart Lung Transplant. 2010;29:1395–404.
37. Subramanian AK, Theodoropoulos NM, Infectious Diseases Community of Practice of the American Society of T. Mycobacterium tuberculosis infections in solid organ transplantation: guidelines from the infectious diseases community of practice of the American Society of Transplantation. Clin Transpl. 2019;33:e13513.
38. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, Keane J, Lewinsohn DA, Loeffler AM, Mazurek GH, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64:111–5.
39. Casas S, Muñoz L, Moure R, Castellote J, Guerra MR, Gonzalez L, Andreu A, Rafecas AG, Alcaide F, Santin M. Comparison of the 2-step tuberculin skin test and the quantiFERON-TB gold in-tube test for the screening of tuberculosis infection before liver transplantation. Liver Transpl. 2011;17:1205–11.
40. Kim SH, Lee SO, Park JB, Park IA, Park SJ, Yun SC, Jung JH, Kim YH, Kim SC, Choi SH, et al. A prospective longitudinal study evaluating the usefulness of a T-cell-based assay for latent tuberculosis infection in kidney transplant recipients. Am J Transplant. 2011;11:1927–35.
41. Hand J, Sigel K, Huprikar S, Hamula C, Rana M. Tuberculosis after liver transplantation in a large center in New York City: QuantiFERON-TB gold-based pre-transplant screening performance and active tuberculosis post-transplant. Transpl Infect Dis. 2018;20:e12845.
42. Morales P, Gil A, Santos M. Mycobacterium abscessus infection in transplant recipients. Transplant Proc. 2010;42:3058–60.
43. Piersimoni C. Nontuberculous mycobacteria infection in solid organ transplant recipients. Eur J Clin Microbiol Infect Dis. 2012;31:397–403.
44. Malinis M. Management of Mycobacterium Other than tuberculosis in solid organ transplantation. Infect Dis Clin North Am. 2018;32:719–32.
45. Knoll BM. Update on nontuberculous mycobacterial infections in solid organ and hematopoietic stem cell transplant recipients. Curr Infect Dis Rep. 2014;16:421.
46. Longworth SA, Daly JS, Practice ASTIDCo. Management of infections due to non-tuberculous mycobacteria in transplant recipients – guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13588.
47. Dupont L. Lung transplantation in cystic fibrosis patients with difficult to treat lung infections. Curr Opin Pulm Med. 2017;23:574–9.
48. Tissot A, Thomas MF, Corris PA, Brodlie M. NonTuberculous mycobacteria infection and lung transplantation in cystic fibrosis: a worldwide survey of clinical practice. BMC Pulm Med. 2018;18:86.
49. Lobo LJ, Chang LC, Esther CR, Gilligan PH, Tulu Z, Noone PG. Lung transplant outcomes in cystic fibrosis patients with pre-operative Mycobacterium abscessus respiratory infections. Clin Transpl. 2013;27:523–9.
50. Qvist T, Pressler T, Thomsen VO, Skov M, Iversen M, Katzenstein TL. Nontuberculous mycobacterial disease is not a contraindication to lung transplantation in patients with cystic fibrosis: a retrospective analysis in a Danish patient population. Transp Proc. 2013;45:342–5.
51. Osmani M, Sotello D, Alvarez S, Odell JA, Thomas M. Mycobacterium abscessus infections in lung transplant recipients: 15-year experience from a single institution. Transpl Infect Dis. 2017;20:1–8.

52. Perez A, Singer JP, Schwartz BS, Chin-Hong P, Shah R, Kleinhenz ME, Gao Y, Venado A, Leard L, Golden JA, et al. Management and clinical outcomes after lung transplantation in patients with pre-transplant Mycobacterium abscessus infection: a single center experience. Transpl Infect Dis. 2019;21:1–10.
53. Olivier KN, Weber DJ, Wallace RJ Jr, Faiz AR, Lee JH, Zhang Y, Brown-Elliot BA, Handler A, Wilson RW, Schechter MS, et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. Am J Respir Crit Care Med. 2003;167:828–34.
54. Leung JM, Olivier KN. Nontuberculous mycobacteria: the changing epidemiology and treatment challenges in cystic fibrosis. Curr Opin Pulm Med. 2013;19:662–9.
55. Levy I, Grisaru-Soen G, Lerner-Geva L, Kerem E, Blau H, Bentur L, Aviram M, Rivlin J, Picard E, Lavy A, et al. Multicenter cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients, Israel. Emerg Infect Dis. 2008;14:378–84.
56. Seddon P, Fidler K, Raman S, Wyatt H, Ruiz G, Elston C, Perrin F, Gyi K, Bilton D, Drobniewski F, et al. Prevalence of nontuberculous mycobacteria in cystic fibrosis clinics, United Kingdom, 2009. Emerg Infect Dis. 2013;19:1128–30.
57. Chien JY, Lai CC, Sheng WH, Yu CJ, Hsueh PR. Pulmonary infection and colonization with nontuberculous mycobacteria, Taiwan, 2000–2012. Emerg Infect Dis. 2014;20:1382–5.
58. Yoo JW, Jo KW, Kim SH, Lee SO, Kim JJ, Park SK, Lee JH, Han DJ, Hwang S, Lee S, et al. Incidence, characteristics, and treatment outcomes of mycobacterial diseases in transplant recipients. Transpl Int. 2016;29:549–58.
59. Namkoong H, Kurashima A, Morimoto K, Hoshino Y, Hasegawa N, Ato M, Mitarai S. Epidemiology of pulmonary nontuberculous mycobacterial disease, Japan. Emerg Infect Dis. 2016;22:1116–7.
60. Morimoto K, Hasegawa N, Izumi K, Namkoong H, Uchimura K, Yoshiyama T, Hoshino Y, Kurashima A, Sokunaga J, Shibuya S, et al. A laboratory-based analysis of nontuberculous mycobacterial lung disease in Japan from 2012 to 2013. Ann Am Thorac Soc. 2017;14:49–56.
61. Prevots DR, Adjemian J, Fernandez AG, Knowles MR, Olivier KN. Environmental risks for nontuberculous mycobacteria. Individual exposures and climatic factors in the cystic fibrosis population. Ann Am Thorac Soc. 2014;11:1032–8.
62. Adjemian J, Olivier KN, Prevots DR. Nontuberculous mycobacteria among patients with cystic fibrosis in the United States: screening practices and environmental risk. Am J Respir Crit Care Med. 2014;190:581–6.
63. Miller R, Assi M, Practice ASTIDCo. Endemic fungal infections in solid organ transplant recipients-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13553.
64. Kauffman CA, Freifeld AG, Andes DR, Baddley JW, Herwaldt L, Walker RC, Alexander BD, Anaissie EJ, Benedict K, Ito JI, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the transplant-associated infection surveillance network (TRANSNET). Transpl Infect Dis. 2014;16:213–24.
65. Gajurel K, Dhakal R, Deresinski S. Diagnosis and treatment of histoplasmosis in solid organ transplant patients. Curr Opin Infect Dis. 2018;31:301–8.
66. Assi M, Martin S, Wheat LJ, Hage C, Freifeld A, Avery R, Baddley JW, Vergidis P, Miller R, Andes D, et al. Histoplasmosis after solid organ transplant. Clin Infect Dis. 2013;57:1542–9.
67. Grim SA, Proia L, Miller R, Alhyraba M, Costas-Chavarri A, Oberholzer J, Clark NM. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. Transpl Infect Dis. 2012;14:17–23.
68. McDonald R, Dufort E, Jackson BR, Tobin EH, Newman A, Benedict K, Blog D. Blastomycosis cases occurring outside of regions with known endemicity – New York, 2007–2017. Morb Mortal Wkly Rep. 2018;67:1077–8.
69. Chapman SW, Dismukes WE, Proia LA, Bradsher RW, Pappas PG, Threlkeld MG, Kauffman CA. Infectious diseases Society of a. clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. Clin Infect Dis. 2008;46:1801–12.

70. de Almeida JN, Pecanha-Pietrobom PM Jr, Colombo AL. Paracoccidioidomycosis in immunocompromised patients: a literature review. J Fungi (Basel). 2018;5:2.
71. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M. Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol. 2011;49:785–98.
72. Fisher MC, Koenig GL, White TJ, Taylor JW. Molecular and phenotypic description of Coccidioides posadasii sp. nov., previously recognized as the non-California population of Coccidioides immitis. Mycologia. 2002;94:73–84.
73. Barker BM, Jewell KA, Kroken S, Orbach MJ. The population biology of coccidioides: epidemiologic implications for disease outbreaks. Ann N Y Acad Sci. 2007;1111:147–63.
74. Teixeira MM, Barker BM. Use of population genetics to assess the ecology, evolution, and population structure of Coccidioides. Emerg Infect Dis. 2016;22:1022–30.
75. Coates SJ, Fox LP. Disseminated coccidioidomycosis as a harbinger of climate change. JAAD Case Rep. 2018;4:424–5.
76. Nguyen C, Barker BM, Hoover S, Nix DE, Ampel NM, Frelinger JA, Orbach MJ, Galgiani JN. Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of coccidioidomycosis. Clin Microbiol Rev. 2013;26:505–25.
77. Blair JE. Approach to the solid organ transplant patient with latent infection and disease caused by Coccidioides species. Curr Opin Infect Dis. 2008;21:415–20.
78. Kirkland TN, Fierer J. Coccidioides immitis and posadasii; a review of their biology, genomics, pathogenesis, and host immunity. Virulence. 2018;9:1426–35.
79. Stockamp NW, Thompson GR. Coccidioidomycosis. Infect Dis Clin North Am. 2016;30:229–46.
80. Mendoza N, Blair JE. The utility of diagnostic testing for active Coccidioidomycosis in solid organ transplant recipients. Am J Transplant. 2013;13:1034–9.
81. Kassis C, Zaidi S, Kuberski T, Moran A, Gonzalez O, Hussain S, Hartmann-Manrique C, Al-Jashaami L, Chebbo A, Myers RA, et al. Role of Coccidioides antigen testing in the cerebrospinal fluid for the diagnosis of Coccidioidal meningitis. Clin Infect Dis. 2015;61:1521–6.
82. Twarog M, Thompson IIIG. Coccidioidomycosis: recent updates. Semin Respir Crit Care Med. 2015;36:746–55.
83. Phonphok K, Beaird O, Duong T, Datta N, Schaenman J, Bunnapradist S. Screening Coccidioides serology in kidney transplant recipients: a 10-year cross-sectional analysis. Transpl Infect Dis. 2018;20:1–8.
84. Kahn A, Carey EJ, Blair JE. Universal fungal prophylaxis and risk of coccidioidomycosis in liver transplant recipients living in an endemic area. Liver Transpl. 2015;21:353–61.
85. Chaudhary S, Meinke L, Ateeli H, Knox K, Raz Y, Ampel NM. Coccidioidomycosis among persons undergoing lung transplantation in the coccidioidal endemic region. Transpl Infect Dis. 2017;19:1–6.
86. Singh N, Sifri CD, Silveira FP, Miller R, Gregg KS, Huprikar S, Lease ED, Zimmer A, Dummer JS, Spak CW, et al. Cryptococcosis in patients with cirrhosis of the liver and posttransplant outcomes. Transplantation. 2015;99:2132–41.
87. Sifri CD, Sun HY, Cacciarelli TV, Wispelwey B, Pruett TL, Singh N. Pretransplant cryptococcosis and outcome after liver transplantation. Liver Transpl. 2010;16:499–502.
88. Singh N, Husain S, De Vera M, Gayowski T, Cacciarelli TV. Cryptococcus neoformans infection in patients with cirrhosis, including liver transplant candidates. Medicine (Baltimore). 2004;83:188–92.
89. Hwang SY, Yu SJ, Lee JH, Kim JS, Yoon JW, Kim YJ, Yoon JH, Kim EC, Lee HS. Spontaneous fungal peritonitis: a severe complication in patients with advanced liver cirrhosis. Eur J Clin Microbiol Infect Dis. 2014;33:259–64.
90. Lin YY, Shiau S, Fang CT. Risk factors for invasive Cryptococcus neoformans diseases: a case-control study. PLoS One. 2015;10:e0119090.
91. Chaturvedi V, Chaturvedi S. Cryptococcus gattii: a resurgent fungal pathogen. Trends Microbiol. 2011;19:564–71.
92. Levinson M. Diseases transmitted by birds. Microbiol Spectr. 2015; 3:IOL5–0004-2015.

93. Abdala E, Miller R, Pasqualotto AC, Munoz P, Colombo AL, Cuenca-Estrella M. Endemic fungal infection recommendations for solid-organ transplant recipients and donors. Transp J. 2018;102:S52–9.
94. Baddley JW, Forrest GN, Practice ASTIDCo. Cryptococcosis in solid organ transplantation-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13543.
95. Saha DC, Goldman DL, Shao X, Casadevall A, Husain S, Limaye AP, Lyon M, Somani J, Pursell K, Pruett TL, et al. Serologic evidence for reactivation of Cryptococcosis in solid-organ transplant recipients. Clin Vaccine Immunol. 2007;14:1550–4.
96. Brunet K, Alanio A, Lortholary O, Rammaert B. Reactivation of dormant/latent fungal infection. J Infect. 2018;77:463–8.
97. Helmi M, Love RB, Welter D, Cornwell RD, Meyer KC. Aspergillus infection in lung transplant recipients with cystic fibrosis: risk factors and outcomes comparison to other types of transplant recipients. Chest. 2003;123:800–8.
98. Lemonovich TL. Mold infections in solid organ transplant recipients. Infect Dis Clin North Am. 2018;32:687–701.
99. Husain S, Solé A, Alexander BD, Aslam S, Avery R, Benden C, Billaud EM, Chambers D, Danziger-Isakov L, Fedson S, et al. The 2015 International Society for Heart and Lung Transplantation guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients executive summary. J Heart Lung Transplant. 2016;35:1–87.
100. Chang A, Musk M, Lavender M, Wrobel J, Yaw M-C, Lawrence S, Chirayath S, Boan P. Epidemiology of invasive fungal infections in lung transplant recipients in Western Australia. Transpl Infect Dis. 2018;21:1–7.
101. Tracy MC, Moss RB. The myriad challenges of respiratory fungal infection in cystic fibrosis. Pediatr Pulmonol. 2018;53:S75–85.
102. Schwarz C, Hartl D, Eickmeier O, Hector A, Benden C, Durieu I, Sole A, Gartner S, Milla CE, Barry PJ. Progress in definition, prevention and treatment of fungal infections in cystic fibrosis. Mycopathologia. 2018;183:21–32.
103. Pierrotti LC, Kotton CN. Transplantation in the tropics: lessons on prevention and management of tropical infectious diseases. Curr Infect Dis Rep. 2015;17:492.
104. van de Peppel RJ, Dekkers OM, von dem Borne PA, de Boer MG. Relapsed and secondary disease drive the risk profile for invasive aspergillosis prior to stem cell transplantation in patients with acute myeloid leukemia or myelodysplastic syndrome. Med Mycol. 2014;52:699–705.
105. Liu YC, Chien SH, Fan NW, Hu MH, Gau JP, Liu CJ, Yu YB, Liu CY, Hsiao LT, Liu JH, et al. Incidence and risk factors of probable and proven invasive fungal infection in adult patients receiving allogeneic hematopoietic stem cell transplantation. J Microbiol Immunol Infect. 2016;49:567–74.
106. Gerull S, Medinger M, Heim D, Passweg J, Stern M. Evaluation of the pretransplantation workup before allogeneic transplantation. Biol Blood Marrow Transplant. 2014;20:1852–6.
107. Clemente WT, Pierrotti LC, Abdala E, Morris MI, Azevedo LS, Lopez-Velez R, Cuenca-Estrella M, Torre-Cisneros J, Petersen E, Camargo LFA, et al. Recommendations for management of endemic diseases and travel medicine in solid-organ transplant recipients and donors: Latin America. Transplantation. 2018;102:193–208.
108. Buchan CA, Kotton CN, Practice ASTIDCo. Travel medicine, transplant tourism, and the solid organ transplant recipient-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13529.
109. Camargo LFA, Kamar N, Gotuzzo E, Wright AJ. Schistosomiasis and Strongyloidiasis recommendations for solid-organ transplant recipients and donors. Transplantation. 2018;102:S27–34.
110. Schär F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, Vounatsou P, Odermatt P. Strongyloides stercoralis: global distribution and risk factors. PLoS Negl Trop Dis. 2013;7:e2288.

111. Clemente WT, Mourao PHO, Lopez-Medrano F, Schwartz BS, Garcia-Donoso C, Torre-Cisneros J. Visceral and cutaneous Leishmaniasis recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102:S8–S15.
112. La Hoz RM, Morris MI, Infectious Diseases Community of Practice of the American Society of T. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, Babesia, Acanthamoeba, Balamuthia, and Naegleria in solid organ transplant recipients-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13546.
113. Fernandez-Sabe N, Cervera C, Fariñas MC, Bodro M, Muñoz P, Gurguí M, Torre-Cisneros J, Martin-Davila P, Noblejas A, Len O, et al. Risk factors, clinical features, and outcomes of toxoplasmosis in solid-organ transplant recipients: a matched case-control study. Clin Infect Dis. 2012;54:355–61.
114. Baran DA, Alwarshetty M, Alvi S, Arroyo L, Lubitz S, Pinney S, Gass A, Zucker M. Is toxoplasmosis prophylaxis necessary in cardiac transplantation? Long-term follow-up at two transplant Centers. J Heart Lung Transplant. 2006;25:1380–2.
115. Mastrobuoni S, Dell'Aquila AM, Herreros J. Fatal toxoplasma gondii dissemination in a heart transplant recipient: description of a case. Case Rep Transplant. 2012;2012:1–3.
116. Derouin F, Pelloux H. Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infect. 2008;14:1089–101.
117. Hamadani M, Craig M, Awan FT, Devine SM. How we approach patient evaluation for hematopoietic stem cell transplantation. Bone Marrow Transplant. 2010;45:1259–68.
118. Locke JE, Durand C, Reed RD, MacLennan PA, Mehta S, Massie A, Nellore A, DuBay D, Segev DL. Long-term outcomes after liver transplantation among human immunodeficiency virus-infected recipients. Transplantation. 2016;100:141–6.
119. Kucirka LM, Durand CM, Bae S, Avery RK, Locke JE, Orandi BJ, McAdams-DeMarco M, Grams ME, Segev DL. Induction immunosuppression and clinical outcomes in kidney transplant recipients infected with human immunodeficiency virus. Am J Transplant. 2016;16:2368–76.
120. Malat GE, Boyle SM, Jindal RM, Guy S, Xiao G, Harhay MN, Lee DH, Ranganna KM, Anil Kumar MS, Doyle AM. Kidney transplantation in HIV-positive patients: a single-center, 16-year experience. Am J Kidney Dis. 2019;73:112–8.
121. Madan S, Patel SR, Saeed O, Sims DB, Shin JJ, Goldstein DJ, Jorde UP. Outcomes of heart transplantation in patients with human immunodeficiency virus. Am J Transplant. 2019;19:1529–35.
122. Chen C, Wen X, Yadav A, Belviso N, Kogut S, McCauley J. Outcomes in human immunodeficiency virus-infected recipients of heart transplants. Clin Transpl. 2019;33:e13440.
123. Ong S, Levy RD, Yee J, Partovi N, Churg A, Romeo P, Chalaoui J, Nador R, Wright A, Manganas H, et al. Successful lung transplantation in an HIV seropositive patient with desquamative interstitial pneumonia: a case report. BMC Pulm Med. 2018;18:162.
124. Schillie S, Harris A, Link-Gellis R, Romero J, Ward J, Nelson N. Recommendations of the advisory committee on immunization practices for use of a hepatitis B vaccine with a novel adjuvant. Morb Mortal Wkly Rep. 2018;67:455–8.
125. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67:1560–99.
126. Fontaine H, Alric L, Labreuche J, Legendre B, Louvet A, Antoine C, Legendre CM, Hazzan M, Kamar N, Dharancy S, et al. Control of replication of hepatitis B and C virus improves patient and graft survival in kidney transplantation. J Hepatol. 2019;70:831–8.
127. Sarmati L, Andreoni M, Antonelli G, Arcese W, Bruno R, Coppola N, Gaeta GB, Galli M, Girmenia C, Mikulska M, et al. Recommendations for screening, monitoring, prevention, prophylaxis and therapy of hepatitis B virus reactivation in patients with haematologic malignancies and patients who underwent haematologic stem cell transplantation-a position paper. Clin Microbiol Infect. 2017;23:935–40.

128. Ankcorn MJ, Ijaz S, Poh J, Elsharkawy AM, Smit E, Cramb R, Ravi S, Martin K, Tedder R, Neuberger J. Toward systematic screening for persistent hepatitis E virus infections in transplant patients. Transplantation. 2018;102:1139–47.
129. von Wulffen M, Westholter D, Lutgehetmann M, Pischke S. Hepatitis E: still waters run deep. J Clin Transl Hepatol. 2018;6:40–7.
130. Vangipuram R, Tyring SK. Epidemiology of Kaposi sarcoma: review and description of the nonepidemic variant. Int J Dermatol. 2019;58:538–42.
131. Garcia-Astudillo LA, Leyva-Cobian F. Human herpesvirus-8 infection and Kaposi's sarcoma after liver and kidney transplantation in different geographical areas of Spain. Transpl Immunol. 2006;17:65–9.
132. Liu Z, Fang Q, Zuo J, Chen Y, Minhas V, Wood C, Zhang T. Global epidemiology of human herpesvirus 8 in men who have sex with men: a systematic review and meta-analysis. J Med Virol. 2018;90:582–91.
133. Andreoni M, Goletti D, Pezzotti P, Pozzetto A, Monini P, Sarmati L, Farchi F, Tisone G, Piazza A, Pisani F, et al. Prevalence, incidence and correlates of HHV-8/KSHV infection and Kaposi's sarcoma in renal and liver transplant recipients. J Infect. 2001;43:195–9.
134. Charpentier C, Delyon J, Glotz D, Peraldi MN, Rerolle JP, Barrou B, Ducroux E, Coilly A, Legeai C, Barete S, et al. Kaposi sarcoma in HIV-positive solid-organ transplant recipients: a French multicentric national study and literature review. Transplantation. 2019;103:e22–8.
135. Pellett Madan R, Hand J, Practice ASTIDCo. Human herpesvirus 6, 7, and 8 in solid organ transplantation: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13518.
136. Armstrong MJ, Corbett C, Rowe IA, Taylor GP, Neuberger JM. HTLV-1 in solid-organ transplantation: current challenges and future management strategies. Transplantation. 2012;94:1075–84.
137. Taylor GP. Human T-lymphotropic virus type 1 infection and solid organ transplantation. Rev Med Virol. 2018;28
138. Gessain A, Cassar O. Epidemiological aspects and world distribution of HTLV-1 infection. Front Microbiol. 2012;3:388.
139. Kaul DR, Sharma TS, Practice AICo. Human T-cell lymphotrophic virus in solid-organ transplant recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13575.
140. Rossi S, Ross TM, Evans JD. West Nile virus. Clin Lab Med. 2010;30:47–65.
141. Anesi JA, Silveira F. Arenaviruses and West Nile virus in solid organ transplant recipients: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13576.
142. Nogueira ML, Estofolete CF, Terzian AC, Mascarin do Vale EP, da Silva RC, da Silva RF, Ramalho HJ, Fernandes Charpiot IM, Vasilakis N, Abbud-Filho M. Zika virus infection and solid organ transplantation: a new challenge. Am J Transplant. 2017;17:791–5.
143. Rosso F, Rodriguez S, Cedano JA, Mora BL, Moncada P, Velez J. Chikungunya in solid organ transplant recipients, a case series and literature review. Transpl Infect Dis. 2018;20:e12978.

Transplant Tourism and the Traveling Transplant Recipient: Infection Mitigation and Treatment Strategies

10

C. Arianne Buchan

Contents

Introduction 201
Part 1: Transplant Tourism 202
Introduction 202
Transplant Tourism and Infectious Complications 202
Conclusion 204
Key Points 204
Part 2: The Traveling Transplant Recipient 205
Introduction 205
Risk of Infection While Abroad 205
Pre-departure Infectious Disease Evaluation and Recommendations 226
Conclusion 229
Key Points 229
References 230

Introduction

Transplant tourism and travel posttransplant both play roles in acquisition of infection in solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients. Transplant tourism refers to travel for the purpose of obtaining an organ or tissue transplant outside of a patient's usual country of residence and is often done through commercial means. Travel posttransplant refers to trips abroad taken by SOT and HSCT recipients for the purposes of business, visiting friends and relatives,

C. A. Buchan (✉)
Divisoin of Infectious Diseases, Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada

The University of Ottawa, Ottawa, ON, Canada

The Ottawa Hospital Research Institute, Ottawa, ON, Canada
e-mail: abuchan@toh.ca

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_12

or leisure. This chapter discusses both topics separately in order to best address risk of infection and strategies for prevention and treatment accordingly.

Part 1: Transplant Tourism

Introduction

The term "transplant tourism" refers to travel of either the organ donor or recipient for purposes of organ transplantation and often occurs in commercial settings [1]. Numerous international transplant organizations, including the World Health Organization (WHO), the Transplantation Society, and the International Society of Nephrology, have made efforts to decrease the purchase and sale of organs on ethical grounds [2–4], and the practice is illegal in many countries [5]. Nonetheless, these comprise an estimated 10% of SOTs worldwide [6]. Studies have found that outcomes of patients who undergo transplant through commercial means experience decreased allograft and patient survival as well as increased complications including higher rates of rejection and higher rates of infectious complications [7–13]. Although this issue is more prominent and regulations are in place to deter the practice in SOT, transplant tourism also occurs in patients seeking HSCT and stem cell therapies. There is growing concern for stem cell tourism, which involves stem cell therapy for a host of diseases as novel or experimental treatments beyond the traditional use for treatment of hematologic malignancies and disorders [14].

Transplant Tourism and Infectious Complications

Several publications and review papers, including guidelines published by the American Society of Transplantation Infectious Disease Community of Practice (AST Guidelines), highlight the risk of infection that comes with procuring organs through transplant tourism and commercial means [12, 15]. The literature raises concern regarding increased risk across all categories of infection including bacterial, viral, fungal, and parasitic. Differences between endemic rates of infection, antimicrobial resistance patterns, transplant practice, and regulation are all reasons to explain this concern. Pretransplant evaluation and screening of both the donor and recipient as well as routine prophylaxis can vary by country and may not be consistent with the accepted practice, thus potentially increasing the risk of reactivation of latent infection, donor-derived infection, and perioperative or hospital-acquired infection. Furthermore, documentation of transplants that occur in foreign countries, especially for commercial purposes, may not be readily available, therefore making it difficult to provide comprehensive care once the patient returns to his/her resident country.

The body of literature addressing this issue ranges from case reports that outline individual infections acquired through foreign or commercial transplantation to single-center studies looking at outcomes of organ transplants performed

in foreign countries [7, 8, 16, 17] and to review articles and guideline recommendations.

In 2013, Chen and Wilson published a review paper outlining the impact of medical tourism on the field of infectious disease. This paper highlights the magnitude of transplant tourism for both transplant and other purposes on a global scale and provides a useful overview of commonly encountered complications and their impact on healthcare. It raises issues regarding specific infections especially bacterial infections with resistant organisms that can be more common overseas. [18] Given the growing concern for multidrug-resistant organisms (MDRO) and antibiotic resistance worldwide, this is a real concern for patients who have undergone transplant abroad.

Several centers from countries including the United States, Canada, Korea, Australia, and Turkey have looked at rates of infection in patients returning from organ transplantation performed overseas compared to transplants done locally [7, 8, 11, 16, 17]. Prasad et al. have published data on outcomes in patients who underwent renal transplant commercially as compared to those done locally in Toronto, Canada [7, 8]. The first publication reported on 20 patients who received 22 allografts obtained abroad and found increased risk of graft loss and death, and reported 11 patients had serious postoperative infections [7]. The same research group later reported on outcomes of 69 patients who received 72 commercial renal transplants which included infectious complications such as active hepatitis (4 patients), seroconversion of hepatitis B or C (7 patients), pulmonary tuberculosis (2 patients), as well as reduced allograft survival and delayed graft function [8]. Similarly, in a study by Gill et al., 33 patients who underwent transplant abroad were compared to a matched cohort done at the University of California Los Angeles (UCLA) and were found to have a more complex posttransplant course with higher incidence of severe infections [10]. Although the overall rates of infectious complications were similar (52% vs. 48.5%), the severity of infection and the need for hospitalization were greater in those who had transplants abroad. Notably, there were more infections with cytomegalovirus (CMV) in the transplant tourism group as the majority did not receive post-transplant CMV prophylaxis when transplant was done abroad. Finally, in this study, one patient died of donor-derived hepatitis B, which highlights concern regarding screening for infection and donor selection [10].

Studies done outside of North America show similar findings in transplant tourists. An Australian review of recipients of commercial transplants done abroad found patients were at risk of transplant-related HIV infection and new hepatitis [16]. As well, a case series from Turkey reported an increased incidence of malaria, invasive fungal infection, and tuberculosis in 115 patients who had undergone commercial transplants in various countries (including India, Iraq, and Iran) as compared to patients who had undergone transplant locally from living-related donors [17].

Finally, it is important to consider that foreign-born transplant recipients may return to their country of origin for organ transplants [19, 20]. Patients will often

overlook increased infection risk given they are returning home and procedures may occur either during trips for this specific purpose or while they are visiting friends and relatives. These patients are at risk for both reactivation of latent infections and acquisition of new endemic infections; knowledge of the specific infections existing in the transplant region can direct further evaluation [21].

Although less published data exists with regard to the infectious risk involved with stem cell therapy procured through medical tourism and commercial means, professional organizations including the American Society for Transplantation and Cellular Therapy have issued advisory statements on this topic [14, 22]. Concerns from an infection perspective include potential lack of pretransplant screening (both donor and recipient), differences in infection control practices, and possible exposure to multidrug-resistant pathogens and other endemic infections which can vary depending on the center [23]. Patients seeking stem cell therapies through medical tourism or commercial means should be cautioned with regard to these concerns. They should also be made aware that due to differences in healthcare regulations in various countries, stem cell therapies may be experimental or may be offered for novel diseases that have not yet been universally approved; thus, caution with regard to efficacy of treatment as well as other noninfectious complications should be taken into consideration [14, 22, 24].

Conclusion

Experience from transplant centers across the world has shown that there is an increased risk of infection when transplants are done through transplant tourism and in commercial settings. Therefore, identification of patients who have undergone transplant abroad and subsequent evaluation for infection upon their return to local transplant centers is important. This evaluation should include obtaining information regarding transplant process, surgical procedure, and any complications from healthcare received abroad, if possible. It is prudent to perform routine infectious disease screening as per local recommendations that may have been omitted abroad (e.g., CMV, EBV, etc.). Additional screening for endemic infections that could be donor-derived or acquired de novo while abroad based on geographic risk (malaria, strongyloides, tuberculosis, etc.), for blood-borne pathogens (HBV, HCV, and HIV), and for multidrug-resistant organisms is important to thoroughly assess. Treatment of any identified active or latent infection as well as initiation of appropriate prophylaxis as required based on routine practice and screening results will optimize care of these patients.

Key Points

- Despite regulations and laws, 10% of organ transplants occur via commercial practices.
- Patients, both SOT and HSCT, should be discouraged from this practice based on ethical reasons, but should also understand differences in outcomes and increased risk of complications.

- Transplant tourism increases risk of infection, and those patients who have received transplant abroad should be evaluated upon return to their country of residence.
- Special consideration should be paid to endemic infection based on geographic risk and should include resistant bacterial infections, mycobacterial infections, viral infections, fungal infections, and parasitic infections.

Part 2: The Traveling Transplant Recipient

Introduction

An increasing number of solid organ transplants (SOTs) and hematopoietic stem cell transplant (HSCT) are performed each year, thus expanding the population of patients who are immunocompromised posttransplant and hope to re-establish life after transplant in a safe manner. Travel is a major component of this. Studies from the United States, Canada, and Europe have shown that at least one-third of SOT patients travel outside their home country or continent [25–27]. HSCT patients also travel outside their home country; one study showed 44% of post-HSCT patients travel with 32% of patients traveling internationally within 2 years after HSCT [28]. As with any person wishing to travel to destinations with higher infective risk, pre-departure travel medicine evaluation is recommended and is especially important in SOT and HSCT patients who are at higher risk of infection. Unfortunately, not all patients seek or receive travel advice pre-departure [25, 27, 28]. This highlights the importance of ensuring patients are aware to seek advice before planning trips especially to destinations with higher infectious risk and the role of physicians to counsel their patients to do so. The recently published AST Guidelines provide recommendations for traveling SOT recipients and emphasize the importance of addressing travel with patients prior to transplant so they are aware of the need to seek medical advice prior to future travel. [15] Similar guidelines for traveling HSCT recipients are not readily available, but the topic of travel post-HSCT is addressed as part of the American Society for Blood and Marrow Transplantation (ASBMT) Guidelines for Preventing Infectious Complications among Hematopoietic Cell Transplantation Recipients: A Global Perspective [29]. This chapter will outline specific infection that pose risk to SOT and HSCT travelers (Table 2) and ways to mitigate risk during travel, as well as provide a summary of recommendations of key components of the pre-departure travel evaluation.

Risk of Infection While Abroad

Viral Infections

Respiratory Viral Infections

Influenza

Influenza virus is a respiratory virus that is spread through respiratory droplets. Influenza causes respiratory infection that usually present with fever and cough;

however, severe infections leading to respiratory failure and death occur. In fact, the WHO estimates that there are up to five million cases of severe influenza and approximately 500,000 per year globally [30]. Influenza viruses cause infections worldwide and can pose a threat to travelers, especially those with a history of transplant given higher risk of complications due to immunosuppressed state. Transplant recipients are at higher risk of severe infections, complications, and death [31, 32]. Goeijenbier et al. highlight the important considerations for the risk of acquiring influenza during travel including increased human-to-human contact while in transit or at mass gatherings, seasonality of influenza varying between hemispheres, and increased risk by geographic destination [33]. Concern exists during travel as patients transit through airports, on and off planes, trains, and other means of transportation. Proximity to potentially infected people, close spaces, and poor ventilation have all been suspected to increase transmission and attack rates. Seasonality of influenza changes by hemisphere. [34] Flu season falls between May and October in the Southern Hemisphere and between October and May in the Northern Hemisphere. In general, seasonal influenza strains in the Southern Hemisphere precede those seen later in the year in the Northern Hemisphere. Finally, tropical regions especially those located around the equator can have multiple influenza peaks and pose risk of transmission throughout the year. [35] Time of year and destination should be considered to ensure patients are up to date with influenza immunizations. High-risk travelers, such as transplant recipients, may warrant receiving the opposite hemisphere influenza vaccine 2 weeks prior to travel or on arrival at their travel destination [36].

Measles

Measles is a viral illness that infects the respiratory tract and leads to high fevers, upper respiratory tract symptoms, and a diffuse rash. Complications can occur, including encephalitis, and are more common in immunosuppressed patients. The virus is highly contagious and spread through respiratory secretions but can remain in the air or on surfaces for up to 2 hours [37]. Measles remains a common problem in developing countries, and despite immunization programs, there are millions of cases globally, with approximately 110,000 deaths reported in 2017. [37] More recently, measles outbreaks have become a growing global concern. The WHO reports 82,596 people in 47 of 53 countries in Europe had contracted measles in 2018. [38] Smaller outbreaks have also been recently reported across the United States and Canada. Measles vaccination is typically administrated through the combined MMR (measles, mumps, rubella) vaccine. Unfortunately, the MMR vaccine is a live vaccine and contraindicated in SOT recipients due to risk of complications including encephalitis [39–44]. Prior to travel to endemic areas or countries with ongoing outbreaks, evidence of immunity against measles should be evaluated in all SOT recipients to assess for the risk. Some transplant centers will screen and immunize prior to transplantation. For those patients who have not undergone this process pretransplant, immune status should be assessed prior to travel. Patients who were born before 1957, who have had two vaccinations, or who have had

clinical disease are likely protected. Serology (measles IgG) can also be checked. [15] Immune globulin can be considered for short-term protection of nonimmune SOT recipients at high risk for exposure [43].

For HSCT recipients, live attenuated vaccines are generally considered contraindicated until 24 months posttransplant provided the patient is not suffering from graft-versus-host disease (GVHD) and is not on immunosuppressive therapy [29, 42, 45–47]. A typical practice is to perform serology (measles IgG) at this time period to assess if a patient requires measles vaccination, which is generally given as the MMR vaccine. Repeat serology is often checked to ensure seroconversion and a second dose of MMR considered if patient remains seronegative. For those HSCT recipients traveling prior to this time, measles serology (IgG) could be performed early to help with risk assessment and counseling; however, reliability of this as a marker of true protection from measles early posttransplant is controversial. Therefore, risk of measles acquisition during travel should be discussed on an individual basis depending on patient, travel destination and trip itinerary factors.

Middle Eastern Respiratory Syndrome

Middle Eastern respiratory syndrome coronavirus (MERS-CoV) belongs to the family *Coronaviridae*. It was first reported in 2012 and is an emerging coronavirus that causes severe acute respiratory illness [48, 49]. Cases mainly occur in the Middle East, and Saudi Arabia has reported 80% of these. Cases outside in other countries are mostly linked with travel. There have been case reports in SOT [50] and HSCT patients [51]. Transmission is poorly understood, but there is evidence to suggest person-to-person spread as well as increased risk if exposed to dromedary camels. There are no specific methods of prevention recommended for travel; however, travelers should be encouraged to take precautions including proper hand hygiene if they have been in contact with animals [48, 49]. The WHO has identified immunocompromised travels, including SOT recipients, to be at high risk of severe illness from MERS-CoV. As such, SOT recipients should avoid contact with camels and should not consume raw, undercooked, or unpasteurized camel products (i.e., meat and dairy) [48].

Gastrointestinal Viral Infections

Norovirus

Norovirus is a cause of gastroenteritis that is spread through fecal-oral routes, either person-to-person contact or from contaminated foods, mainly raw or undercooked shellfish. Norovirus has been associated as a cause of traveler's diarrhea and is more frequently seen in outbreaks linked to crowded spaces such as cruise ships [52, 53]. While typically norovirus causes a short, self-limited gastroenteritis, transplant patients are at higher risk of complications and can go on to develop chronic norovirus [54]. Prevention involves proper hand hygiene as well as avoidance of raw or undercooked shellfish.

Hepatitis A

Hepatitis A is a frequent cause of food-borne illness and is spread through fecal-oral routes. It is associated with contaminated food and water as well as poor sanitation [55]. Clinical syndromes can range from mild illness to fulminant hepatic failure. Patients who are immunocompromised have higher risk of severe illness, and consequences can be significant.

Geographic distribution is classified as high, medium, or low risk based on endemic rates of infection. The risk of contracting hepatitis A in nonimmune travelers to high-risk areas is estimated at 1 in 1000 per week for those on tourist routes but increases to 1 in 200 for those on more remote itineraries [56].

Transplant recipients traveling to areas with risk of hepatitis A should be protected and should be aware of risk reduction strategies with regard to food and water precautions. [57] Unfortunately, hepatitis A vaccine is less effective in SOT recipients, and studies have shown lower rates of seroconversion [58]. Therefore, when possible, evaluation for hepatitis A serology testing should be done during transplant workup, and nonimmune patients who wish to travel posttransplant should be vaccinated prior to transplant. For HSCT recipients who plan to travel in the future, hepatitis A vaccine can be added to the standard posttransplant immunization schedule.

For those posttransplant patients whose immune status is unknown, hepatitis A serology should be performed. For those nonimmune transplant recipient travelers, two doses of hepatitis A vaccine 6–12 months apart should be given. If the travel departure date does not allow for both doses to be administered, the second dose should still be given upon return in order to confer longer-term protection especially for those repeat travelers. For patients who have already had one dose of vaccine previously, a second dose should be given prior to departure. Finally, for nonimmune transplant recipients who do not have adequate time prior to departure or who are unlikely to respond to immunization, pooled immunoglobulin should be given [45, 59]. Intramuscular pooled immune globulin provides 85–90% protection against hepatitis A [59]. Guidelines are available with regard to administration and dosing specifics [59].

Hepatitis B

Hepatitis B can cause illnesses ranging from an acute hepatitis to a chronic infection that can predispose to cirrhosis, liver failure, and hepatocellular carcinoma. It is transmitted through contact with infected blood or body fluids, and routes of transmission can include maternal-child, sexual contact, unsafe injections, or blood transfusions [60]. Hepatitis B, specifically chronic hepatitis B, affects over 250 million people worldwide with the highest prevalence in the Western Pacific area and Africa. [61] For SOT recipients who were not vaccinated prior to transplant, immunization against hepatitis B before travel is indicated for travelers with higher risk of exposure including those who will be living or traveling in endemic areas for extended periods; those who are likely to need medical care, procedures, or blood products while abroad; and those who anticipate having new sexual partners [15]. Again, if the patient has not been immunized prior to transplant, they can be

immunized prior to departure; however, immune response to the standard hepatitis B vaccine posttransplant is poor. The high-dose vaccination series may be more effective in this population; therefore, immunization with a vaccine containing 40 mcg of hepatitis B surface antigen given in a three- or four-dose schedule is recommended [62]. Anti-HB titers can be measured to assess vaccine efficacy, and a titer of >10 IU/ml is considered protective [42, 62]. Although the need for booster doses is controversial in SOT recipients, they can be considered for high-risk travelers. Hepatitis B vaccination series is typically given over a 6-month span; however, accelerating the schedule to give each vaccine dose 4 weeks apart is possible if travel departure date limits time [15].

For HSCT recipients, hepatitis B vaccine is part of the standard posttransplant immunization schedule [29, 42, 45–47]. Most schedules include a minimum of three doses of hepatitis B vaccine, and anti-HB titers can be measured to assess if booster or repeat vaccine series is required. High-dose vaccine may be more effective in this population.

The combination hepatitis A and B vaccine (such as Twinrix®) is sometimes used for those needing both vaccines; however, the response is often poor in this patient population, and giving the components (A and B) separately is advised for SOT recipients.

Hepatitis E

Hepatitis E is another viral cause of hepatitis that is often self-limiting, but in some cases more severe infections can occur including fulminant hepatitis leading to liver failure, and cases of chronic hepatitis E have been seen in immunocompromised patients. [63] It is spread through fecal-oral route and associated with contaminated drinking water and poor sanitation and rarely has been transmitted through ingestion of infected animals. It is found worldwide but more common in resource-limited countries with the highest prevalence in East and South Asia. [63] There is no specific vaccine for prevention; however, patients should practice food and water precautions and avoid eating raw or undercooked meat products especially in high-risk areas [64].

Polio

Poliovirus is a neurotrophic virus that causes poliomyelitis and can lead to irreversible paralysis. It is spread through fecal-oral transmission, typically person to person, but can also be contracted through contaminated water [65]. Wild-type poliovirus has been eradicated from the Western Hemisphere; however, wild-type virus still exists in regions of sub-Saharan Africa and South Asia, and to this day, Afghanistan, Nigeria, and Pakistan remain endemic countries [66]. On occasion, outbreaks of vaccine-associated poliomyelitis have also occurred.

In efforts to eradicate polio worldwide, the majority of the population has received polio vaccine during childhood immunization series. For HSCT recipients, inactivated polio vaccine is part of the standard posttransplant schedule. Patients typically receive at least three doses and thus should be up to date once they have completed this schedule [29, 42, 45–47]. For adult SOT travelers, this should be

verified, and they should also receive a booster when traveling to endemic areas. [67, 68] For subsequent travel, booster immunization may be prudent for individuals traveling to locations with circulating wild-type polioviruses or polio outbreaks.

Careful attention should be paid to the fact that two forms of the polio vaccine are available: the live oral poliovirus vaccine (OPV) and the injected inactivated poliovirus vaccine (IPV). The oral, live attenuated vaccine is contraindicated in transplant recipients. Also, household contacts of immunocompromised individuals should not receive the OPV as it is possible to spread vaccine strain poliovirus through fecal-oral contact.

Vector-Borne Viral Infections

Yellow Fever

Yellow fever (YF) virus is a flavivirus that can cause hemorrhagic fever with a 20% mortality rate. [69] It is spread through bites from mosquitos, typically the *Aedes* and *Haemagogus* species [70]. As of 2018, 47 countries in South America and Africa either were endemic or had areas of endemicity, with the latest outbreak occurring in Brazil [70].

The yellow fever vaccine (YFV) is an effective vaccine that offers lifelong immunity with one dose [65]. However, it contains a live attenuated viral strain and is contraindicated in SOT recipients. [41, 43, 71–73] Interestingly, a study of 53 SOT patients who received yellow fever vaccine prior to transplantation demonstrated persistence of yellow fever antibodies posttransplant; detectable antibodies persisted after a median duration of 13 years. [74] Therefore, those who plan to travel to or have reasons to potentially need to travel to yellow fever areas should be immunized pre-transplantation. Antibody testing can be done posttransplant to ensure persistent immunity, with a suggested threshold of a YF neutralization test >10 U/l required for travel to high-risk areas. [74]

Those SOT recipients who have not been vaccinated or were vaccinated pre-transplant but do not demonstrate protective antibodies should be advised not to travel to endemic regions. They should not receive the yellow fever vaccine post-transplant. If avoiding travel is not possible, they should not go in peak season. [43, 75]

For HSCT recipients, data is limited. As per the *Guidelines: Vaccination of hematopoietic cell transplant recipients*, the risk-benefit of yellow fever vaccine should be considered [45]. Since it is a live attenuated vaccine, patients should be >24 months posttransplant with no active GVHD and off immunosuppressive therapy [45]. A retrospective study of 21 post-allogeneic (allo)-HSCT recipients immunized with yellow fever vaccine, a median of 39 [21–86] months post-transplant, reported no side effects [76]. Thus, this vaccine could be considered for long-term survivors of HSCT who are traveling to high-risk areas, and consultation with travel medicine expert familiar with immunocompromised patient would be advised.

When yellow fever vaccine has not been given, patients should travel with a yellow fever vaccine waiver letter stating the contraindication to vaccination.

Patients should be counseled to present this letter when entering countries that require proof of yellow fever vaccine and are generally acceptable to most governments.

Family members of immunosuppressed patients may receive yellow fever vaccine.

Japanese Encephalitis

Japanese encephalitis (JE) virus of the genus *Flavivirus* is an arbovirus that causes encephalitis. It causes up to 10,000 deaths annually in Asia and can lead to long-term neuropsychiatric sequelae in survivors [77, 78]. JE is transmitted by mosquitoes (*Culex* species) and is the most common vaccine-preventable encephalitis in Asia [78]. It is more common in rural and agricultural areas specifically those with significant irrigation such as rice paddies. Peak seasons occur in the summer and fall, but can vary based on rain patterns [78].

Immunization against Japanese encephalitis with two doses of inactivated JE vaccine can be considered for individuals with intense rural travel in areas of Asia endemic for JE, especially during periods of increased transmission [78, 79]. It is recommended for travelers with trips planned for longer than 1 month or with plans for extensive rural travel in endemic areas [80]. Although vaccine efficacy data in SOT and HSCT recipients is limited, inactivated JE vaccine is recommended in transplant recipients for the same indications. A booster dose should be given for re-exposure or continued JE risk if the primary series was administered $\geq$1 year previously. [77–79] Some JE vaccines available in Asia contain live virus; these are contraindicated in SOT recipients and those post-HSCT who do not meet LAV criteria. Patients should be aware they should not receive such vaccines [15].

Dengue, Chikungunya, and Zika

Dengue, chikungunya, and Zika are all arboviruses or arthropod-borne viruses transmitted by mosquitos, most commonly the *Aedes aegypti*. Although dengue is the most common mosquito-borne virus, increasing cases of chikungunya and Zika are being seen in tropical and subtropical regions [81–83]. They can all pose a risk to immunocompromised hosts including traveling transplant recipients [84]. To date, no vaccines currently exist to prevent these viral illnesses, and patients should be counseled on mosquito protection.

Dengue virus is of the genus *Flavivirus*. It typically causes a self-limited flu-like illness, but serious complications including dengue hemorrhagic fever and dengue shock syndrome can be fatal. Patients who have previously suffered from or been exposed to dengue fever are at increased risk of serious complications such as dengue hemorrhagic fever or dengue shock syndrome during a second infection. This is especially concerning in immunocompromised hosts [81]. Clinical course and outcomes in SOT recipients may be similar to that of immunocompetent patients, [85, 86] but studies have raised concern for worse outcomes including an increased risk of dengue shock syndrome leading to death as well as graft dysfunction. [87, 88] Limited data exists regarding dengue in HSCT patients, although case

series of post-HSCT recipients suggests prolonged viremia in this patient population [89, 90].

Chikungunya is of the *Alphavirus* genus. It presents with some unique symptoms which helps to distinguish it from other arboviruses. Febrile illness with abrupt fevers, maculopapular rash, and arthralgias are characteristic. The disease is typically self-limited, but literature does report that SOT recipients can develop chronic polyarthritis from this viral infection [81, 91].

Zika is another *Flavivirus*. It was first reported in Uganda, and there have been outbreaks in Africa, Asia, the Americas, and the Pacific. Most recently, in 2015, Brazil reported association between Zika, pregnancy, and microcephaly. Since then, Zika has spread throughout the Americas prompting travel recommendations for women who are or may wish to become pregnant and those interested in preventing sexual transmission of Zika [92, 93]. In adult patients, it presents as a non-specific viral illness but has been linked to Guillain-Barré syndrome (GBS). Data is limited in transplant recipients, and further studies are needed to appreciate the impact on this patient population [81, 90, 94, 95].

Rabies

Rabies (*Rhabdoviridae*, genus *Lyssavirus*) is a neurotropic virus that causes acute encephalomyelitis. The WHO reports 59,000 deaths per year from rabies, with most cases occurring in Africa and Asia. Rabies is transmitted through the saliva from infected animal bites [96]. All mammals are susceptible to the virus, with most common being terrestrial carnivores and bats [96]. The majority of human cases occur from transmission via dog bites in endemic areas, but wild animals including bats worldwide are of concern [96, 97]. All travelers should be counseled regarding the risk of animal bites while abroad, especially in rabies-endemic countries, and in general should avoid contact with free-roaming and wild animals. Pre-exposure rabies vaccine can be given pre-departure to select patients with high risk of exposure. For example, it may be considered in long-term travelers to endemic areas, individuals expecting significant animal exposure, and individuals who plan to be in remote areas without access to medical care [15, 96].

In the event of an animal bite while traveling, all transplant recipient should seek medical care. For those with rabies exposure or potential rabies exposure, patients should receive postexposure prophylaxis. This involved thorough and immediate cleansing of the wound with soap and water. Those who have not previously been immunized require human rabies immune globulin (HRIG) injected into the wound directly with the remainder given intramuscularly and postexposure prophylaxis with multidose vaccine regimen [97]. Those who have received pre-exposure prophylaxis require two more doses of the vaccine, but do not require HRIG. Since SOT recipients may not mount adequate antibody responses to the rabies vaccine, some experts recommend administration of HRIG after all at-risk exposures [80, 97, 98].

Bacterial Infections

Salmonella enterica Serovar Typhi

Salmonella enterica serovar Typhi is a bacterial infection that causes typhoid fever. It affects up to 20 million people and leads to 160,000 related deaths worldwide each year [99]. *Salmonella typhi* is spread through contaminated food and water and infects the gastrointestinal tract. Typhoid fever classically presents with systemic illness including high fevers, abdominal pain, and altered bowel movements. Some patients will develop blood stream infections. SOT recipients are at risk of severe complications [100].

Treatment for *Salmonella typhi* involves antibiotic therapy; typically, fluoroquinolones have been used, but with increasing resistance especially in South and Southeast Asia, alternatives may be needed. If possibility of resistance is high, third-generation cephalosporins are favored [101].

Given the risk of severe illness, immunization is recommended for travel to endemic areas. Two vaccines are available. The oral live attenuated vaccine is contraindicated in immunocompromised patients. Transplant recipients should receive the injectable polysaccharide vaccine. This should be given at least 2 weeks prior to departure and repeated every 2 years for ongoing travel or future trips to endemic areas. Travelers should be counseled that the immune response to this vaccine may be suboptimal. Travelers should be counseled about food and water precautions.

Vibrio cholerae

Vibrio cholerae is a gram-negative bacillus that infects the intestinal tract; infections in humans are most often caused by serogroups O1 and O139 which are the toxigenic strains. It is most often acquired from contaminated water but has also been associated with fish and shellfish consumption. Person-to-person transmission is possible but rare. It is generally associated with poor sanitation. *Vibrio cholera* is endemic in approximately 50 countries worldwide, mainly in Africa and South and Southeast Asia. It is often associated with natural disasters. For example, more recently, it has been seen more frequently in the Caribbean with a large outbreak in Haiti associated with the 2010 earthquake. Cholera presents as watery diarrhea, and when severe or if left untreated, it can cause severe fluid loss and life-threatening dehydration [102].

The mainstay of treatment is rehydration. In most countries, doxycycline is the first-line antibiotic, but patterns of resistance are emerging, and antibiotic susceptibility testing on the specimen is recommended.

Prevention of infection in travelers is mainly through food and water precautions as well as proper hand hygiene. Three inactivated oral cholera vaccines exist and include the inactivated, oral travelers' diarrhea and cholera vaccine (Dukoral®). This vaccine offers protection against *Vibrio cholera* serogroup O1 [103, 104]. In general, this vaccine is not recommended routinely for travelers, but physicians may wish to consider it on an individual basis for those at high risk of exposure. [105–107] It is

administered in two doses 1 week apart with a booster every 2 years if ongoing travel [103]. It is not available within the United States, but is available in other countries such as Canada. Within the United States, the oral cholera vaccine (Vaxchora®) is a live vaccine; it is not recommended for transplant recipients as safety and efficacy have not been established in immunocompromised patients.

Enterotoxigenic *Escherichia coli* (ETEC)

Traveler's diarrhea is the most common travel-related illness. Bacterial pathogens are the most common cause, accounting for up to 90% of cases [108]. The enterotoxigenic strain of *E. coli* or ETEC is the most common cause of traveler's diarrhea. Highest-risk areas include Asia, the Middle East, Africa, Mexico, and Central and South America [109]. The route of spread is fecal-oral and mainly is acquired through contaminated food or water. Clinical presentation involves acute watery diarrhea that can become severe. The mainstay of treatment involves rehydration. Antibiotics that can be used include fluoroquinolones and macrolides; therefore, many travelers will travel with antibiotics on hand to be started based on physician instructions depending on severity and duration of traveler's diarrhea. Prevention mainly entails food and water precautions as well as proper hand hygiene. The inactivated, oral travelers' diarrhea and cholera vaccine (Dukoral®) has not shown sufficient evidence to support reliable protection against travelers' diarrhea caused by enterotoxigenic *E. coli* [103, 104].

Neisseria meningitidis

Neisseria meningitidis is an encapsulated, gram-negative diplococcus spread person to person through aerosolized droplets or secretions. There are eight main serovars that cause invasive infections in humans. Clinical disease includes meningitis, bacteremia, and shock with high case fatality rates (5–15%). Suspected infections require prompt evaluation, diagnosis, and treatment; typically beta-lactam antibiotics such as penicillin or ceftriaxone are recommended.

Immunization against meningococcal disease is recommended for travelers to endemic areas, such as the "meningitis belt" of sub-Saharan Africa, and for those traveling to Saudi Arabia for the Muslim pilgrimages of *hajj* or *umra*, where proof of vaccination is required [110]. SOT recipients traveling to areas of risk should receive two doses of the conjugated quadrivalent vaccine (MenACWY) prior to departure with booster repeated every 5 years. For HSCT recipients, quadrivalent meningococcal disease may be part of the standard posttransplant schedule as per country recommendations [29, 42, 45–47]. HSCT recipients who are not vaccinated and traveling to areas of risk should be immunized prior to departure. The conjugated vaccine protects against serotypes A, C, W, and Y; traditionally serotype A was of most concern for travelers, but more recently outbreaks with C and W have been seen [110]. Separate serogroup B meningococcal vaccines (MenBV) are now also available and can be considered in transplant recipients. This vaccine is not yet generally recommended for travel [110], but should be considered for transplant recipients traveling to endemic areas [80].

Streptococcus pneumoniae

Streptococcus pneumoniae is a gram-positive coccus, and infection with this bacterium causes clinical presentations such as pneumonia, bacteremia, and meningitis. It is one of the most common causes of community-acquired pneumonia worldwide. Developing countries have higher incidence [111]. Treatment of infection involves antibiotic therapy with beta-lactam or macrolide; the antibiotic choice, route, and duration depend on diagnosis and severity of the illness. For prevention, two pneumococcal vaccines are available, and transplant recipients should be up to date prior to travel if these have not been administered within the past 5 years. SOT recipients should have received at least one dose of the conjugated pneumococcal vaccine (Prevnar®13, PCV13) followed by the polysaccharide pneumococcal vaccine (Pneumovax®23, PPSV23) [42, 44]. HSCT recipients should complete pneumococcal vaccines as part of the standard posttransplant schedule, receiving multiple doses of the conjugated vaccine and finishing with the polysaccharide [29, 42, 45–47].

Clostridium tetani

Clostridium tetani is an anaerobic gram-positive bacillus that causes tetanus. It is distributed worldwide in the environment, but is more common in rural areas [112]. It is transmitted through contact with broken skin barrier, often occurring during an injury. Tetanus causes acute spasms and muscle rigidity, often starting locally but can become generalized and lead to death. Treatment involves wound care, tetanus immune globulin, antibiotic therapy typically with metronidazole, tetanus toxoid vaccine, and supportive management. Infection can be prevented through immunization, which is universally recommended. Although tetanus is rare among travelers, all transplant recipients should have a tetanus booster if they are not up to date before traveling. Patients can be immunized with combination vaccine for tetanus, diphtheria, and acellular pertussis (Tdap) [42].

Corynebacterium diphtheriae

Corynebacterium diphtheriae causes diphtheria; most commonly respiratory diphtheria can cause inflammation of pseudomembranes which can lead to fatal airway obstruction. Mortality occurs in 5–10% of cases. It is transmitted person to person through droplets. In tropical countries, cutaneous diphtheria can occur through direct skin contact. Diphtheria continues to be endemic in many countries worldwide with outbreaks occurring. It is more common in resource-poor regions. Treatment requires diphtheria antitoxin and antibiotic therapy, usually penicillin or macrolides. Diphtheria toxoid vaccine is universally recommended, and travelers, including transplant recipients, should be up to date. A booster should be administered prior to travel to endemic areas or those with outbreaks if patients were last vaccinated more than 10 years previously. Patients can be immunized with combination vaccine for tetanus, diphtheria, and acellular pertussis (Tdap) [42].

Bordetella pertussis

Bordetella pertussis is a gram-negative aerobic bacillus that causes pertussis or more casually known as whooping cough. It is highly communicable with transmission

via respiratory droplets. Unfortunately, worldwide incidence of pertussis is on the rise, with 90% of pertussis occurring in developing countries. Treatment is with antibiotic therapy with macrolides, specifically erythromycin, recommended first line. [113] All travelers including transplant recipients should be protected [114]. Patients can be immunized with combination vaccine for tetanus, diphtheria, and acellular pertussis (Tdap) [42].

Mycobacterial Infections

Mycobacterium tuberculosis

Mycobacterium tuberculosis is an acid-fast bacillus that causes tuberculosis (TB). It is transmitted person to person through airborne particles. TB can lead to both active, pulmonary and extrapulmonary, and latent infections. In 2017, the WHO reported an estimated ten million new cases of TB and 1.6 million deaths [115]. Immunocompromised patients, including transplant recipients, are at higher risk of TB. In these patients, most cases are felt to be reactivation of latent infection from prior exposure; however, new infection can be acquired posttransplant with higher risk in endemic countries. Clinical manifestation of TB in SOT recipients can present differently, and mortality rates are high [116]. Treatment of active TB involves multidrug regimen with careful consideration for interactions with immunosuppressive therapy.

Although there are no specific recommendations for prevention of TB exposure in travelers, transplant recipients should be cognizant of risk while traveling in endemic areas for prolonged periods of time or if their itinerary involves high-risk exposure. For example, use of an appropriate mask (fitted N95) for those working or volunteering in high-risk healthcare settings in endemic regions should be considered [15]. If there is concern for TB exposure while abroad, a post-travel tuberculosis skin tests with the purified protein derivative (PPD) or an interferon-gamma release assay may be helpful; however it should be noted that immunocompromised patients are at risk of false-negative tests. TB screening in asymptomatic patients should only be done if there is a known exposure to active tuberculosis infection or if there has been prolonged exposure in a high-risk environment such as working or volunteering in a healthcare setting in an endemic area [117]. The bacillus Calmette-Guérin (BCG) vaccine is a live attenuated strain of *M. bovis* and is contraindicated in transplant recipients due to risk of disseminated BCG infection.

Fungal Infections

SOT and HSCT recipients are at higher risk for fungal infection compared to the immunocompetent population. Although this risk is universal and not limited to travel exposures, certain fungal infections are known to be linked to geographic areas. [118, 119] Transmission or acquisition of infection is generally through environmental exposures and most commonly through inhalation or cutaneous inoculation. Clinical presentation is variable and depends on organ system involved, but can include pulmonary disease, cutaneous disease, rhino-orbital-cerebral disease, and disseminated infection among others. Optimal antifungal therapy depends on the

fungal species responsible for infection, and surgical excision or debridement should be considered when appropriate or necessary for source control. Prevention revolves around avoidance of high-risk environmental exposures.

Certain geographic areas pose higher risk of endemic fungal infections. In 2002, Ericsson et al. published a review of fungal infections among returning travelers that had been reported through the GeoSentinel network [120]. This study reported up to 0.5% monthly morbidity of illness in returning travelers was due to fungal infection and specifically review cases due to histoplasmosis, coccidioidomycosis, and penicilliosis (now talaromycosis). In 2013, Lortholary et al. reviewed the published literature of fungal infections in immunocompromised travelers [121]. Endemic fungal infections, including histoplasmosis, blastomycosis, and coccidioidomycosis in North America, paracoccidioidomycosis in South America, and talaromycosis due to *Talaromyces* (formerly *Penicillium*) *marneffei* infection in Southeast Asia, can be acquired during travel [21, 80, 119–124]. More details on the geographic distribution of these endemic fungal infections are summarized in Table 1.

Transplant recipients should be counseled to be diligent with regard to avoiding high-risk exposures for respiratory exposures such as visiting caves (spelunking). If exposures cannot be avoided, surgical masks can be worn to reduce risk of inhalational exposure. Other fungal infections can be transmitted through direct skin inoculation from the environment; thus gloves or skin covering can be donned to minimize direct skin inoculation.

Parasitic Infections

A wide variety of parasites exist worldwide and are generally classified as helminths, protozoa, and ectoparasites. They can cause infection of the blood, tissue, and intestinal tract in human hosts. In SOT recipients, parasite infections can occur through reactivation, donor-derived acquisition, or de novo acquisition, especially in patients who live or spend prolonged periods of time in endemic areas. A Canadian study found 29% of 3,528 returning travelers, and new immigrants had parasitic infection [125]. Travelers who were visiting friends and relatives (VFR) seemed to be at high risk. With increasing travel among SOT recipients, the incidence of parasitic infections in returning travelers is expected to grow [126] (Tables 3 and 4).

Vector-Borne Parasite Infections

Vectors, mainly insects, can spread parasite infections to human hosts. These include through mosquitos (*Plasmodium* spp.), sand flies (*Leishmania* spp.), triatomine bugs or tsetse flies (*Trypanosoma* spp.), and ticks (*Babesia* spp.) [127].

Plasmodium Species

Five species of *Plasmodium*, including *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*, cause malaria. Malaria remains a large global health problem with most recent estimates of 219 million infections in 87 countries and 435,000 deaths worldwide [128]. It is transmitted through mosquitos (female *Anopheles*). Malaria is an acute febrile illness that can quickly progress to severe illness and death if left

Table 1 Geographic distribution of endemic fungal infections

Fungal infection	Geographic distribution
Blastomycosis	
Blastomyces dermatitidis	Midwestern, South Central, and Southeastern United States and Canada (especially Ohio and Mississippi Rivers, the Great Lakes, Saint Lawrence River, and Northern Ontario)
Coccidioidomycosis	
Coccidioides immitis ***Coccidioides posadasii***	Southwestern United States (Arizona, California, Nevada, New Mexico, Texas, Utah), Mexico, and Central and South America
Cryptococcosis	
Cryptococcus gattii	Pacific Northwest United States (Washington, Oregon) and Canada (British Columbia and Vancouver Island), Australia, Papua New Guinea Also seen in parts of Africa, Asia, Europe, Mexico, and South America
Histoplasmosis	
Histoplasma capsulatum	Worldwide especially river valleys in the United States (Ohio and Mississippi Rivers), Canada (Ontario, Quebec especially Saint Lawrence River) Also seen in Central and South America, Africa, Asia, and Australia
Paracoccidioides	
Paracoccidioides brasiliensis	Central and South America (Brazil, Venezuela, Colombia, and Argentina)
Talaromycosis	
Talaromyces marneffei	Southeast Asia, southern China, eastern India

untreated. Like all travelers to endemic areas, transplant recipients are at risk. Treatment involves prompt diagnosis and administration of antimalarials; the choice and route depend on causative species, area of acquisition along with known resistance patterns, level of parasitemia, and severity of illness. Supportive care and close monitoring are especially important in severe disease.

Patients traveling to malaria areas should be provided prophylaxis prior to departure and be counseled on insect protection including use of bed nets. Transplant recipients should be provided prophylaxis against malaria as per routine recommendations based on area of travel and resistance patterns, but careful attention needs to be paid to drug interactions with immunosuppressive therapy [129]. The *Center for Disease Control (CDC) Yellow Book* among other resources provides country-specific guidelines and patient-related considerations for selecting a prophylactic agent [130]. The AST Guidelines provide additional information including a summary of antimalarial interactions with common medications taken by SOT recipients including calcineurin inhibitors, mTOR inhibitors, and/or trimethoprim-sulfamethoxazole [15], some of which are also by HSCT recipients. This information has been adapted from several references and summarized in Table 5.

Table 2 Travel-related infections by organism

Viral
Chikungunya virus
Dengue virus
Hepatitis A, B, and E viruses
Influenza virus
Japanese encephalitis virus
Measles virus
Middle Eastern respiratory syndrome coronavirus
Norovirus
Poliovirus
Rabies virus
Yellow fever virus
Zika virus
Bacterial
Bordetella pertussis
Burkholderia pseudomallei
Clostridium tetani
Enterotoxigenic
Escherichia coli Leptospira spp.
Neisseria meningitidis Rickettsia spp.
Salmonella enterica serovar
Typhi
Streptococcus pneumoniae Vibrio cholerae Yersinia pestis
Mycobacteria
Mycobacterium tuberculosis
Fungal
Blastomyces dermatitidis
Coccidioides immitis
Coccidioides posadasii
Cryptococcus gattii
Histoplasma capsulatum
Paracoccidioides brasiliensis
Talaromyces marneffei
Parasites
Cryptosporidium spp.
Cyclospora cayetanensis
Babesia spp.
Brugia spp.
Entamoeba spp.
Fasciola spp.
Giardia intestinalis
Leishmania spp.
Onchocerca volvulus
Schistosoma spp.
Strongyloides stercoralis
Taenia spp.
Toxoplasma gondii
Wuchereria bancrofti

Table 3 Recommendations for vaccination for solid organ transplant travelers [15], 44, 72]

Vaccine	Recommendations for adults
Routine immunizations	
Parenteral influenza	Yearly Should be considered prior to travel for patients traveling to opposite hemisphere during flu season
Pneumococcal conjugated and polysaccharide	Recommended; booster after 5 years
Tetanus/diphtheria	Recommended; booster every 10 years
Pertussis	Recommended in combination with tetanus and diphtheria (Tdap) once
Measles, mumps, and rubella (MMR)[a]	Contraindicated If serostatus or immunization history is unknown, perform serology prior to travel to areas of outbreak
Varicella[a]	Contraindicated
Travel immunizations	
Hepatitis A	Recommended when indicated
Hepatitis B	Recommended when indicated
Meningococcal conjugate	Recommended when indicated
Meningococcal serogroup B	Recommended when indicated
Inactivated polio vaccine (IPV)	Recommended when indicated
Rabies	Recommended when indicated
Japanese encephalitis (inactivated)	Recommended when indicated
Cholera vaccine	Recommended when indicated; non-live available in Canada and elsewhere; live vaccine contraindicated
Typhim Vi	Recommended when indicated
S. typhi Ty21a[a]	Contraindicated
Oral polio vaccine (OPV)[a]	Contraindicated in patients and family/household members
Bacillus Calmette-Guérin (BCG)[a]	Contraindicated
Yellow fever vaccine (YFV)[a]	Contraindicated

Adapted from the Centers for Disease Control and Prevention "Recommended Adult Immunization Schedule — United States, 2018," [114] "Advising Travelers with Specific Needs: The Immunocompromised Traveler" [43] in Centers for Disease Control and Prevention's "Health Information for International Travel," "Guidelines for vaccination of solid organ transplant candidates and recipients" [44], and the AST Guidelines [15]

[a]*Live attenuated*

Leishmania Species

Leishmania species cause protozoan infection known as leishmaniasis that can be classified as cutaneous, mucocutaneous, or visceral. An estimated one million new cases occur globally each year [131]. Visceral leishmaniasis most often occurs in

Table 4 Recommendations for vaccination for hematopoietic stem cell transplant travelers

Vaccine	Recommendations for adults
Routine immunizations	
Parenteral influenza	Yearly Should be considered prior to travel for patients traveling to opposite hemisphere during flu season
Pneumococcal conjugated and polysaccharide	Recommended as part of post-transplant immunization series; booster after 5 years
Tetanus/diphtheria	Recommended as part of post-transplant immunization series; booster every 10 years
Pertussis	Recommended as part of post-transplant immunization series
Hepatitis B	Recommended as part of post-transplant immunization series
Inactivated polio vaccine (IPV)	Recommended as part of post-transplant immunization series
Meningococcal conjugate	Recommended as part of post-transplant immunization series
Measles, mumps, and rubella (MMR)[a]	Typically given if serology indicates nonimmune at 24 months post-transplant if patient meets criteria to receive live attenuated vaccine (LAV)[b]
Varicella[a]	Typically given if serology indicates nonimmune at 24 months post-transplant if patient meets criteria to receive live attenuated vaccine (LAV)[b]
Travel immunizations	
Hepatitis A	Recommended when indicated
Meningococcal serogroup B	Recommended when indicated
Rabies	Recommended when indicated
Japanese encephalitis (inactivated)	Recommended when indicated
Cholera vaccine	Recommended when indicated; non-live available in Canada and elsewhere; live vaccine contraindicated
Typhim Vi	Recommended when indicated
S. typhi Ty21a[a]	Contraindicated; an inactivated vaccine exists and is recommended for these patients
Oral polio vaccine (OPV)[a]	Contraindicated; an inactivated vaccine exists and is recommended for these patients
Bacillus Calmette-Guérin (BCG)[a]	Contraindicated
Yellow fever vaccine (YFV)[a]	Contraindicated, however, may be considered if indicated if patient is 24 months post-transplant and meets criteria to receive live attenuated vaccine (LAV)[b]

Adapted from the Centers for Disease Control and Prevention "Recommended Adult Immunization Schedule — United States, 2018," [114] "Advising Travelers with Specific Needs: The Immunocompromised Traveler" [43] in Centers for Disease Control and Prevention's "Health Information for International Travel," and ASBMT: Guidelines for Preventing Infectious Complications among Hematopoietic Cell Transplantation Recipients: A Global Perspective [29]

[a] *Live attenuated*

[b] *Criteria to receive LAV: ≥ 24 months post-HSCT, no active GVHD, no immunosuppressive therapy*

Table 5 Travel-related medications, adult dosages, duration, and drug interactions

	Dose	Duration	Change for reduced GFR	Interactions with calcineurin inhibitors (CNI): tacrolimus and cyclosporine	Interactions with mTOR inhibitors: sirolimus, everolimus
Travelers' diarrhea treatment					
Ciprofloxacin	500 mg bid	For 3–7 days	Yes	May cause QT prolongation when used in combination May ↑CNI levels (cyclosporine)	
Levofloxacin	500 mg daily	For 3–7 days	Yes	May cause QT prolongation when used in combination May ↑CNI levels (cyclosporine)	
Azithromycin	500 mg daily	For 3–7 days	Use caution if CrCl<10 mL/minute	May ↑CNI levels	May ↑ levels
Malaria prophylaxis					
Atovaquone-proguanil	Atovaquone/ proguanil 250 mg/ 100 mg daily	Start 1–2 days prior to entering a malaria-endemic area, continue throughout the stay and for 7 days after leaving malarial area	No dose adjustment for mild to moderate renal impairment; avoid if severe renal impairment occurs (CrCl <30 ml/min)	No significant interactions	
Mefloquine	250 mg weekly	Begin 2 weeks before, arrival in endemic area, continuing weekly during travel and for	No	Can interact with tacrolimus to prolong QT	Everolimus may increase mefloquine levels

		4 weeks after leaving malarial area			
Doxycycline	100 mg daily	Start 1–2 days prior to entering a malaria-endemic area, continue throughout the stay and for 28 days after leaving malarial area	No	No significant interactions	
Chloroquine	500 mg/week (300 mg base)	Begin 1–2 weeks prior to exposure; continue for 4 weeks after leaving malarial area	Administer 50% of dose if CrCl<10 mL/minute	May ↑ cyclosporine levels Can interact with tacrolimus to prolong QT	May ↑ cyclosporine levels Can interact with tacrolimus to prolong QT
Hydroxychloroquine	400 mg/week	Begin 1–2 weeks prior to exposure; continue for 4 weeks after leaving malarial area	No, but may need to consider reduction	May ↑ cyclosporine levels	
Primaquine	52.6 mg/daily (30 mg base)	Begin 1–2 days before exposure; continue for 7 days after leaving malarial area	No	May ↑ cyclosporine levels Can interact with tacrolimus to prolong QT	

Adapted from Chap. 3 "Malaria" in the Centers for Disease Control and Prevention's Yellow Book [130], the AST Guidelines [15], Lexi-Comp®Lexi-Drugs, and Lexi-Comp® Drug Interactions

Brazil, Southeast Asia, and East Africa, whereas cutaneous leishmaniasis is more common in the Mediterranean Basin, Middle East, and Central Asia [131]. Transmission is through sandfly bites. Immunocompromised patients, including SOT recipients, are at higher risk of developing disease and more severe infection [132]. Treatment requires prompt diagnosis and treatment, with options including amphotericin B, pentavalent antimonials, miltefosine, and paromymycin [132]. Although rare, travelers can be exposed when visiting endemic regions. Transplant recipients should take precautions to prevent sandfly bites including use of protective clothing, insect repellent, and treated bed nets and minimize activities during dusk hours [82, 133].

Trypanosoma Species

Trypanosoma species are protozoan parasites that cause two specific disease entities.

In Africa, *Trypanosoma brucei* is transmitted through the tsetse fly and causes African trypanosomiasis or sleeping sickness. Fortunately, due to control efforts, the WHO reports a significant drop in the number of cases and is targeting elimination by 2020 [134]. Therefore, this is unlikely to pose a significant risk to travelers; however, transplant recipients should still take precautions to avoid tsetse fly bites while traveling to endemic areas.

Of more significant concern, in the Americas, *Trypanosoma cruzi* is transmitted through feces of triatomine insects and causes American trypanosomiasis or Chagas disease. It can also be transmitted through blood transfusions and organ transplantation. It is endemic in Mexico and Central and South America. The WHO reports an estimated seven million people worldwide are infected [135]. The disease can cause an acute phase and chronic phase, with the chronic phase leading to cardiomyopathy and disease involving the gastrointestinal tract and nervous system [135, 136]. Treatment requires trypanocidal therapy with benznidazole [136]. The risk of acquiring de novo infection can occur while traveling; however, the incidence of this occurrence is thought to be quite low. Nonetheless, transplant recipients should take precautions to minimize exposure to triatomine bugs by avoiding sleeping in thatched, mud, or adobe housing, by avoiding consumption of products that may have been contaminated with insect feces, and by being cautious of blood transfusions in endemic areas [136, 137].

Babesia Species

Babesia species are protozoan parasites which are transmitted through bites by *Ixodes* ticks. Cases have been reported worldwide; however, they are seen mostly in North America and Europe. Areas of high risk within North America include the Northeast and Midwest United States. Clinical infection can range from asymptomatic to flu-like illness to hemolytic anemia and thrombocytopenia. SOT recipients are at higher risk of severe and fatal infection even with proper treatment [138]. Other immunocompromised patients, including HSCT recipients and especially patients with asplenia, can also have more severe disease and are at risk for prolonged or refractory infection [139, 140]. Treatment consists of combination therapy with atovaquone plus azithromycin or clindamycin plus quinine; duration

of therapy may need to be extended in transplant recipients. In some patients, exchange transfusion may be indicated. Transplant recipients traveling to endemic areas, especially those with plans for significant outdoor activities, should take steps to prevent tick exposure including wearing long sleeves and using insect repellent (DEET). Tick checks should be performed on a regular basis [127].

Environmentally Transmitted Parasite Infections

Parasites can be transmitted through penetration of the skin during environmental exposures such as direct contact with soil or water. These parasites include hookworm (*Ancylostoma duodenale* and *Necator americanus*), *Strongyloides stercoralis*, and *Schistosoma* species.

Strongyloides stercoralis

Strongyloides stercoralis is a helminth or worm. Upwards of 100 million people worldwide are infected. It is found mainly in tropical and subtropical regions but has been reported in temperate climates as well. It is acquired by direct contact with contaminated soil. It can lead to asymptomatic infection or can cause strongyloidiasis affecting the intestinal tract, lungs, and skin. Patients with immunocompromising conditions, including SOT and HSCT recipients, are at higher risk of severe disease including hyperinfection syndrome and disseminated strongyloidiasis. This can lead to critical illness including shock and multi-organ failure with high rates of mortality. Treatment is with ivermectin, and duration depends on the type and severity of infection. Albendazole is an alternative. The risk for short-term travel is low but can increase with prolonged time spent in endemic areas or participating in activities that would increase exposure. Transplant recipients should wear appropriate footwear/shoes while in endemic areas especially if walking in areas that could be contaminated with human feces [141, 142].

Schistosoma Species

Schistosoma spp. are helminths that cause schistosomiasis. Species vary between geographic regions, and clinical syndrome can depend on which species is involved. The majority of the cases occur in Africa, and most common species include *S. mansoni* and *S. haematobium*. Other affected areas include the Middle East (*S. haematobium*) and Asia (*S. japonicum*, *S. mekongi*). Travelers often acquire infection in sub-Saharan Africa. Exposure is through freshwater in endemic areas with infection acquired when swimming, bathing, or wading in contaminated water. Clinical presentation of acute infection, known as Katayama syndrome, consists of fever, headache, diarrhea, and respiratory symptoms. Chronic disease can develop and depends on the type of species causing the infection with *S. mansoni* and *S. japonicum*, whereas *S. haematobium* affects the urinary tract. Treatment is with praziquantel. Travelers to endemic areas should avoid freshwater exposures including swimming, bathing, wading, water adventure sports, etc. [142, 143]

Ingested Parasitic Infections

Parasite infections can be acquired through ingestion of contaminated food or water or accidental ingestion of contaminated dirt/soil. These parasites include amebiasis (*Entamoeba* spp.), cryptosporidiosis (*Cryptosporidium* spp.), cyclosporiasis (*Cyclospora cayetanensis*), fascioliasis (*Fasciola* spp.), giardiasis (*Giardia intestinalis*), taeniasis (*Taenia* spp.), and toxoplasmosis (*Toxoplasma gondii*) among others. Geographic distribution, syndromes, and treatments are infection specific, and more information can be found in Chap. 3 of the *CDC Yellow Book* [144] and in the AST Guidelines on Parasitic Infections in Solid Organ Transplantation [126]. Generally speaking, for all of these parasitic infections, prevention centers around precautions to prevent food- and water-borne illnesses. Transplant recipients traveling to endemic areas or areas with poor sanitation should avoid eating raw or undercooked meats and fish and raw fruits and vegetables that they cannot peel themselves and should ensure safe drinking water. They should not drink from freshwater sources such as lakes, rivers, and streams.

Pre-departure Infectious Disease Evaluation and Recommendations

SOT and HSCT recipients who plan to travel should seek advice from a travel medicine specialist. All transplant recipients should be counseled on the importance of safe travel posttransplant and value of pre-departure assessment; this should be included as part of their pretransplant education and teaching.

SOT recipients should be advised not to travel to high-risk areas during times of increased immunosuppression such as the first year posttransplant or after treatment for rejection. [43, 80, 145] Recommendations for timing of travel post-HSCT depend on the type of transplant. Allo-HSCT recipients should be advised not to travel to high-risk areas for the first 6–12 months posttransplant or if on treatment for graft-versus-host disease (GVHD) with immunosuppressive therapy, whereas autologous (auto)-HSCT recipients may be able to travel as soon as 3–6 months posttransplant [146]. All patients should consult with their transplant physician(s) to ensure it is safe to travel as decisions should be made on an individual basis. Although patients may be out of the highest-risk period, it is important to note the post-HSCT immunization schedule is generally not completed until 24 months posttransplant date. Thus, this should be taken into consideration when determining timing of travel posttransplant and when providing recommendations for minimizing the risk of infection while traveling.

Infectious risk depends on the travel itinerary by both destination and type of travel, and recommendations for prevention of infection will vary accordingly. Important elements of the pre-departure evaluation include review of past medical history, current medications, and travel itinerary including destinations, accommodation type, and potential exposures; counseling on risk reduction strategies; counseling on when and where to seek medical attention should they fall ill while away; administration of recommended vaccinations; and prescribing of prophylactic medications. Patients should verify travel insurance coverage prior to departure.

Finally, a review of important items in the form of a packing list may also be helpful for travelers. Examples of such lists can be found in the *CDC Yellow Book* and the AST Guidelines [15, 147].

Vaccine Recommendations

Patients should be advised to seek pre-travel advice early when planning trips as full vaccine series may require several months prior to departure. Passive immunization with immune globulin can be considered for urgent travel situations. When possible, if patients indicate plans to travel after SOT, travel-related vaccines can be considered pretransplant. This is especially important if there is a need for live attenuated vaccines which are contraindicated posttransplant. SOT recipients should be up to date with their routine immunizations as immunocompromised individuals are at risk of common infections both at home and abroad. Recommendations are available through the AST Guidelines, the CDC, and other publications on routine immunizations in SOT recipients. [44, 114, 148, 149] More specific information regarding travel-related vaccines in immunocompromised individuals can be found in the *Yellow Book* [43] and other publications including recommendations for SOT recipients who travel specifically [15, 72, 80, 148, 150, 151]. Recommended immunizations have been summarized based on these recommendations in Table 3. Patients should travel with their immunization records as well as a waiver for yellow fever immunization indicating contraindication.

Recommended immunizations for HSCT recipients differ from those recommended for SOT. Typically, HSCT patients receive extensive immunization schedule posttransplant as it is believed their previous immunity to vaccine-preventable diseases (VPDs) may have been eliminated during the transplant process. Although post-HSCT immunization schedules differ between allo- and auto-HSCT recipients, both take approximately 18 months to complete and typically finish around 24 months posttransplant [42, 45, 47, 146, 152]. The last immunizations in these series are the live attenuated vaccines, including measles, mumps, and rubella (MMR) and varicella. For those patients who plan to travel once deemed safe to do so, common travel-related vaccines, such as hepatitis A, can be built into the standard immunization schedule. Specific travel-related vaccines should be considered on an individual basis and take into consideration the destination and trip itinerary [29, 47]. Recommended immunizations have been summarized in Table 4.

Risk Reduction of Non-vaccine-Preventable Infections

Education and counseling of patients prior to departure plays an important role in minimizing risk during travel. The AST Guidelines provide further information and helpful summary tables on important travel-related topics for SOT [15]. Similarly, the ASBMT Guidelines provide information for HSCT patients [29]. Basic infectious precautions including hand hygiene, avoiding sick contacts, and use of procedure mask while in transit (e.g., airplanes) or participating in high-risk activities should be reviewed.

Prevention of Food-Borne Illnesses and Diarrhea

Diarrhea is the most common illness of travelers, especially in travelers to low-resource countries. Although, in general, many episodes can be mild and self-limited, travelers' diarrhea can lead to complications and can even be life-threatening. Transplant recipients should be instructed on appropriate food and water precautions [105, 153–155]. They should avoid beverages that contain local water or ice and only drink boiled or bottled water. Other high-risk food and drink including unpasteurized dairy products, raw meats and fish, and undercooked foods should be avoided. Consumption of fruits and vegetables should be restricted to those they can peel themselves. All travelers should carry appropriate antibiotics for self-treatment of traveler's diarrhea. The choice of antibiotic depends on local resistance patterns of enteric pathogens, [105, 106, 156] and interactions with immunosuppressive agents should be taken into consideration (Table 5). Patients should be counseled on when to seek medical attention, such as non-resolving diarrhea, fever, or bloody stools.

Respiratory Infections

Respiratory infections are the second most common infection affecting travelers [56]. High-risk periods of travel include while in transit through public spaces such as airports or while on airplanes. Masks may be helpful in preventing respiratory infections. Active outbreaks of respiratory infections may warrant deferring travel.

Insect Protection

Travelers to areas endemic for mosquito- and tick-borne diseases should be counseled about minimizing insect bites. Strategies including use of repellents containing DEET (N,N-diethyl-3-methylbenzamide), protective clothing with long sleeves, and permethrin-impregnated clothing and bed nets should be considered [43, 105]. Efforts should be made to choose accommodation with air conditioning and screens.

Environmental Exposures

Infections that exist in the environment can pose a threat and include those that transmit via direct contact and those that transmit via respiratory particles or spores.

Water and soil exposure can pose a risk of infections through direct contact. Travelers should avoid swimming or bathing in freshwater when traveling in areas endemic for *Schistosoma* and should always wear footwear when in regions endemic for *Strongyloides stercoralis* [82].

Exposure to endemic fungal infections occurs through inhalation of spores found in the environment. Transplant recipients should avoid activities that place them at higher risk of exposure, and use of procedural masks may help prevent acquisition of infection.

Blood-Borne Pathogens

Travelers should avoid contact with non-sterile needles, syringes, and other medical equipment. If travel is planned to regions where healthcare settings may not be reliable, travelers should consider a first aid kit equipped with sterile needles, syringes, and sutures in case required while abroad.

Sexually Transmitted Infections (STI)

Travelers may be more likely to engage in sexual activity with new partners, counseling should be provided with regard to safe sex practices, and patients should be encouraged to have STI screening performed upon return home should they engage with new partners while abroad.

Post-travel Evaluation

Transplant recipients should contact their transplant center and be evaluated following return from travel if they develop new symptoms of infection. Workup and investigations will depend on evaluation of symptoms and timing of onset, as well as travel history with specific attention paid to destinations, activities, and exposures. In general, travelers who are well on return do not need to be seen after foreign travel. However, if they have been in high-risk situations or have a known or potential exposure to infection, then evaluation for necessary screening should be pursued. Examples of such may include working or volunteering in a healthcare setting, exposure to blood-borne pathogen, new sexual partner(s), etc.

Conclusion

Travel is an important component of many people's lives, whether it is for business, visiting friends and relatives, or leisure purposes. In 2017, the United Nations World Travel Organization documented 1.326 billion international tourist arrivals worldwide [157]. This number is expected to continue to increase. Among those traveling to international destinations include transplant recipients, who are immunosuppressed and at increased risk of infection. Transplant recipients should be counseled on the importance of pre-departure travel assessment when planning trips abroad, especially to areas of higher infection risk. They should seek medical advice before departure, and risk mitigation will depend on destination(s) and trip itineraries and should be tailored to the patients taking into consideration time since transplant, type of transplant, and immunosuppressive therapy.

Key Points

- Importance of safe travel should be discussed during the pretransplant evaluation.
- All transplant recipients should be seen by a travel medicine specialist prior to travel to regions with higher rates of infection.
- SOT patients should not travel to areas with higher infection risk during the first year posttransplant and/or during times of increased immunosuppression.
- HSCT patients should not travel to areas with higher infection risk during the first 3–12 months posttransplant depending on the transplant type and/or during times of increased immunosuppression and/or presence of GVHD.
- Travel recommendations, immunizations, and prophylaxis depend on destination and itinerary. Country-specific recommendations should be followed.

- Vaccine-preventable illnesses:
  - Patients should be up to date with routine immunizations:
    - Travel-related immunizations depend on travel destination.
  - Live vaccines are *contraindicated* in SOT recipients.
  - Live vaccines are contraindicated in HSCT recipients until 24 months post-transplant, no active GVHD, and they are no longer taking immunosuppressive therapy:
    - Patients traveling to countries with yellow fever immunization requirements should travel with an official waiver.
- Prophylaxis:
  - Necessary prophylaxis should be assessed by trip destination and itinerary.
  - One of the most important and common infections that requires prophylaxis when traveling is malaria.
    - Malaria prophylaxis should be based on country-specific guidelines.
    - Assess for drug interactions with immunosuppressant agents.
- Counseling:
  - Topics including prevention of food- and water-borne illness, respiratory infections, insect-borne illnesses, blood-borne infections, and sexually transmitted infections should be covered during the pre-travel visit.
- Patients should travel with adequate supply of medications (especially immunosuppressants) and copies of prescriptions.
- Patients should be aware of transplant centers at which they could seek medical attention if they become ill while away.
- Patients should have adequate travel medical insurance.
- Patients should be aware to seek medical attention if they develop signs and symptoms of infection upon return home after travel.

References

1. Kotton CN, Hibberd PL. Travel medicine and transplant tourism in solid organ transplantation. Am J Transplant. 2013;13(s4):337–47. https://doi.org/10.1111/ajt.12125.
2. *Human Organ and Tissue Transplantation*. 2004. https://www.who.int/transplantation/en/A57_R18-en.pdf. Accessed 25 Feb 2019.
3. Participants in the International Summit on Transplant Tourism and Organ Trafficking Convened by the Transplantation Society and International Society of Nephrology in Istanbul, Turkey, April 30–May 2, 2008. The declaration of Istanbul on organ trafficking and transplant tourism. Transplantation. 2008;86(8):1013–8. https://doi.org/10.1097/TP.0b013e318185ffc9.
4. *The Declaration of Istanbul on Organ Trafficking and Transplant Tourism (2018 Edition)*. 2018. https://www.declarationofistanbul.org/images/Policy_Documents/2018_Ed_Do/2018_Edition_of_the_Declaration_of_Istanbul_Final.pdf. Accessed 30 July 2018.
5. Caulfield T, Zarzeczny A. Curbing transplant tourism: Canadian physicians and the law. CMAJ. 2016;188(13):935–6. https://doi.org/10.1503/cmaj.160497.
6. WHO | WHO proposes global agenda on transplantation. *WHO*. 2010. http://www.who.int/mediacentre/news/releases/2007/pr12/en/. Accessed 1 Aug 2018.
7. Prasad GVR, Shukla A, Huang M, D'A Honey RJ, Zaltzman JS. Outcomes of commercial renal transplantation: a Canadian experience. Transplantation. 2006;82(9):1130–5. https://doi.org/10.1097/01.tp.0000241072.03400.11.

8. Prasad GVR, Ananth S, Palepu S, Huang M, Nash MM, Zaltzman JS. Commercial kidney transplantation is an important risk factor in long-term kidney allograft survival. Kidney Int. 2016;89(5):1119–24. https://doi.org/10.1016/j.kint.2015.12.047.
9. Geddes CC, Henderson A, Mackenzie P, Rodger SC. Outcome of patients from the west of Scotland traveling to Pakistan for living donor kidney transplants. Transplantation. 2008; 86(8):1143–5. https://doi.org/10.1097/TP.0b013e318187cdd0.
10. Gill J, Madhira BR, Gjertson D, et al. Transplant tourism in the United States: a single-center experience. Clin J Am Soc Nephrol. 2008;3(6):1820–8. https://doi.org/10.2215/CJN.02180508.
11. Cha R-H, Kim YC, Oh YJ, et al. Long-term outcomes of kidney allografts obtained by transplant tourism: observations from a single center in Korea. Nephrology. 2011; 16(7):672–9. https://doi.org/10.1111/j.1440-1797.2011.01480.x.
12. Babik JM, Chin-Hong P. Transplant tourism: understanding the risks. Curr Infect Dis Rep. 2015;17(4):18. https://doi.org/10.1007/s11908-015-0473-x.
13. Quach K, Sultan H, Li Y, Famure O, Kim SJ. Outcomes of kidney transplantation abroad. Prog Transplant. 2016;26(1):5–12. https://doi.org/10.1177/1526924816632133.
14. American Society for Transplantation and Cellular Therapy. Stem cell therapy and medical tourism – American Society for Transplantation and Cellular Therapy. https://www.asbmt.org/practice-resources/policy-statements/stem-cell-therapy-and-medical-tourism. Accessed 7 Oct 2019.
15. Buchan CA, Kotton CN. Travel medicine, transplant tourism, and the solid organ transplant recipient – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13529. https://doi.org/10.1111/ctr.13529.
16. Kennedy SE, Shen Y, Charlesworth JA, et al. Outcome of overseas commercial kidney transplantation: an Australian perspective. Med J Aust. 2005;182(5):224–7. http://www.ncbi.nlm.nih.gov/pubmed/15748132. Accessed 30 July 2018
17. Sever MŞ, Kazancioğlu R, Yildiz A, et al. Outcome of living unrelated (commercial) renal transplantation. Kidney Int. 2001;60(4):1477–83. https://doi.org/10.1046/j.1523-1755.2001.00951.x.
18. Chen LH, Wilson ME. The globalization of healthcare: implications of medical tourism for the infectious disease clinician. Clin Infect Dis. 2013;57(12):1752–9. https://doi.org/10.1093/cid/cit540.
19. Merion RM, Barnes AD, Lin M, et al. Transplants in foreign countries among patients removed from the US transplant waiting list. Am J Transplant. 2008;8(4p2):988–96. https://doi.org/10.1111/j.1600-6143.2008.02176.x.
20. Gill J, Diec O, Landsberg DN, et al. Opportunities to deter transplant tourism exist before referral for transplantation and during the workup and management of transplant candidates. Kidney Int. 2011;79(9):1026–31. https://doi.org/10.1038/ki.2010.540.
21. Martín-Dávila P, Fortún J, López-Vélez R, et al. Transmission of tropical and geographically restricted infections during solid-organ transplantation. Clin Microbiol Rev. 2008; 21(1):60–96. https://doi.org/10.1128/CMR.00021-07.
22. Marks PW, Witten CM, Califf RM. Clarifying stem-cell therapy's benefits and risks. N Engl J Med. 2017;376(11):1007–9. https://doi.org/10.1056/NEJMp1613723.
23. Caulfield T, Zarzeczny A, Toronto Stem Cell Working Group TSCW. Stem cell tourism and Canadian family physicians. Can Fam Physician. 2012;58(4):365–8, e182–5. http://www.ncbi.nlm.nih.gov/pubmed/22611603. Accessed 7 Oct 2019
24. FDA | FDA warns about stem cell therapies. https://www.fda.gov/consumers/consumer-updates/fda-warns-about-stem-cell-therapies. Accessed 3 Dec 2019.
25. Boggild AK, Sano M, Humar A, Gilman M, Salit I, Kain KC. Travel patterns and risk behavior in solid organ transplant recipients. J Travel Med. 2004;11:37–43.
26. Hochberg NS, Barnett ED, Chen LH, et al. International travel by persons with medical. Mayo Clin Proc. 2013;88(11):1231–40. https://doi.org/10.1016/j.mayocp.2013.07.018.
27. Roukens AHE, van Dissel JT, de Fijter JW, Visser LG. Health preparations and travel-related morbidity of kidney transplant recipients traveling to developing countries. Clin Transpl. 2007;21(4):567–70. https://doi.org/10.1111/j.1399-0012.2007.00691.x.

28. Mikati T, Griffin K, Lane D, Matasar M, Shah MK. International travel patterns and travel risks for stem cell transplant recipients. J Travel Med. 2015;22(1):39–47. https://doi.org/10.1111/jtm.12166.
29. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective recommendations. Biol Blood Marrow Transplant. 2009;15(10):1143–238. https://doi.org/10.1016/j.bbmt.2009.06.019.Guidelines.
30. WHO | Fact Sheet: Influenza (Seasonal). https://www.who.int/en/news-room/fact-sheets/detail/influenza-(seasonal). Accessed 7 Apr 2019.
31. Ison MG. Influenza prevention and treatment in transplant recipients and immunocompromised hosts. Influenza Other Respir Viruses. 2013;7:60–6. https://doi.org/10.1111/irv.12170.
32. Engelhard D, Mohty B, de la Camara R, Cordonnier C, Ljungman P. European guidelines for prevention and management of influenza in hematopoietic stem cell transplantation and leukemia patients: summary of ECIL-4 (2011), on behalf of ECIL, a joint venture of EBMT, EORTC, ICHS, and ELN. Transpl Infect Dis. 2013;15(3):219–32. https://doi.org/10.1111/tid.12054.
33. Goeijenbier M, van Genderen P, Ward BJ, Wilder-Smith A, Steffen R, Osterhaus ADME. Travellers and influenza: risks and prevention. J Travel Med. 2017;24(1) https://doi.org/10.1093/jtm/taw078.
34. Boggild AK, Castelli F, Gautret P, et al. Latitudinal patterns of travel among returned travelers with influenza: results from the GeoSentinel Surveillance Network, 1997–2007. J Travel Med. 2012;19(1):4–8. https://doi.org/10.1111/j.1708-8305.2011.00579.x.
35. Hirve S, Newman LP, Paget J, et al. Influenza seasonality in the tropics and subtropics – when to vaccinate? PLoS One. 2016;11(4):e0153003. https://doi.org/10.1371/journal.pone.0153003.
36. WHO | International travel and health: Seasonal influenza. http://www.who.int/ith/vaccines/seasonal_influenza/en/. Accessed 30 July 2018.
37. WHO | Fact Sheets: Measles. WHO. http://www.who.int/en/news-room/fact-sheets/detail/measles. Accessed 25 Mar 2019.
38. Measles in Europe: record number of both sick and immunized. February 2019. http://www.euro.who.int/en/media-centre/sections/press-releases/2019/measles-in-europe-record-number-of-both-sick-and-immunized. Accessed 25 Mar 2019.
39. Avery RK, Ljungman P. Prophylactic measures in the solid-organ recipient before transplantation. Clin Infect Dis. 2001;33(s1):S15–21. https://doi.org/10.1086/320899.
40. Molrine DC, Hibberd PL. Vaccines for transplant recipients. Infect Dis Clin N Am. 2001;15(1):273–305. https://doi.org/10.1016/S0891-5520(05)70279-8.
41. Duchini A, Goss JA, Karpen S, Pockros PJ. Vaccinations for adult solid-organ transplant recipients: current recommendations and protocols. Clin Microbiol Rev. 2003;16(3):357–64. https://doi.org/10.1128/CMR.16.3.357-364.2003.
42. Chong PP, Avery RK. A comprehensive review of immunization practices in solid organ transplant and hematopoietic stem cell transplant recipients. Clin Ther. 2017;39(8):1581–98. https://doi.org/10.1016/j.clinthera.2017.07.005.
43. Kotton CN, Kroger AT, Feedman D. Immunocompromised travelers – Chapter 8 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/advising-travelers-with-specific-needs/immunocompromised-travelers. Published 2017. Accessed 30 July 2018.
44. Danziger-Isakov L, Kumar D, the AST Infectious Diseases Community of Practice. Vaccination in solid organ transplantation. Am J Transplant. 2013;13(s4):311–7. https://doi.org/10.1111/ajt.12122.
45. Ljungman P, Cordonnier C, Einsele H, et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant. 2009;44(8):521–6. https://doi.org/10.1038/bmt.2009.263.

46. Carpenter PA, Englund JA. How I vaccinate blood and marrow transplant recipients. Blood. 2016;127(23):2824–32. https://doi.org/10.1182/blood-2015-12-550475.
47. L'Huillier AG, Kumar D. Immunizations in solid organ and hematopoeitic stem cell transplant patients: a comprehensive review. Hum Vaccin Immunother. 2015; 11(12):2852–63. https://doi.org/10.1080/21645515.2015.1078043.
48. WHO | Fact Sheet: Middle East respiratory syndrome coronavirus (MERS-CoV). https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov). Accessed 30 Mar 2019.
49. Watson JT, Gerber SI. Middle East Respiratory Syndrome (MERS) – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/middle-east-respiratory-syndrome-mers. Accessed 30 Mar 2019.
50. AlGhamdi M, Mushtaq F, Awn N, Shalhoub S. MERS CoV infection in two renal transplant recipients: case report. Am J Transplant. 2015;15(4):1101–4. https://doi.org/10.1111/ajt.13085.
51. Motabi IH, Zaidi SZA, Ibrahim MH, et al. Report of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in four patients with hematological malignancies treated at King Fahad Medical City, Riyadh, Saudi Arabia. Blood. 2016;128(22):4903. http://www.bloodjournal.org/content/128/22/4903. Accessed 21 Sept 2019
52. Hall AJ, Lopman B. Norovirus – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/norovirus. Accessed 8 Apr 2019.
53. Rosen J. Travel medicine and the solid organ transplant recipient. Infect Dis Clin NA. 2013; 27(2):429–57. https://doi.org/10.1016/j.idc.2013.02.009.
54. Bok K, Green KY. Norovirus gastroenteritis in immunocompromised patients. N Engl J Med. 2013;368(10) https://doi.org/10.1056/NEJMc1301022.
55. WHO | Fact Sheet: Hepatitis A. https://www.who.int/news-room/fact-sheets/detail/hepatitis-a. Accessed 25 Mar 2019.
56. Ryan ET, Kain KC. Health advice and immunizations for travelers. N Engl J Med. 2000; 342(23):1716–25. https://doi.org/10.1056/NEJM200006083422306.
57. Nelson NP. Hepatitis A – Chapter 3 – 2018 Yellow Book | Traveler's Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/hepatitis-a. Published 2017. Accessed 21 Sept 2018.
58. Jeon HJ, Ro H, Jeong JC, et al. Efficacy and safety of hepatitis A vaccination in kidney transplant recipients. Transpl Infect Dis. 2014;16(3):511–5. https://doi.org/10.1111/tid.12217.
59. Nelson NP. Updated dosing instructions for immune globulin (human) GamaSTAN S/D for hepatitis A virus prophylaxis. MMWR Morb Mortal Wkly Rep. 2017;66(36):959–60. https://doi.org/10.15585/mmwr.mm6636a5.
60. Te H, Doucette K. Viral hepatitis: guidelines by the American Society of Transplantation Infectious Disease Community of Practice. Clin Transpl. 2019;33(9):e13514. https://doi.org/10.1111/ctr.13514.
61. WHO | Fact Sheet: Hepatitis B. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. Accessed 25 Mar 2019.
62. Canadian Immunization Guide: Part 3: Vaccination of specific populations. Immunization of immunocompromised persons. https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-3-vaccination-specific-populations/page-8-immunization-immunocompromised-persons.html. Published 2017. Accessed 31 May 2017.
63. WHO | Fact sheet: Hepatitis E. https://www.who.int/news-room/fact-sheets/detail/hepatitis-e. Accessed 25 March 2019.
64. Hepatitis E – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/hepatitis-e. Accessed 25 Mar 2019.
65. WHO | Fact Sheet: Poliomyelitis. https://www.who.int/news-room/fact-sheets/detail/poliomyelitis. Accessed 25 Mar 2019.

66. Global Polio Eradication Initiative – Endemic Countries. http://polioeradication.org/where-we-work/polio-endemic-countries/. Accessed 30 July 2018.
67. Brandão LGP, Santoro-Lopes G, Oliveira S de S, da Silva EE, do Brasil PEAA. Seroprevalence of antibodies against the three serotypes of poliovirus and IPV vaccine response in adult solid organ transplant candidates. Vaccine. 2018;36(31):4681–6. https://doi.org/10.1016/J.VACCINE.2018.06.031.
68. Brandão LGP, do Brasil PEAA, Oliveira S de S, da SEE, Lopes GS. Seronegativity to polio viruses among previously immunized adult candidates to solid organ transplantation. Braz J Infect Dis. 2018;22(2):150–2. https://doi.org/10.1016/J.BJID.2018.02.003.
69. CDC | Global Health – Newsroom – Yellow Fever. https://www.cdc.gov/globalhealth/newsroom/topics/yellowfever/index.html. Published 2018. Accessed 30 July 2018.
70. WHO | Fact Sheet – Yellow Fever. http://www.who.int/news-room/fact-sheets/detail/yellow-fever. Accessed 30 July 2018.
71. Fishman JA. Smallpox and live-virus vaccination in transplant recipients. Am J Transplant. 2003;3(7):786–93. https://doi.org/10.1034/j.1600-6143.2003.00145.x.
72. Kotton CN, Ryan ET, Fishman JA. Prevention of infection in adult travelers after solid organ transplantation. Am J Transplant. 2005;5(1):8–14. https://doi.org/10.1111/j.1600-6143.2004.00708.x.
73. Staples J, Gershman M, Fischer M. Yellow fever vaccine recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2010;59 No. RR-7:1–27. www.cdc.gov/mmwr. Accessed 30 July 2018.
74. Wyplosz B, Burdet C, Fran H. Persistence of yellow fever vaccine – induced antibodies after solid organ transplantation. Am J Transplant. 2013;13:2458–61. https://doi.org/10.1111/ajt.12338.
75. Gershman M, Staples J. Yellow Fever – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/yellow-fever. Published 2018. Accessed 30 July 2018.
76. Sicre de Fontbrune F, Arnaud C, Cheminant M, et al. Immunogenicity and safety of yellow fever vaccine in allogeneic hematopoietic stem cell transplant recipients after withdrawal of immunosuppressive therapy. J Infect Dis. 2018;217(3):494–7. https://doi.org/10.1093/infdis/jix564.
77. Canadian Immunization Guide: Part 4: Active vaccines: Japanese encephalitis vaccine. https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/page-11-japanese-encephalitis-vaccine.html. Accessed 2 Aug 2018.
78. Hills, S.L; Rabe, I.BF; Fischer M. Japanese encephalitis – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/japanese-encephalitis. Published 2018. Accessed 30 July 2018.
79. Fischer M, Lindsey N, Staples JE, Hills S, Centers for Disease Control and Prevention (CDC). Japanese encephalitis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep Morb Mortal Wkly Rep Recomm Rep. 2010;59(RR-1):1–27. http://www.ncbi.nlm.nih.gov/pubmed/20224546. Accessed 30 July 2018
80. Trubiano JA, Johnson D, Sohail A, Torresi J. Travel vaccination recommendations and endemic infection risks in solid organ transplantation recipients. J Travel Med. 2016;23(6):1–15. https://doi.org/10.1093/jtm/taw058.
81. Darrigo LG, de Sant'Anna Carvalho AM, Machado CM. Chikungunya, Dengue, and Zika in immunocompromised hosts. Curr Infect Dis Rep. 2018;20(4):5. https://doi.org/10.1007/s11908-018-0612-2.
82. Clemente WT, Pierrotti LC, Abdala E, et al. Recommendations for management of endemic diseases and travel medicine in solid-organ transplant recipients and donors. Transplantation. 2018;102(2):193–208. https://doi.org/10.1097/TP.0000000000002027.
83. Mourão PHO, Gavalda J, Salvador F, Molina I. Recommendations for management of endemic diseases and travel medicine in solid-organ transplant recipients and donors. Transplantation. 2018;102(2S Suppl 2):S81. https://doi.org/10.1097/TP.0000000000002031.

84. Morris MI, Grossi P, Nogueira ML, Azevedo LS. Arboviruses recommendations for solid-organ transplant recipients and donors. Transplantation. 2018;102:S42–51. https://doi.org/10.1097/TP.0000000000002011.
85. Fernandes PFCBC, Siqueira RA, Girão ES, et al. Dengue in renal transplant recipients: clinical course and impact on renal function. World J Transplant. 2017;7(1):57. https://doi.org/10.5500/wjt.v7.i1.57.
86. Costa SD, da Silva GB, Jacinto CN, et al. Dengue fever among renal transplant recipients: a series of 10 cases in a tropical country. Am J Trop Med Hyg. 2015;93(2):394–6. https://doi.org/10.4269/ajtmh.15-0038.
87. Prasad N, Bhadauria D, Sharma RK, Gupta A, Kaul A, Srivastava A. Dengue virus infection in renal allograft recipients: a case series during 2010 outbreak. Transpl Infect Dis. 2012;14(2):163–8. https://doi.org/10.1111/j.1399-3062.2011.00699.x.
88. Nasim A, Anis S, Baqi S, Akhtar SF, Baig-Ansari N. Clinical presentation and outcome of dengue viral infection in live-related renal transplant recipients in Karachi. Pakistan Transpl Infect Dis. 2013;15:516–25. https://doi.org/10.1111/tid.12114.
89. de Souza Pereira BB, Darrigo Junior LG, de Mello Costa TC, et al. Prolonged viremia in dengue virus infection in hematopoietic stem cell transplant recipients and patients with hematological malignancies. Transpl Infect Dis. 2017;19(4):e12721. https://doi.org/10.1111/tid.12721.
90. Machado CM, Pereira BB de S, Felix AC, et al. Zika and chikungunya virus infections in hematopoietic stem cell transplant recipients and oncohematological patients. Blood Adv. 2017;1(10):624–7. https://doi.org/10.1182/bloodadvances.2016003285.
91. Girão ES, Santos BGR, Amaral ES, et al. Chikungunya infection in solid organ transplant recipients. Transplant Proc. 2017;49(9):2076–81. https://doi.org/10.1016/j.transproceed.2017.07.004.
92. Zika Virus | CDC. https://www.cdc.gov/zika/index.html. Accessed 13 Sept 2018.
93. Polen KD, Gilboa SM, Hills S, et al. Update: interim guidance for preconception counseling and prevention of sexual transmission of Zika virus for men with possible Zika virus exposure – United States, August 2018. MMWR Morb Mortal Wkly Rep. 2018;67(31):868–71. https://doi.org/10.15585/mmwr.mm6731e2.
94. Nogueira ML, Estofolete CF. Zika virus infection and solid organ transplantation: a new challenge. Am J Transpl. 2017;17(3):791–5. https://doi.org/10.1111/ajt.14047.
95. Kotton CN. Zika virus and solid organ transplantation. Transplantation. 2016;100(5):970–2. https://doi.org/10.1097/TP.0000000000001179.
96. Wallace MW, Peterson BW, Shlim DR. Rabies – Chapter 3 – 2018 Yellow Book | travelers' health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/rabies. Accessed 25 Mar 2019.
97. WHO | Rabies vaccines: WHO position paper – April 2018. *WHO*. 2018;16(93):201–220. http://www.who.int/rabies/resources/who_wer9316/en/. Accessed 30 July 2018.
98. Gibbons RV, Rupprecht CE. Postexposure rabies prophylaxis in immunosuppressed patients. JAMA. 2001;285(12):1574–5. http://www.ncbi.nlm.nih.gov/pubmed/11268256. Accessed 30 July 2018
99. WHO | Typhoid. *WHO*. 2018. https://www.who.int/mediacentre/factsheets/typhoid/en/. Accessed 26 Mar 2019.
100. Kotton CN. Travel and transplantation. Curr Opin Organ Transplant. 2012; p 1. https://doi.org/10.1097/MOT.0b013e328359266b.
101. Judd MC, Mintz ED. Typhoid & Paratyphoid fever – chapter 3 – 2018 yellow book | travelers' health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/typhoid-paratyphoid-fever. Accessed 26 Mar 2019.
102. Wong KK, Burdette E, Mintz ED. Cholera – chapter 3 – 2018 yellow book | travelers' health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/cholera. Accessed 27 Mar 2019.
103. Canadian Immunization Guide: Part 4: Active Vaccines: Cholera and Enterotoxigenic Escherichia Coli (ETEC) Travellers' Diarrhea Vaccine. https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/

page-3-cholera-enterotoxigenic-escherichia-coli-travellers-diarrhea-vaccine.html. Published 2017. Accessed 30 July 2018.
104. Ahmed T, Bhuiyan TR, Zaman K, Sinclair D, Qadri F. Vaccines for preventing enterotoxigenic Escherichia coli (ETEC) diarrhoea. Cochrane Database Syst Rev. 2013;7:CD009029. https://doi.org/10.1002/14651858.CD009029.pub2.
105. Hill DR, Ericsson CD, Pearson RD, et al. The practice of travel medicine: guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2006;43(12):1499–539. https://doi.org/10.1086/508782.
106. Committee to Advise on Tropical Medicine and Travel (CATMAT). An Advisory Committee Statement (ACS): Statement on Travellers' Diarrhea. Public Health Agency of Canada. https://www.canada.ca/en/public-health/services/travel-health/about-catmat/statement-travellers-diarrhea.html. Published 2015. Accessed 9 Aug 2018.
107. Global Task Force on Cholera Control (GTFCC). WHO | the use of oral cholera vaccines for international workers and travelers to and from cholera-affected countries; 2016. http://www.who.int/cholera/vaccines/OCV_use_International_Workers_Travelers_Technical_Note.pdf. Accessed 9 Aug 2018.
108. Connor BA. Travelers' Diarrhea – Chapter 2 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/the-pre-travel-consultation/travelers-diarrhea. Accessed 27 Mar 2019.
109. O'Reilly CE, Iwamoto M, Griffin PM. Escherichia coli, diarrheagenic – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/escherichia-coli-diarrheagenic#5166. Accessed 27 Mar 2019.
110. MacNeil JR, Meyer SA. CDC | Meningococcal Disease – Chapter 3 – 2018 Yellow Book | Travelers' Health. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/meningococcal-disease. Published 2017. Accessed 1 Aug 2018.
111. Lessa FC. Pneumococcal Disease – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/pneumococcal-disease. Accessed 27 Mar 2019.
112. Tiwari TS. Tetanus – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/tetanus. Accessed 28 Mar 2019.
113. von Konig C-HW. Use of antibiotics in the prevention and treatment of Pertussis. Pediatr Infect Dis J. 2005;24(Supplement):S66–8. https://doi.org/10.1097/01.inf.0000160916.47479.22.
114. CDC | Combined Recommended Immunization Schedule for Adults Aged 19 Years or Older, United States. 2018. https://doi.org/10.7326/M17-3439.
115. *TUBERCULOSIS Global Tuberculosis Report 2018*; 2018. www.who.int/tb. Accessed 28 Mar 2019.
116. Subramanian AK, Theodoropoulos NM. *Mycobacterium tuberculosis* infections in solid organ transplantation: guidelines from the infectious diseases community of practice of the American Society of Transplantation. Clin Transpl. 2019;33(9):e13513. https://doi.org/10.1111/ctr.13513.
117. Lobue P. Tuberculosis – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/tuberculosis. Published 2017. Accessed 9 Oct 2018.
118. Shoham S, Dominguez EA. Emerging fungal infections in solid organ transplant recipients: guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13525. https://doi.org/10.1111/ctr.13525.
119. Miller R, Assi M. Endemic fungal infections in solid organ transplantation. Am J Transplant. 2013;13(s4):250–61. https://doi.org/10.1111/ajt.12117.
120. Panackal AA, Hajjeh RA, Cetron MS, Warnock DW, Cetron MS, Warnock DW. Fungal infections among returning travelers. Clin Infect Dis. 2002;35(9):1088–95. https://doi.org/10.1086/344061.
121. Lortholary O, Charlier C, Lebeaux D, Lecuit M, Consigny PH. Fungal infections in immunocompromised travelers. Clin Infect Dis. 2013;56(6):861–9. https://doi.org/10.1093/cid/cis935.

122. Rappo U, Beitler JR, Faulhaber JR, et al. Expanding the horizons of histoplasmosis: disseminated histoplasmosis in a renal transplant patient after a trip to Bangladesh. Transpl Infect Dis. 2010;12(2):155–60. https://doi.org/10.1111/j.1399-3062.2009.00466.x.
123. Hart J, Dyer JR, Clark BM, McLellan DG, Perera S, Ferrari P. Travel-related disseminated Penicillium marneffei infection in a renal transplant patient. Transpl Infect Dis. 2012; 14(4):434–9. https://doi.org/10.1111/j.1399-3062.2011.00700.x.
124. Types of Fungal Diseases | Fungal Diseases | CDC. https://www.cdc.gov/fungal/diseases/index.html. Accessed 5 Apr 2019.
125. Boggild AK, Yohanna S, Keystone JS, Kain KC. Prospective analysis of parasitic infections in Canadian travelers and immigrants. J Travel Med. 2006;13(3):138–44. https://doi.org/10.1111/j.1708-8305.2006.00032.x.
126. Schwartz BS, Mawhorter SD. Parasitic infections in solid organ transplantation. Am J Transplant. 2013;13(s4):280–303. https://doi.org/10.1111/ajt.12120.
127. La Hoz RM, Morris MI. Tissue and blood protozoa including toxoplasmosis, Chagas disease, Leishmaniasis, Babesia, Acanthamoeba, Balamuthia, and Naegleria in solid organ transplant recipients – guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation. Clin Transpl. 2019;33(9):e13546. https://doi.org/10.1111/ctr.13546.
128. WHO | Fact Sheet: Malaria. https://www.who.int/news-room/fact-sheets/detail/malaria. Accessed 28 Mar 2019.
129. Pierrotti LC, Levi ME, Di Santi SM, Segurado AC, Petersen E. Malaria disease recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102:S16–26. https://doi.org/10.1097/TP.0000000000002017.
130. Arguin PM, Tan KR. CDC | malaria – chapter 3 – 2018 yellow book | travelers' health. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/malaria. Published 2017. Accessed 30 July 2018.
131. WHO | Fact Sheet: Leishmaniasis. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed 30 Mar 2019.
132. Clemente WT, Mourão PHO, Lopez-Medrano F, Schwartz BS, García-Donoso C, Torre-Cisneros J. Visceral and cutaneous leishmaniasis recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102(2S Suppl 2):S8–S15. https://doi.org/10.1097/TP.0000000000002018.
133. Herwaldt BL, Magill AJ. Leishmaniasis, visceral – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/leishmaniasis-visceral. Accessed 30 Mar 2019.
134. WHO | Fact Sheet: Trypanosomiasis, human African (sleeping sickness). https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness). Accessed 30 Mar 2019.
135. WHO | Fact Sheet: Chagas disease (American trypanosomiasis). https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis). Accessed 30 Mar 2019.
136. Pierrotti LC, Carvalho NB, Amorin JP, Pascual J, Kotton CN, López-Vélez R. Chagas disease recommendations for solid-organ transplant recipients and donors. Transplantation. 2018;102: S1–7. https://doi.org/10.1097/TP.0000000000002019.
137. Montgomery S. Trypanosomiasis, American (Chagas Disease) – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/trypanosomiasis-american-chagas-disease. Accessed 30 Mar 2019.
138. Kotton CN, Lattes R. Parasitic infections in solid organ transplant recipients. Am J Transplant. 2009;9(SUPPL. 4) https://doi.org/10.1111/j.1600-6143.2009.02915.x.
139. Jarque I, Salavert M, Pemán J. Parasitic infections in hematopoietic stem cell transplantation. Mediterr J Hematol Infect Dis. 2016;8(1):e2016035. https://doi.org/10.4084/MJHID.2016.035.
140. Lubin AS, Snydman DR, Miller KB. Persistent babesiosis in a stem cell transplant recipient. Leuk Res. 2011;35(6):e77–8. https://doi.org/10.1016/j.leukres.2010.11.029.

141. Abanyie F. Strongyloidiasis – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/strongyloidiasis. Accessed 30 Mar 2019.
142. Camargo LFA, Kamar N, Gotuzzo E, Wright AJ. Schistosomiasis and Strongyloidiasis recommendations for solid-organ transplant recipients and donors. Transplantation. 2018;102:S27–34. https://doi.org/10.1097/TP.0000000000002016.
143. Montgomery S. Schistosomiasis – Chapter 3 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/schistosomiasis. Accessed 30 Mar 2019.
144. Table of Contents | 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/table-of-contents. Accessed 30 Mar 2019.
145. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med. 2007;357:2601–14. https://doi.org/10.1016/j.semnephrol.2007.03.006.
146. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238. https://doi.org/10.1016/j.bbmt.2009.06.019.
147. Winter K. Travel Health Kits – Chapter 2 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/the-pre-travel-consultation/travel-health-kits. Published 2017. Accessed 31 Aug 2018.
148. Avery RK, Michaels M. Update on immunizations in solid organ transplant recipients: what clinicians need to know. Am J Transplant. 2008;8(1):9–14. https://doi.org/10.1111/j.1600-6143.2007.02051.x.
149. Canadian Immunization Guide: Part 3: Vaccination of Specific Populations. https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-3-vaccination-specific-populations.html. Accessed 7 Jan 2018.
150. Aung AK, Trubiano JA, Spelman DW. Travel risk assessment, advice and vaccinations in immunocompromised travellers (HIV, solid organ transplant and haematopoeitic stem cell transplant recipients): a review. Travel Med Infect Dis. 2015;13:31–47. https://doi.org/10.1016/j.tmaid.2014.12.007.
151. Committee to Advise on Tropical Medicine and Travel (CATMAT). An Advisory Committee Statement (ACS): the immunocompromised traveller. Canada Commun Dis Rep. 2007;33 (ACS-4):1–24. https://www.canada.ca/content/dam/phac-aspc/migration/phac-aspc/publicat/ccdr-rmtc/07pdf/acs33-04.pdf. Accessed 30 July 2018
152. Rubin LG, Levin MJ, Ljungman P, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58(3):e44–e100. https://doi.org/10.1093/cid/cit684.
153. Griffin PM, Hlavsa MC, Yoder JS. Food and water precautions – Chapter 2 – 2018 Yellow Book | Travelers' Health | CDC. https://wwwnc.cdc.gov/travel/yellowbook/2018/the-pre-travel-consultation/food-water-precautions. Published 2017. Accessed 1 Aug 2018.
154. Eat and drink safely – travel health and safety. Government of Canada. https://travel.gc.ca/travelling/health-safety/food-water. Published 2018. Accessed 1 Aug 2018.
155. Beeching NJ, Carratalà J, Razonable RR, Oriol I, Vilela EG. Traveler's Diarrhea recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102:S35–41. https://doi.org/10.1097/TP.0000000000002015.
156. Riddle MS, Connor BA, Beeching NJ, et al. Guidelines for the prevention and treatment of travelers ' diarrhea: a graded expert panel report. J Travel Med. 2018;24(Suppl 1):S63–80. https://doi.org/10.1093/jtm/tax026.
157. UNWTO Tourism Highlights. https://doi.org/10.18111/9789284419876.

Preventative Health in Solid Organ Transplant: Safe Living Post-transplant

11

Barbra M. Blair

Contents

Introduction 239
Pre-transplant Evaluation 240
Vaccination 241
Everyday Living Strategies 241
Activities of Leisure 243
Sexual Health 244
Food and Water Safety 245
Animal and Insect Precautions 247
Travel Advice 248
Conclusion 248
Key Points 249
References 249

Introduction

In order to minimize a solid organ transplant (SOT) recipient's risk for infection and injury, it is important to anticipate the risks post-transplantation inherent in routine and not so routine activities of daily living. It is invaluable to orient potential SOT recipients to these risks, as often leading up to transplant they may likely experience debilitation due to chronic illness, yet after SOT can go on to live healthy, fruitful lives with increased exposures and risks. Potential transplant recipients should update their vaccinations, with a particular eye toward ensuring live virus vaccines are prioritized. Education should commence regarding the importance of food and water safety after transplant so recipients may plan accordingly. While often pet ownership is beneficial due to the animal-human bonding, the risks of such

B. M. Blair (✉)
Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston, MA, USA

Harvard Medical School, Boston, MA, USA
e-mail: bblair@bidmc.harvard.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_10

ownership due to animal exposure require careful discussion and planning to minimize potential exposure to zoonotic pathogens post-transplant. Additionally, realistic expectations should be set with regard to travel, occupational environment, and participation in recreational activities. Incorporating such counseling and planning routinely into pre- and post-transplant visits can empower SOT recipients and assure their knowledge of both the risks inherent to everyday living and the best strategies to mitigate such risks.

Pre-transplant Evaluation

Ensuring optimal health after solid organ transplant (SOT) mandates conscious preparation pre-transplantation. Part of this preparation should involve evaluation of home environment including water supply, potential future travel, returning to work, hobbies, and other social activities. Table 1 lists the topic areas that should be part of the pre-transplant evaluation in order to address areas that should be identified, improved upon, and planned for subsequent to transplant.

The pre-transplant evaluation can assist with identifying other potential risks to the SOT recipient including the vaccination status and philosophy of their household contacts. The CDC's Morbidity and Mortality Weekly Report from January 2018 to May 2019 reported on outbreaks of hepatitis A, hepatitis B, measles, and mumps, reinforcing the need to optimize community protection against vaccine-preventable illness, especially for those living with immunocompromised hosts [1–4]. Counseling the SOT recipient pre-transplant hopefully ensures an open dialogue for assessment of the acquisition of new lifestyle infectious risks allowing for continued intervention post-transplant.

Table 1 Pretransplant evaluation

Travel history
Country of birth
Previous international travel
Potential travel after transplant
Home Environment
Household members
Pets
Water supply
Occupation/Occupational exposures
Hobbies/Recreation
Piercings/Tattoos/Nail Salons
Marjuana use
Recreational water use
Vaccination history

Vaccination

As discussed above, optimization of a SOT recipient's vaccine status is ideal in order to prevent morbidity and mortality post-transplant both in everyday life and in planning for potential travel. Preferably, this is initiated pre-transplant and should be reassessed post-transplant with the patient to verify vaccine statuses are staying current. The CDC Advisory Committee on Immunization Practices (ACIP) updates recommendations regarding routine and special vaccinations for adults at least yearly and is located at www.cdc.gov/vaccines/schedules/hcp/imz/adult.html. It is important to review vaccination status frequently of the SOT recipient to ensure updated completeness. In addition, see ▶ Chap. 12, "Vaccination Strategies in Solid Organ and Stem Cell Transplant" for a more complete review of this topic specific to the SOT recipient. Likewise, the American Society of Transplantation published guidelines in 2019 updating general principals and use of specific vaccines in solid organ transplant recipients [5]. Although use of live vaccines is contraindicated in adult SOT recipients post-transplant, significant indirect benefit of live virus vaccination of household contacts can be achieved [6]. Aside from potential risk of transmission of live attenuated influenza vaccine (LAIV), smallpox vaccine, and oral polio vaccines, there is no concern for transmission of live virus vaccines from vaccinated household contacts to their immunocompromised contacts [6]. Smallpox vaccine is no longer routine, and most countries have switched or are switching to the inactivated polio vaccine [7]. In 2019, the World Health Organization (WHO) unveiled a new strategic plan to face multiple global health challenges, including "vaccine hesitancy" – the reluctance to vaccinate despite the availability of vaccines. This is particularly relevant for an immunocompromised host who benefits from "herd immunity" and is at increased risk for the acquisition of vaccine-preventable diseases in the current climate where illnesses such as measles have seen a 30% increase globally [8]. Moreover, within developed countries such as the United States, authors have identified a strong association between vaccine refusal and vaccine-preventable disease outbreaks such as measles and pertussis [9].

Everyday Living Strategies

As the American Society of Transplantation (AST) notes, even after the immediate post-transplant period, the risk of infection remains for the SOT recipient for as long as they are immunocompromised, with periods of enhanced susceptibility [10]. As most pathogens are acquired via direct contact via hands, ingestion, or inhalation, frequent handwashing and avoidance of those with respiratory illnesses are essential ways to minimize acquisition of infectious pathogens [10]. There have been numerous studies performed to evaluate the effect of handwashing in decreasing gastrointestinal and respiratory illnesses. One meta-analysis evaluated the efficacy of different methods of hand hygiene interventions in the prevention of illness and found that education combined with handwashing with antibacterial soap decreased gastrointestinal illness by 50% [11]. In this same analysis, the utilization of

benzalkonium chloride-based hand sanitizers prevented 40% of respiratory illnesses [11]. As well outlined in the AST recommendations for safe living after transplantation and summarized in Table 2, frequent handwashing is strongly recommended but especially after certain activities/exposures. While the WHO and CDC recommend that alcohol-based hand sanitizers (ABHS) not be used on visibly soiled hands, the lack of readily available soap and water in some situations could impede clean hands. As such, investigators at Stanford University investigated the efficacy of ABHS against experimentally soiled hands with dirt, cooking oil, or both after inoculation with 10^7 *E. coli.* They observed that despite participants feeling that their soiled hands were less clean than their unsoiled hands, the ABHS reduced the levels of *E. coli* as well on clean or soiled hands [12]. While noting that laboratory conditions may not entirely reflect everyday life, these data support the use of ABHS as a reasonable temporizing substitute in select circumstances to handwashing until access to soap and water is attained. Practically, in everyday life, ABHS could be utilized while out in public places such as retail locations, recreational facilities, or places of religious worship until proper handwashing can be employed. Similar to recognizing where hand hygiene practices are most important, attention to risks inherent in other everyday activities is imperative to reducing risk for acquisition of infections. As summarized in Table 2, the AST provides guidance on the types of activities to avoid. Certain occupations may require planning in advance of transplantation especially in cases where occupational exposures are inherent and serious consideration given to modification or discontinuation of such employment. In particular, occupations such as farming, bird breeding, construction involving excavation and demolition, landscaping, and arboriculture place SOT recipients at risk for inhalational exposure to fungal infections such as histoplasmosis, coccidioidomycosis, blastomycosis, aspergillosis, and cryptococcosis [13]. Subsequent to transplant if recipients must remain employed in these fields, precautionary measures including N95 particulate respirators and personal protective equipment including non-vented goggles, gloves, and coveralls should be utilized [14]. Exposure to herbivores such as horses can result in *Rhodococcus* infections, which can be

Table 2 Ways to reduce risk of infection from every day activities.

Employ hand washing after	Avoid
Eating or Preparing food	Close contact with persons with respiratory viruses
Changing diapers (if not unavoidable)	Prolonged contact with crowded areas
Touching plants or dirt	Tobacco and Marjuana smoking
Using/cleaning the restroom	Visiting areas with increased risk of exposure to tuberculosis (prisons, homeless shelters, certain healthcare facilities)
Touching animals	Construction areas/areas of excavation
Touching items in contact with animal or human bodily fluids (litter boses/tissues)	Areas with possible exposure to fungal spores: caves, bird cages/coops, soil aerosols via mulching
Collecting or deposing of garbage	Self-piercing, tatooing or needle sharing
Going outdoors or to a public place	
Adapted from [10]	

complicated to diagnose and manage in SOT recipients [15]. Relatedly, recipients should be discouraged from engaging in such activities for leisure.

Akin to avoiding inhalation of decayed vegetation in soil through leisure activities or as part of an occupation, SOT recipients should be counseled against smoking, vaping, and ingestion of cannabis plant-based materials, "medical" or otherwise. This is particularly relevant nowadays with the legalization of recreational marijuana in many regions [16]. Investigators have evaluated the microbiome of cannabis samples obtained from marijuana dispensaries. These samples grew *Pseudomonas aeruginosa* in up to 12% of samples, *Aspergillus fumigatus* in 12% of samples, as well as numerous other Gram-negative bacteria known to be capable of necrotizing lung infections such as *Klebsiella* as well as other fungi and molds such as *Mucor* and *Cryptococcus* in smaller overall amounts [17, 18]. While it's not clear that the presence of such organisms is associated with actual infection, there are several cases in the literature of *Pseudomonas aeruginosa* [19] and *Aspergillus* [20] inhaled via either "bongs," water pipes, or vaping causing necrotizing lung infections and some of these in less immunocompromised hosts than the SOT recipient. There have been few studies evaluating the effect of sterilization to alleviate or abrogate this risk. Some experts recommend heat treatment of dried marijuana leaves to kill mold spores. The SOT recipient should be aware of potential drug interactions with their transplant medications and a higher risk of graft loss due to rejection in those who use cannabis products [21]. Thus, there is no way to safely recommend or approve of the utilization of cannabis plant-based materials in the SOT recipient due to the unquantifiable risk of infection with opportunistic pathogens. Manufactured "edibles" may be a safer alternative as far as decreasing the infection risk, although these products have not yet been adequately studied.

Activities of Leisure

Strategies listed above as recommendations about modifying employment also apply to leisure activities. As outlined, because of the fungi inherent in soil, landscaping and gardening need to be approached with caution. Recreational water exposure-related illnesses can be significant. There have been increasingly recognized *Cryptosporidium* outbreaks, now the leading cause of swimming pool-associated diarrheal illness [22]. SOT recipients should be counseled that chlorine can kill many infectious pathogens but some (especially *Cryptosporidium)* can persist in spite of chlorination. Thus, they should avoid getting water in their mouths and noses when swimming in lakes, ponds, oceans, rivers, and swimming pools [22]. The CDC in 2018 reported 140 separate outbreaks of illness associated with non-treated recreational water from 2000 to 2014 and 493 outbreaks from treated recreational water from the same time period [23, 24]; Table 3 lists the major pathogen causes and the frequency of each in the reported outbreaks. Not surprisingly, the peak incidence of outbreaks in treated and non-treated water is the summer months but with a relatively steady number of outbreaks in treated water all year round [23, 24]. Hot tubs have

Table 3 Recreational water-associated outbreaks: Major pathogen causes and the frequency of each in the reported outbreaks associated with both treated and untreated recreational water from 2000 to 2014. (Data from the CDC [23, 24])

Recreational water associated outbreaks		
Etiology	Treated water	Untreated water
Bacteria	26%	31%
	E. coli>*Shigella*>*Leptospirosis*	*Legionella*>*Pseudomonas*>*Shigella*
Parasite	45%	18%
	Cryptosporidium>Giardia	Cryptosporidium>Avian Schistomiasis>Naegleria fowleri
Virus	3%	16%
	Norovirus	Norovirus
Toxin	4%	4%
	Chlorine	Algae blooms
Unidentified	22%	26%

been associated with *Pseudomonas* and other pathogens (including mycobacteria) and should be avoid after transplant.

Activities including body piercing, tattooing, and pedicures have been associated with acquisition of infections, especially non-tuberculous *Mycobacterium* (NTM). Body piercing can lead to blood-borne infections (especially hepatitis C), although cellulitis is more common and can lead to bacteremia (even endocarditis), Ludwig's angina, Fournier's gangrene, and sepsis or toxic shock [25]. Tattoo ink contaminated with *Mycobacterium chelonae* has caused an outbreak of cutaneous NTM infection in immunocompetent hosts in multiple US states in 2012 [26]. Cutaneous mold infections have also been reported after tattooing in the SOT recipient [27]. There have been several outbreaks of rapid-growing *Mycobacterium* spp. associated with whirlpool footbaths in mostly immunocompetent individuals with one case of fatal cutaneous *Mycobacterium chelonae* in a relatively immunocompromised host [28–30]. The authors of that report caution about pedicures in the setting of open wounds or skin trauma. Additionally, the source of the mycobacteria was felt to be the drains associated with the footbaths with recommendations for salons to utilize a 10% bleach solution to adequately disinfect such drains [28, 29]. As such, SOT recipients should be cautioned of such risk and advised to avoid whirlpool footbaths especially if the integrity of the cleaning cannot be assured.

Sexual Health

Sexual health concerns in the SOT recipients likely exist pre-transplant and based on scant studies in the literature exist post-transplant [31]. One study in liver transplant recipients found that about 25% of men and women experienced sexual health

concerns pre-transplant that increased to 51% and 40%, respectively, post-transplant [31]. Most of the concerns are related to sexual function, desire, and dyspareunia. Transplant clinicians may become the primary physician many SOT recipients see after transplant and candid discussion about sex (especially safer sex for those not in monogamous relationships) may be important to ensure avoidance of sexually transmitted infections (STI). There have been reports of HIV acquisition through sex after transplantation [32]. In addition, acquisition of other STIs such as syphilis has been described as well. Due to low infectious doses, inadvertent consumption of fecal material during sexual activities involving oral-anal and unprotected genital-anal contact can lead to human to human transmission of *Shigella, Giardia*, and *Entamoeba histolytica* [33]. Counseling should therefore be provided about avoidance of such sexual practices post-SOT to minimize risk of sexually transmitted enteritis. While most transplant recipients will be adequately vaccinated for hepatitis B prior to transplant and thus protected against sexual transmission, SOT recipients should be counseled about other viral infections such as CMV, HSV, and EBV and their enhanced susceptibility if seronegative prior to transplant and avoidance of sex with partners who may be manifesting symptoms of such infections. Testing provided in sexual health clinics may be useful for those entering longer-term relationships. Sexual activity when pursued safely can be fulfilling and infectious risk can be minimized.

Food and Water Safety

Access to safe water is essential after SOT because the most common causes of water-associated disease outbreaks due to private water sources (i.e., private wells) per the CDC as of 2010 are hepatitis A, *Giardia, Campylobacter, Shigella, E. coli, Cryptosporidium, Salmonella,* and *Yersinia enterocolitica* [34]. As such, assessment of water sources prior to solid organ transplant can assist recipients with advance planning especially if they have a private water source associated with their home. Municipal water supplies in most regions where transplants are done would be safe, both well chlorinated and tested as the provision of safe water both in the United States and internationally is a defining characteristic of a developed country [35]. In the United States, the Safe Drinking Water Act of 1994 enacted many state- and federal-level controls governing municipal water supplies [36]. Groundwater supplies half the US's water supply and can become easily contaminated. Further, it is estimated that over 13 million US households rely on private wells [37]. Ensuring patients are aware to be cognizant of water use after intense rainstorms is extremely important as in the event of extreme weather especially significant rainfall; groundwater may contaminate private wells increasing risk for infectious pathogens. In addition, in certain highly populated areas, common sewer overflow during times of extreme precipitation can dump untreated overflow into drinking water sources, and in such areas, there has been association with GI illnesses after such events [38]. Thus, SOT recipients should

be instructed to attend to boil water advisories after both periods of significant storms and any disruption in usual water supply to prevent ingestion of potentially contaminated drinking water. More concerning, in addition to the issues related to contaminated water, studies have found contamination of drinks obtained from beverage dispensers such as soda fountains with coliform bacteria [39]. Because of this, SOT recipients should be cautioned to avoid such sources for consumption. Finally, many SOT recipients may choose bottled water instead of using tap or boiled water. The FDA does regulate bottled water as a packaged food. Unless bottled water is distilled, filtered through a 1 micron unit, or treated with ozonation or reverse osmosis, organisms such as *Cryptosporidium* can persist [40]. According to the Centers for Disease Control and Prevention, 48 million persons get sick, 128,000 are hospitalized, and 3,000 die from foodborne infection and illness in the United States each year [41]. In the United Kingdom, the Government National Food Survey collects population-based food consumption data and can estimate the incidence and effect of foodborne infection; data collected from 1996 to 2000 estimated 1,724,315 cases of indigenously acquired foodborne infection with 4% of these being attributable to human transmission through food handlers [42]. Infection attributed to ingestion of chicken accounted for 30% of the foodborne illnesses likely accounting for the *Campylobacter* and nontyphoidal *Salmonella* cases. Not surprisingly, *Listeria monocytogenes* and *E. coli* 0157: H7, while only accounting for 0.1% of all cases of foodborne illness, unfortunately accounted for 15% of the deaths [42]. Authors from the CDC in the United States have published data compiled from surveillance from 2000 to 2008 and population data as of 2006 estimating the incidence of foodborne illness [43]. It was necessary to perform some modeling, and the types of potential pathogens may be underreported due to data being extrapolated from laboratory surveillance and not due to active disease surveillance [43]. The authors found that in the United States, similar to the UK data, norovirus caused the most illnesses; nontyphoidal *Salmonella* spp., norovirus, *Campylobacter* spp., and *T. gondii* caused the most hospitalizations; and nontyphoidal *Salmonella* spp., *T. gondii*, *L. monocytogenes*, and norovirus caused the most deaths [43]. These data are particularly important when counseling the SOT recipient due to their enhanced susceptibility to severe or persistent infections with pathogens such as *Listeria monocytogenes* and norovirus, respectively. In preparation for SOT and after transplantation, recipients should be educated about the risk of illness due to foodborne infections. The US Federal Drug Administration (FDA) with the US Department of Agriculture and the US Department of Health and Human Services have a published guide available from 2006 and updated in 2011 that it is downloadable from the FDA's website that outlines safe handling of food, safer food choices if eating outside of home, temperature and hygiene guidance, as well as a list of the most common foodborne pathogens and associated foods and the most common symptoms of illness with such pathogens [44]. In addition, this guide is available in both English and Spanish and contains resources with instructions on procedures if foodborne illness is suspected and tip lines to contact with questions. Consideration should be given to providing such a guide to

recipients in addition to verbal instructions provided by transplant team members especially nutritionists. Further reinforcing food and water safety as a part of ongoing post-transplant care can ensure such issues are not minimized or lose their importance as the time increases from receipt of an organ transplant.

Animal and Insect Precautions

As the human population expands with increasing contact with animals and the environment and as the One Health Initiative purports, an integrative effort of multiple disciplines working locally, nationally, and globally is necessary to ensure the health and preservation of all [45]. One Health is working to survey infections in domestic and wild animals as others have reported that approximately 75% of newly reported human infections have emerged from animal reservoirs [46]. This is of particular importance in the field of solid organ transplantation as zoonotic infections have become additionally recognized and reported [47]. In addition, trends are emerging where exotic or unconventional pets are being captured locally or imported, and with these unconventional pets, unusual diseases such as salmonellosis, tularemia, monkeypox, LCMV, and yersiniosis are being transmitted to their human owners [48]. In addition, there have been several reports of pet store-acquired birds leading to psittacosis [49]. As such, ownership of exotic pets should be discouraged, and this should be discussed pre-transplant and reiterated subsequently.

Dogs, cats, and fish are generally acceptable pets for SOT recipients. When possible, adopting older animals may be preferable as there may be fewer bites and scratches. Transplant recipients should not change kitty litter, nor should they wash fish tanks. In general, other animals are not recommended. All pet animals should receive routine veterinary care. Attention should be provided to pet's diet, avoiding raw meat or eggs and discouraging consumption of toilet or other non-potable water [47]. The CDC provides guidance entitled "Healthy Pets, Healthy People" that offers helpful information and instruction for both SOT recipients and care providers about the care of domestic animals and avoidance of certain animals as pets and in their natural environments [50].

When addressing zoonoses, it is imperative to also address vector-borne illness, as animals may serve as the reservoirs for such diseases for which humans become infected. Some domestic animals may provide a means for vectors such as ticks to access humans, i.e., a dog that brings a tick from outdoors into a human's bed when sleeping with its owner. There are cases in the literature of SOT recipients with infections due to vector-borne infections such as babesiosis, anaplasmosis, and West Nile virus but little about the overall incidence [51, 52]. Regardless, the clinical courses reported are typically more severe than in non-SOT hosts; thus, avoidance of insect bites through the use of DEET application, clothing, avoidance, etc. should be routinely practiced. In addition, if traveling to malaria-endemic regions, SOT recipients should be prescribed malaria prophylaxis and advised about bed netting in addition to the other measures advocated for daily living [51].

Travel Advice

Pre-transplant counseling about future travel is essential and may be of particular importance to the foreign-born SOT recipient. Although only 13% of the US population is foreign-born, 37% of international travelers from the United States list visiting friends and relatives (VFR) as their reason for travel [53]. Furthermore, in a large travel clinic study in Canada from 2001, two thirds of the SOT recipient travelers surveyed were foreign-born [54]. Additionally, when foreign-born SOT travelers are VFRs, they experience higher risk for acquisition of travel-associated infections most often due to lack of awareness, potential last minute travel (i.e., returning for family illness or death), travel for longer duration, and living a local lifestyle with exposure to less safe food and water and mosquito netting [53]. Pre-transplant evaluation of foreign-born SOT recipients allows for updating routine vaccines, ensuring immunity to vaccine-preventable illnesses, and assessing need for live vaccines such as MMR, varicella, and yellow fever prior to immunosuppression. Furthermore, for any SOT recipient, education regarding safe eventual travel can be initiated and advice given around seeking future pre-travel counseling visits. Good habits with regard to food and water safety should continue to be followed, with emphasis placed on not eating street food or raw fruits or vegetables that can't be peeled. In addition, routine vaccinations such as influenza vaccine should be updated, and consideration for non-live travel vaccines such as inactivated polio, typhoid vaccine, meningococcal vaccine, and hepatitis A vaccine should be made depending on the area of travel. For those traveling to yellow fever regions, they should be supplied with a Yellow Card stating that they cannot undergo yellow fever vaccination given their immunosuppression. The CDC recommends that several key education points be discussed with immunocompromised travelers: developing an illness contingency plan with an identified clinic/hospital; bringing extra medications in case of travel delay; avoiding procuring medications during travel due to risk of counterfeit; use of sun protection; vigilant food and water precautions; and travel with a health kit [55]. In addition to these measures, travel to high-risk destinations should be postponed until at least a year after transplantation [55]. Malaria chemoprophylaxis should be prescribed for SOT recipient travelers to endemic areas [55]. Strict mosquito and other insect precautions should be emphasized as diseases like dengue, Zika, chikungunya, Chagas, and leishmaniasis can cause major illness in immunocompromised hosts [56]. While such extensive travel counseling may be perceived as excessive especially by those foreign-born who are traveling to visit friends and relatives, such measures should be planned for and discussed pre-transplant and emphasized subsequent to receipt of organ transplantation.

Conclusion

The benefits of longevity by virtue of organ transplantation need to be closely protected by education before, during, and after transplantation about potential infectious risks and measures to mitigate such exposures. Additionally, SOT

recipients cannot stand alone in their efforts toward modifying their risks. Household contacts should be considered and advice offered toward moderating their contribution to risk by encouraging updating their vaccines and encouraging them to assume responsibility of daily tasks/chores that should be avoided by SOT recipients such as direct animal care and gardening. Additionally, while many activities are still able to be enjoyed after transplant such as career activities, travel, and recreation, careful preparation should be employed and reiterated by care providers to mitigate any infectious risks inherent in such activities and implement risk reduction behaviors if such activities are embarked upon. A longitudinal preventative health strategy based on the topics addressed here can assist with maintenance of health and avoidance of infection and its associated morbidity and mortality.

Key Points

- Safe living after solid organ transplant (SOT) involves preparation pre-transplant.
- Vaccine status evaluation should be continually reassessed for the SOT recipient and household contacts before and after transplant.
- Avoidance of environmental pathogens is ideal, but if it cannot be avoided, then personal protective equipment should be consistently used.
- Guidance exists and should be made readily available to the SOT recipient about food and water safety to minimize risk for foodborne illness.
- Animal-human bonding may be beneficial but certain animals as pets engender greater risk than others and such ownership should be avoided.

References

1. Foster M, Ramachandran S, Myatt K, Donovan D, Bohm S, Fiedler J, et al. Hepatitis A virus outbreaks associated with drug use and homelessness – California, Kentucky, Michigan, and Utah, 2017. MMWR. 2018;67(43):1208–10.
2. Comer M, Matthias J, Nicholson G, Asher A, Holmberg S, Wilson C. Notes from the field: increase in acute hepatitis B infections – Pasco County, Florida, 2011–2016. MMWR. 2018;67(7):230–1.
3. Avramovich E, Indenbaum V, Haber M, Amitai Z, Tsifanski E, Farjun S, et al. Measles outbreak in a highly vaccinated population – Israel, July–August 2017. MMWR. 2018;67(42):1186–1188a.
4. Golwalkar M, Pope B, Stauffer J, Snively A, Clemmons N. Mumps outbreaks at four universities – Indiana, 2016. MMWR. 2018;67(29):793–7.
5. Danzinger-Isakov L, Dumar D. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;19:e13563.
6. Kamboj M, Sepkowitz KA. Risk of transmission associated with live attenuated vaccines given to healthy persons caring for or residing with an immunocompromised. Infect Control Hosp Epidemiol. 2007;28(6):702–7.
7. World Health Organization. Replacing trivalent OPV with bivalent IPV. [Cited June 14, 2019]. https://www.who.int/immunization/diseases/poliomyelitis/endgame_objective2/oral_polio_vaccine/en/

8. World Health Organization. Ten threats to global health in 2019. [Cited June 06 2019]. www.who.int/emergencies/ten-threats-to-global-health-in-2019
9. Phadke VK, Bednarczyk RA, Salmon DA, Omer SB. Association between vaccine refusal and vaccine-preventable diseases in the United States: a review of measles and pertussis. JAMA. 2016;315(11):1149–58.
10. Avery RK, Michaels ME. Strategies for safe living following solid organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13519.
11. Aiello AE, Coulborn RM, Perez V, Larson EL. Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. Am J Public Health. 2008;98:1372–81.
12. Pickering AJ, Davis J, Boehm AB. Efficacy of alcohol-based hand sanitizer on hands soiled with dirt and cooking oil. J Water Health. 2011;9(3):429–33.
13. Sipsas NV, Kontoyiannis DP. Occupation, lifestyle, diet, and invasive fungal infections. Infection. 2008;36(6):515–25.
14. Doss H, Tilma C. Personal protective equipment necessary for some farm jobs. National Ag Safety Database. [Cited May 20, 2019]. http://nasdonline.org/1035/d000833/personal-protective-equipment-necessary-for-some-farm-jobs.html
15. Vergidis P, Ariza-Heredia EJ, Nellore A, Kotton CN, Kaul DR, Morris MI, et al. *Rhodococcus* infection in solid organ and hematopoietic stem cell transplant recipients. Emerg Infect Dis. 2017;23(3):510–2.
16. Rein JL. The nephrologist's guide to cannabis and cannabinoids. Curr Opin Nephrol Hypertens. 2020;29(2):248–57.
17. McHardy I, Romanelli A, Harris LJ, Opp G, Gaudino R, Torres A, et al. Infectious risks associated with medicinal cannabis: potential implications for immunocompromised patients? J Inf Secur. 2018;76(5):500–1.
18. Thompson GR, Tuscano JM, Dennis M, Singapuri A, Libertini S, Gaudino R, et al. A microbiome assessment of medical marijuana. Clin Microbiol Infect. 2017;23:269–70.
19. Kumar AN, Soo CI, Ng BH, Hassan T, Ban AY, Manap RA. Marijuana "bong" pseudomonas lung infection: a detrimental recreational experience. Respirol Case Rep. 2018;6(2):1–3.
20. Remington TL, Fuller J, Chiu I. Chronic necrotizing aspergillosis in a patient with diabetes and marijuana use. CMAJ. 2015;187(17):1305–8.
21. Fabbri KR, Anderson-Haag TL, Spenningsby AM, Israni A, Nygaard RM, Stahler PA. Marijuana use should not preclude consideration for kidney transplantation. Clin Transpl. 2019;33:e13706.
22. Center for Disease Control and Prevention. Healthy swimming: recreational water illnesses [updated January 25, 2017; cited May 25, 2019]. www.cdc.gov/healthywater/swimming/swimmers/rwi.htm
23. Graciaa DS, Cope JR, Roberts VA, Cikesh BL, Kahler AM, Vigar M, et al. Outbreaks associated with untreated recreational water – United States, 2000–2014. MMWR. 2018;67(25):701–6.
24. Hlvasa MC, Cikesh BL, Roberts VA, Kahler AM, Vigar M, Hilborn ED, et al. Outbreaks associated with treated recreational water – United States, 2000–2014. MMWR. 2018;67(19):547–51.
25. Van Remoortel H, Moorkens D, Avau B, Compernolle V, Vandekerekhove P, De Buck E. Is there a risk of transfusion-transmissible infections after percutaneous needle treatments in blood donors? A systematic review and meta-analysis. VoxSanguinis. 2019;114:297–309.
26. Bedard B, Kennedy B, Escuyer V, Mitchell K, Duchin JS, Pottinger P, et al. Tattoo-associated nontuberculous mycobacterial skin infections–multiple states, 2011–2012. MMWR Morb Mortal Wkly Rep. 2012;61(33):653–6.
27. Trinh SA, Angarone MP. *Purpureocillium lilacinum* tattoo-related skin infection in a kidney transplant recipient. Transpl Inf Dis. 2017;19:e12689.
28. Stout JE, Gadkowski B, Rath S, Alspaugh JA, Miller MB, Cox GM. Pedicure-associated rapidly growing mycobacterial infection: an endemic disease. Clin Infect Dis. 2011;53:787–92.
29. Winthrop KL, Albridge K, South D, Albrecht P, Abrams M, Samuel MC, et al. The clinical management and outcome of nail salon acquired Mycobacterium fortuitum skin infection. Clin Infect Dis. 2004;38:38–44.

30. Schmidt AN, Zic JA, Boyd AS. Pedicure associated *Mycobacterium chelonae* infection in a hospitalized patient. J Am Acad Dermatol. 2014;71(6):e248–9.
31. Ho JK, Ko HH, Schaeffer DF, Erb SR, Wong C, Buczkowski AK, et al. Sexual health after orthotopic liver transplantation. Liver Transpl. 2006;12:1478–84.
32. Cristelli MP, Mazolin MA, Manzardo C, Ribeiro MSJ, Cofan F, Santos DWS, et al. Sexual acquisition of HIV infection after solid organ transplantation: late presentation and potentially fatal complications. Transpl Infect Dis. 2018;20(4):e12894.
33. Narayan S, Galanis G, BC STEI Group. Are enteric infections sexually transmitted in British Columbia? Can Commun Rep. 2016;42(2):24–9.
34. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Diseases, Division of foodborne, waterborne and environmental diseases. Drinking water. [Updated December 8, 2017; cited May 19, 2019]. https://www.cdc.gov/healthywater/drinking/index.html
35. Ashbolt NJ. Microbial contamination of drinking water and human health from community water systems. Curr Environ Health Rep. 2015;2:95–106.
36. National Association of local boards of health. Drinking water factsheet. [Updated 2011; cited May 28, 2019]. https://www.cdc.gov/nceh/ehs/nalboh/factsheets/drinking_water.pdf
37. United States Environmental Protection Agency. Private drinking water wells. [Updated April 26, 2019; cited May 28, 2019]. https://www.epa.gov/privatewells
38. Jagai JS, Li Q, Wang S, Messier KP, Wade TJ, Hilborn ED. Extreme precipitation and emergency room visits for gastrointestinal illness in areas with and without combined sewer systems: an analysis of Massachusetts data, 2003–2007. Environ Health Perspect. 2015;123:873–9.
39. White AS, Godard RD, Belling C, Kasza V, Beach RL. Beverages obtained from soda fountain machines in the U.S. contain microorganisms, including coliform bacteria. Int J Food Microbiol. 2010;137(1):61–6.
40. United States Environmental Protection Agency. Water health series bottled water basics. [Updated 2005; cited June 14, 2019]. https://nepis.epa.gov
41. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Diseases, Division of Foodborne, Waterborne and Environmental Diseases. Foodborne illnesses and germs. [Updated February 16, 2018; cited June 14, 2018]. https://www.cdc.gov/foodsafety/foodborne-germs.html
42. Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien SJ. Disease risks from foods, England and Wales, 1996–2000. Emerg Infect Dis. 2005;11(3):365–72.
43. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Middowson M, Rosy SL, et al. Foodborne illness acquired in the United States-major pathogens. Emerg Infect Dis. 2011;17(1):7–15.
44. United States Food and Drug Administration. Food safety for transplant recipients. [Updated September 2011; cited June 16, 2019. https://www.fda.gov/food/people-risk-foodborne-illness/food-safety-transplant-recipients
45. American Veterinary Medical Association. One health-what is one health. [Updated 2019; cited June 16, 2019]. https://www.avma.org/KB/Resources/Reference/Pages/One-Health94.aspx
46. Day MJ, Breitschwendt E, Cleaveland S, Karkare U, Khanna C, Kirpensteijn J, et al. Surveillance of zoonotic infectious disease transmitted by small companion animals. Emerg Infect Dis. 2012;18(12):e1.
47. Kotton CN. Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. Clin Infect Dis. 2007;44:857–66.
48. Chomel BB. Diseases transmitted by less common house pets. Microbiol Spectr. 2015;3(6): IOL5–0012-2015.
49. Halsby KD, Walsh AL, Campbell C, Hewitt K, Morgan D. Healthy animals, healthy people: zoonosis risk from animal contact in pet shops, a systematic review of the literature. PLoS One. 2014;9(2):e89309.
50. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Diseases. Healthy pets, healthy people: organ transplant patients. [Updated April 30, 2014; cited June 16, 2019]. https://www.cdc.gov/healthypets/specific-groups/organ-transplant-patients.html

51. Kotton CN, Lattes R, AST Infectious Diseases Community of Practice. Parasitic infections in solid organ transplant recipients. Am J Transplant. 2009;9(Suppl 4):S234–51.
52. Kleinschmidt-DeMasters BK, Marder BA, Levi ME, Laird SP, McNutt JT, Escott EJ, et al. Naturally acquired West Nile Virus encephalomyelitis in transplant recipients clinical, laboratory, diagnostic, and neuropathological features. Arch Neurol. 2004;61:1210–20.
53. Keystone JS. Immigrants returning home to visit friends and relatives (VFR) in Yellow Book [Internet];[Updated June 13, 2017; cited May 19, 2019]. https://wwwnc.cdc.gov/travel/yellowbook/2018/advising-travelers-with-specific-needs/immigrants-returning-home-to-visit-friends-relatives-vfrs
54. Boggild AK, Sano M, Humar A, Gilman M, Salit I, Kain KC. Travel patterns and risk behavior in solid organ transplant recipients 2004; 11: 37-43.
55. Kotton CN, Kroger AT and Freedman DO. Immunocompromised travelers in Yellow Book [Internet]; [Updated June 13, 2017; cited June 16, 2019]. https://wwwnc.cdc.gov/travel/yellowbook/2018/advising-travelers-with-specific-needs/immunocompromised-travelers
56. Patel RP, Liang SY, Koolwal SY, Kuhlmann FM. Travel advice for the immunocompromised traveler: prophylaxis, vaccination, and other preventive measures. Ther Clin Risk Manag. 2015;11:217–28.

Vaccination Strategies in Solid Organ and Stem Cell Transplant

12

Shilpa R. Jain and Deepali Kumar

Contents

Introduction to Vaccination in Transplant Recipients 253
Specific Vaccine Recommendations 255
Vaccination Against Influenza 255
Vaccination Against Pneumococcal Disease 259
Vaccination Against Meningococcal Disease 262
Vaccination Against Hepatitis B Virus 264
Vaccination Against Hepatitis A Virus 266
Vaccination Against Human Papillomavirus 267
Vaccination Against Varicella Zoster Virus 269
Vaccination Against Measles, Mumps, and Rubella 274
Other Routine Vaccines 275
Special Considerations in Vaccination of the Transplant Recipient 275
Other Specifics Regarding Vaccine Timing 275
Travel Vaccines 276
Immunization of Household Contacts 277
Conclusion 277
Key Points 278
Cross-References 278
References 278

Introduction to Vaccination in Transplant Recipients

Patients who have undergone solid organ transplant (SOT) or hematopoietic stem cell transplant (HSCT) are at a higher risk than the general population of contracting a vaccine-preventable illness, by virtue of their immunosuppressed state. It follows

S. R. Jain · D. Kumar (✉)
Transplant Infectious Diseases, University Health Network, Toronto, ON, Canada
e-mail: shilpa.jain@medportal.ca; Deepali.Kumar@uhn.ca

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_11

that vaccination in the transplant patient is an important strategy in mitigating the risk of infectious complications and mortality post-transplant. Given the significant differences in immunogenicity post-SOT and HSCT, the optimal strategy for vaccination in each of these two populations differs.

Recipients of SOT are chronically at risk of infections due to their lifelong use of immunosuppressive medications. The ideal strategy regarding vaccination in the SOT population is to ensure the patient is up-to-date on their immunizations *prior* to the transplant occurring. There are two important principles that govern this recommendation. First, live vaccines are generally contraindicated once a patient has undergone SOT, given the risk of enhanced viral replication in the immunosuppressed patient [1, 2]. Thus, when possible it is important to ensure all live vaccines are administered $\geq$4 weeks prior to transplantation. Second, while inactivated vaccines are generally considered safe in the SOT population [3–5], it is well-known that the immune response to vaccines in this population can be dampened by the use of immunosuppressive medications. Therefore, administration of all recommended vaccines prior to transplant is ideal. In certain situations, however, there may not be enough time to fully immunize a patient prior to undergoing SOT, or certain vaccines might require annual or booster doses in the future. In these cases, careful timing of vaccination post-transplant is critical. Guidelines generally recommend administration of inactivated vaccines begins 3–6 months following organ transplantation, with certain exceptions [6]. This is to avoid vaccinating soon after receipt of induction immunosuppression or while on high doses of immunosuppressive medications that can impair T- and B-cell responses and thus hinder the ability to mount an appropriate immunogenic response. In the absence of graft rejection or other complications, immunosuppression has generally been tapered to maintenance doses by 3–6 months post-transplantation, making immunization more ideal after this time period.

The basic principle behind HSCT involves eliminating disease using cytotoxic therapy in conjunction with immune replacement using a donor's or one's own disease-free stem cells. By way of this process, much of the transplant recipient's immunity to specific pathogens is lost. Thus, in this population, regardless of a patient's vaccination history prior to HSCT, all or most immunizations must be readministered in a carefully timed schedule following HSCT. However, in contrast to SOT patients who require lifelong immunosuppression, HSCT recipients are expected to eventually reconstitute their immune system. It can take up to 3 months before B- and T-cell counts begin to recover post-HSCT. B-cell count recovery can be further delayed in patients who receive rituximab post-transplant. Counts generally recover to a level acceptable enough to mount an immune response to vaccines by 6–12 months following HSCT, barring any complications such as graft-versus-host disease (GvHD) or graft rejection [7]. Thus, in usual circumstances, guidelines recommend the reintroduction of inactivated vaccines 6–12 months following HSCT [7]. Given HSCT patients are expected not to require lifelong immunosuppression, there is no lifelong contraindication to live vaccines as there is in the SOT population. At 24 months post-HSCT, a patient is generally considered immunocompetent again, provided there has been no history of GvHD or other issues requiring

prolonged immunosuppressive therapy. Thus, guidelines suggest administration of live vaccines is safe starting at 24 months post-HSCT, as long as the patient is free of GvHD and is off all immunosuppressive medications [7]. Although the process of immune recovery differs between autologous and allogeneic HSCT recipients, guidelines recommend the same vaccination schedule should apply for all HSCT recipients, regardless of type [7].

In summary, timing of vaccination following SOT and HSCT is critical in order to maximize immune response and safety post-transplant. A general outline of optimal timing of immunizations in SOT and HSCT recipients is presented in Tables 1 and 2, respectively. Unless otherwise specified, vaccination *prior* to SOT and/or HSCT is recommended in accordance with guidelines for the general population. Details and exceptions pertaining to each vaccine will be discussed further in the text that follows.

Specific Vaccine Recommendations

Vaccination Against Influenza

Influenza remains a major public health concern, and each year contributes significantly to morbidity and mortality in the general population. It is well-known that the risks associated with influenza are even higher in certain vulnerable populations, including in those who have undergone SOT or HSCT. For example, progression to lower respiratory tract infection or pneumonia is reportedly more common in transplant recipients than in the general population, with the incidence of influenza pneumonia ranging from 22.1 to 32.5% [8–11]. Vaccination against influenza is associated with lower viral loads at the onset of influenza infection, lower incidence of progression to pneumonia, and a lower rate of ICU admission [8]. Transplant recipients may still be at risk of developing infection secondary to influenza due to reduced immunogenicity of the vaccine as compared to the general population; however, in this subset of patients who do develop disease despite vaccination, disease severity is significantly reduced. Thus, annual vaccination against influenza is strongly recommended in both the SOT and HSCT populations [6, 7, 12].

There are two main types of influenza vaccine available: the live attenuated influenza vaccine (LAIV) and the inactivated influenza vaccine (IIV). The LAIV is contraindicated in transplant recipients given the theoretical risk of viral replication following its administration. The IIV is currently available either as a trivalent (containing three virus strains) or quadrivalent (containing four virus strains) formulation. Among these, the trivalent vaccine has been best studied in the transplant population and is available in standard-dose, high-dose, and adjuvanted formulations. Specific recommendations with respect to influenza vaccination in both the SOT and HSCT populations will be discussed below.

Recommendations in SOT. As previously mentioned, administration of inactivated vaccines is generally recommended to begin 3–6 months post-transplant in the SOT population [6]. However, given the significant risks associated with

Table 1 Recommended timing of vaccination in SOT patients

Vaccine	Recommended schedule
Influenza	Inactivated vaccine recommended annually beginning as early as 1 month post-SOT depending on timing of transplant relative to timing of local influenza season
Streptococcus pneumoniae	Recommended as a three-dose schedule: Conjugated vaccine followed by polysaccharide vaccine ≥8 weeks later, followed by polysaccharide booster vaccine 5 years later[a]
Neisseria meningitidis	Recommended in accordance with guidelines for the general population, except in certain high-risk scenarios
Haemophilus influenzae B	Recommended in accordance with guidelines for the general population
Tetanus, diphtheria, pertussis	Recommended in accordance with guidelines for the general population
Hepatitis B	Recommended as a three-dose schedule ideally at time 0, 1, and 6 months; alternatively may be administered as an accelerated schedule at time 0, 1, and 2 months or time 0, 7, and 21–28 days with a booster dose at 1 year
	Recommended in non-immune patients pre-transplant, but can also be administered beginning 3–6 months post-transplant; consider administration of high-dose rather than standard-dose vaccine in patients with end-stage kidney or liver disease
Hepatitis A	Recommended as a two-dose schedule given prior to liver transplant in non-immune candidates; if unable to administer prior to transplant, can administer post-transplant beginning at 3–6 months
	In all other organ transplant candidates/recipients, vaccination is recommended only if exposure risk is high (e.g., travel, lifestyle factors) as is recommended in the general population[b]
Human papillomavirus	Recommended as a three-dose schedule of quadrivalent or nonavalent vaccine at time 0, 2, and 6 months ideally prior to transplant; if unable to complete series prior to transplant, immunization can occur post-transplant beginning at 3–6 months
	Recommended for men and women up to age 45
Varicella	One dose of live attenuated viral vaccine is recommended to be given ≥4 weeks prior to transplant in non-immune patients, followed by a second dose if seroconversion has not occurred after one dose[c]
	Contraindicated post-transplant in adult patients
Herpes zoster	VZV-seropositive patients ≥ age 50 can receive one dose of live attenuated vaccine ≥4 weeks prior to transplant; alternatively vaccination with inactivated recombinant subunit vaccine can be administered as a two-dose schedule ≥8 weeks apart beginning 3–6 months post-SOT
	Live attenuated zoster vaccine is contraindicated post-SOT
Measles, mumps, rubella	One dose of live attenuated viral vaccine is recommended in non-immune patients ≥4 weeks prior to SOT, followed by a second dose if seroconversion has not occurred after one dose
	Contraindicated post-transplant in adult patients

[a]This is the recommendation in pneumococcal vaccine-naïve patients; see Table 3 for recommendations in other scenarios

[b]Given current outbreak of hepatitis A virus in the United States, consider vaccination of all non-immune transplant candidates

[c]This recommendation is based on guidelines published by the American Society of Transplantation; guidelines by the Infectious Diseases Society of America suggest administration of two doses of varicella vaccine in non-immune transplant candidates, with each dose 4 weeks apart.

Table 2 Recommended timing of vaccination in HSCT patients

Vaccine	Recommended schedule
Influenza	Inactivated vaccine recommended annually beginning as early as 3 months post-HSCT depending on timing of transplant relative to timing of local influenza season
Streptococcus pneumoniae	Recommended as a three-dose schedule of conjugated vaccine beginning at 3–6 months post-HSCT with each dose administered 1 month apart, followed by one dose of polysaccharide vaccine at 12 months post-HSCT in those without GvHD; in those with GvHD, a fourth dose of conjugated vaccine can be administered instead
Neisseria meningitidis	Recommended as a two-dose schedule of quadrivalent conjugated vaccine beginning at 6 months post-HSCT in those aged 11–18 years; one booster dose at age 16–18 when the first dose was given between 11 and 15 years of age
Haemophilus influenzae B	Recommended as a three-dose schedule of conjugated vaccine beginning at 6–12 months post-HSCT
Tetanus, diphtheria, pertussis	Recommended as a three-dose schedule of full-dose diphtheria toxoid vaccine beginning 6–12 months post-HSCT
Hepatitis B	Recommended as a three-dose schedule in non-immune patients beginning at 6–12 months post-HSCT at time 0, 1, and 6 months
Hepatitis A	Recommended in accordance with guidelines for the general population
Human papillomavirus	Recommended in accordance with guidelines for the general population
Varicella	Recommended as a two-dose schedule given $\geq$4 weeks apart starting $\geq$2 years following HSCT in non-immune patients as long as patient is off all immunosuppressive therapies with no evidence of active GvHD
Herpes zoster	Live attenuated vaccine is contraindicated post-HSCT. There is not enough data to recommend use of inactivated recombinant subunit vaccine post-HSCT; if eligible, vaccination should occur in accordance with recommendations for the general population prior to transplant
Measles, mumps, rubella	One dose of live attenuated viral vaccine is recommended in non-immune patients beginning at $\geq$2 years post-HSCT, as long as the patient is off all immunosuppression, and there is no evidence of active GvHD

development of influenza infection in this population, vaccination against influenza specifically is recommended as early as 1 month post-SOT if the timing of transplant coincides with influenza season [6, 12, 13]. Anecdotal concerns have been raised about the potential for triggering organ rejection with early administration of influenza vaccine post-SOT; however, several studies that have looked at the development of HLA alloantibodies and rejection rates post-vaccination have refuted this finding [3, 4, 14, 15].

Regarding immunogenicity of the influenza vaccine, studies report seroprotection and seroconversion rates between 15% and 93% with use of the trivalent IIV in the SOT population [16]. This varying response rate depends on a number of factors, including type of transplant, timing from transplant, and type and intensity of immunosuppression. Given the overall suboptimal response to influenza vaccination in SOT recipients, numerous methods have been studied in

an attempt to increase immunogenicity to the vaccine in this population. For example, a randomized controlled trial (RCT) comparing a high-dose intradermal influenza vaccine to the standard-dose trivalent intramuscular vaccine revealed no significant difference in response rates to the two vaccines in SOT patients [14]. In fact, similar to the standard-dose trivalent vaccine, response rates to the high-dose intradermal vaccine remained quite a bit lower in the SOT population than those observed in the immunocompetent population [14, 17]. Another trial compared MF59-adjuvanted IIV to non-adjuvanted IIV in a cohort of SOT patients [18]. Both vaccines elicited similar seroprotection and seroconversion rates in the overall cohort. However, a subgroup analysis found that the group aged 18–65 appeared to respond significantly better to the adjuvanted vaccine. A recent RCT examined the efficacy of one dose of IIV as compared to a booster strategy whereby two doses were administered 5 weeks apart [19]. In the per-protocol analysis, short-term seroconversion rates to all three vaccine strains were significantly higher in the group who received a booster dose of vaccine; this result was not, however, demonstrated in the modified intention to treat analysis. Most promising is the recent evidence examining the immunogenicity of a high-dose trivalent IIV, containing four times the dose of each antigen as the standard-dose vaccine (60 μg vs. 15 μg). In the general population, the high-dose inactivated influenza vaccine is approved for use in patients ≥65 years of age. Recent data in the SOT population suggest significantly higher seroconversion rates with use of the high-dose trivalent IIV as compared to the standard-dose vaccine [20]. Anecdotally, some transplant centers are now suggesting administration of the high-dose trivalent IIV for all solid organ transplant recipients ≥18 years of age.

Recommendations in HSCT. Influenza infection in the HSCT population can carry with it an even higher mortality risk than that of the SOT population [8]. Thus, although the general recommendations for administration of inactivated vaccines suggest beginning at 6–12 months post-HSCT, influenza vaccination presents an exception. Guidelines recommend influenza vaccination as early as 3–4 months after HSCT, depending on the timing of transplant in relation to influenza season [7, 12, 21]. Because immune reconstitution usually occurs earlier in autologous than allogeneic HSCT recipients, it is possible that earlier administration of influenza vaccine in autologous recipients might produce an effective immune response; however, there are no specific data confirming this. Similar to the SOT population, it is important to strike a balance between early protection of the individual against influenza infection through vaccination and vaccinating at a point where the immune system has shown some signs of recovery, so as to elicit a greater immunogenic response.

Safety of the influenza vaccine has not been a major concern in the HSCT population. However, immunogenicity of the standard-dose trivalent IIV has been shown to be lower in the HSCT population as compared to the general population [22]. Response rates to seasonal influenza vaccine are greater as the interval between HSCT and vaccination increases, with rates approaching those of immunocompetent individuals at around 2 years post-transplantation [23]. In

addition to time from transplant, other factors associated with a lower vaccine response rate include lymphopenia, hypogammaglobulinemia, GvHD, type and intensity of immunosuppression, and the use of rituximab [22–26]. Various strategies have been trialed in order to improve influenza vaccine immunogenicity in the HSCT population. An RCT comparing an adjuvanted trivalent IIV with the non-adjuvanted trivalent IIV showed similar vaccine response rates between groups [24]. Another RCT comparing one versus two standard doses of influenza vaccine given 4 weeks apart revealed no significant increase in the immunogenic response following receipt of the second vaccine [27]. Overall, the use of granulocyte-macrophage colony-stimulating factor along with influenza vaccination in HSCT recipients has not been shown to increase response rates, although, in patients vaccinated at <1 year post-HSCT, and in allogeneic HSCT recipients, a higher response rate to the influenza B antigen was demonstrated [28]. In a study evaluating the effectiveness of HSCT donor or recipient pre-transplant vaccination, pre-transplant recipient vaccination resulted in protective titers up until day 60 post-transplant [29]. Finally, an RCT evaluating the high-dose trivalent IIV as compared to the standard-dose trivalent IIV in the HSCT population resulted in greater immunogenicity against influenza in those who received the high-dose vaccine [30]. Thus, use of high-dose IIV may be the optimal strategy for protection against influenza in the HSCT population, although the data are derived from small numbers of patients and not yet incorporated into guidelines [7, 21].

Vaccination Against Pneumococcal Disease

Pneumococcal disease, or infection secondary to *Streptococcus pneumoniae*, is a vaccine-preventable illness that can cause invasive disease in the form of bacteremia, pneumonia, or meningitis. It causes significant disease in the immunocompetent population, but the risk of invasive pneumococcal disease is much higher in both SOT and HSCT recipients [31, 32].

One of the major virulence factors used by *S. pneumoniae* is its capsular polysaccharide, which plays an important role in the prevention of phagocytosis, and complement activation. To date there have been over 92 serotypes of *S. pneumoniae* identified, differing from each other on the basis of antigenic variations in their capsular polysaccharides. There are two main pneumococcal vaccines available. The 23-valent pneumococcal polysaccharide vaccine (PPSV-23) has coverage against 23 serotypes of *S. pneumoniae*, whereas the 13-valent pneumococcal conjugate vaccine (PCV-13) covers 13 serotypes of *S. pneumoniae*. Guidelines recommend SOT and HSCT recipients receive both vaccines as each has its own advantages [6, 12, 21]. Protein-conjugated vaccines such as PCV-13 typically provide greater immunogenicity. They do so by inducing a T-cell-dependent immune response that leads to the formation of memory B-cells conferring long-term protection. In turn, polysaccharide vaccines such as PPSV-23 typically result in a poor memory B-cell response; however, the advantage of the PPSV-23 vaccine is that it

includes a broader range of pneumococcal serotypes (23 vs. 13 serotypes). The specific recommendations with respect to vaccination against pneumococcal disease for SOT and HSCT recipients will be discussed in detail below.

Recommendations in SOT. Studies have shown the risk of contracting invasive pneumococcal disease in the SOT population is as high as 13–41 times that of the general population, depending on the transplanted organ [33]. In a 10-year prospective population-based surveillance study performed in order to better understand the epidemiology of invasive pneumococcal disease in the SOT population, it was found that 85.0% of invasive pneumococcal infections in SOT recipients were due to a serotype included in PPSV-23 [31]. In this study only 23.8% of SOT recipients who developed invasive pneumococcal disease had been vaccinated using PPSV-23 within the past 5 years. These data further confirm the importance of immunization against pneumococcal disease in the SOT population. Despite this fact, pneumococcal vaccination rates among SOT recipients have been reported to be as low as 40% [34].

In the SOT population, studies have not demonstrated an increased immunogenic response to conjugated pneumococcal vaccination as compared to the polysaccharide vaccine [34, 35]. In an RCT designed to evaluate the immunogenicity of a conjugated as compared to a polysaccharide pneumococcal vaccine in 60 kidney transplant recipients, overall antibody titers were similar between groups at 8 weeks post-vaccination, although they were greater for serotype 23F in the conjugated vaccine group [34]. In a long-term follow-up of this study, it was noted that antibody titers for both groups declined significantly and were similar to baseline titers after 3 years [35]. More recently, in a prospective cohort study examining kidney transplant recipients immunized with PCV-13, 27% of patients seroconverted to 0 serotypes, 58% to 1–8 serotypes, and 16% to $\geq$9 serotypes [36]. As an alternate solution, experts have suggested a prime-boost strategy may improve vaccine immunogenicity in SOT patients, whereby the conjugated pneumococcal vaccine is given initially followed by a boost with the polysaccharide vaccine a minimum of 8 weeks later. An RCT designed to evaluate this method in adult liver transplant recipients revealed no significant immunogenicity benefit of the prime-boost strategy as compared to those receiving a single dose of "unprimed" polysaccharide vaccine [37]. Similarly, an RCT performed in adult renal transplant recipients revealed no significant difference in immune response with use of the prime-boost strategy as compared to the group that received two doses of polysaccharide vaccine 1 year apart [38]. There are prospective data in the pediatric SOT population to suggest that boosting with one dose of polysaccharide vaccine following completion of a primary series with conjugated vaccine enhances the immunogenic response in heart and liver transplant recipients; however, this has not yet been demonstrated in the adult SOT population [39]. Nevertheless, the Advisory Committee on Immunization Practices (ACIP) does recommend use of the prime-boost strategy for pneumococcal vaccination in immunocompromised hosts, including in adult SOT recipients [40]. This recommendation is also reflected in the most current guidelines regarding vaccination in SOT recipients [6, 12]. Additionally, given antibody titers are known to wane post-vaccination of the SOT recipient, guidelines also recommend

Table 3 Timing of PCV-13 and PPSV-23 vaccination in SOT recipients

Previous doses of pneumococcal vaccine	Recommended vaccination schedule
No previous pneumococcal vaccine	PCV-13 followed by PPSV-23 ≥8 weeks later, followed by PPSV-23 booster 5 years later
Previous PPSV-23	Wait ≥1 year prior to giving PCV-13 followed by PPSV-23 ≥8 weeks later (as long as 5 years has elapsed since previous PPSV-23 dose)
Previous PCV-13	Wait ≥8 weeks prior to giving PPSV-23 followed by PPSV-23 booster 5 years later

a booster dose of PPSV-23 5 years after receipt of the first dose [6, 40]. Table 3 outlines specific recommendations regarding timing of each vaccine in relation to one another in a number of different scenarios.

Recommendations in HSCT. HSCT recipients are at high risk of developing invasive pneumococcal disease; studies have shown the incidence is as high as 30.2 times that of the general population [41]. The risk appears to be higher in allogeneic than autologous HSCT recipients, and among allogeneic HSCT recipients, the risk is highest in those with chronic GvHD [42]. In the allogeneic HSCT population, this increased risk can be attributed in part to impaired reactivity to polysaccharide antigens, IgG2 deficiency, and decreased production of *S. pneumoniae*-specific opsonizing antibodies, which are all important in the regulation of pneumococcal infection. Chronic GvHD impairs immune reconstitution and requires treatment with immunosuppressive medications, further impairing the host's immune response. Although some HSCT patients may have been vaccinated against pneumococcal disease prior to transplantation, there is a gradual loss of acquired immunity that occurs after HSCT. Thus, re-vaccination post-HSCT is an important strategy in order to mitigate the risk of invasive pneumococcal disease.

A retrospective study examining the epidemiology of invasive pneumococcal disease in hematology patients in France revealed that 84% of serotypes causing disease would have been covered by PCV-13 and 92% by PPSV-23 [43]. Another study examining epidemiology of invasive pneumococcal disease in HSCT recipients in Toronto, Canada, revealed that 100% of serotypes causing disease would have been covered by PPSV-23 [41]. In this study, only 21.4% of HSCT recipients who developed invasive pneumococcal disease had been vaccinated in the past 5 years. Vaccination with PPSV-23 alone has been shown to result in protective antibody responses in as few as 19% of HSCT recipients after 2 years [44]. In an RCT comparing HSCT donor and recipient vaccination with polysaccharide vaccine as compared to donor and recipient vaccination with conjugated vaccine, where donors were vaccinated prior to stem cell harvest, and recipients 6 months post-HSCT, immunization with conjugated vaccine was found to be more immunogenic than vaccination with polysaccharide vaccine [45]. Knowing that a significant proportion of infections secondary to *S. pneumoniae* occurs within the first year post-HSCT, Cordonnier et al. performed an RCT examining early versus late immunization with conjugated pneumococcal vaccine in allogeneic HSCT

recipients. The results suggested that vaccination with three doses of conjugated pneumococcal vaccine beginning at 3 months post-HSCT was non-inferior to vaccination beginning at 9 months post-HSCT when measuring antibody titers 1 month after completion of the three-dose series [46]. However, at 24 months post-HSCT, response rates were significantly higher in the late vaccination group, suggesting faster waning of antibody titers in the early vaccination group. Additionally, the effect of a booster dose of PPSV-23 following the primary series was greater in the late vaccination group. Interestingly, of the patients who did not respond at all to three doses of conjugated vaccine, 41% appeared to respond to the antigens in the conjugated vaccine after receiving a booster dose of PPSV-23. Additionally, PPSV-23 vaccination as a booster following a primary vaccination series with conjugated pneumococcal vaccine has been shown to confer protection against additional antigens not found in the conjugated formulation [47]. Finally, in a study examining allogeneic HSCT recipients age $\geq$2 years, three doses of PCV-13 administered 1 month apart, beginning at 3–6 months post-HSCT, resulted in protective antibody responses [48]. An additional fourth dose of PCV-13 elicited antibody levels similar to those achieved with a single dose of PCV-13 in immunocompetent individuals.

With all of the available literature, current guidelines suggest administration of three doses of PCV-13 to HSCT recipients, beginning at 3–6 months post-transplantation. At 12 months post-HSCT, a dose of PPSV-23 is recommended provided the patient does not have chronic GvHD. In those with GvHD, given the known poor immunogenic response to PPSV-23 in this population, a fourth dose of PCV-13 is recommended at the 12-month mark instead [12, 21].

Vaccination Against Meningococcal Disease

Meningococcal disease, or infection secondary to *Neisseria meningitidis*, most commonly manifests as a form of bacterial meningitis, pneumonia, or bacteremia. The most common serotypes causing disease in humans are serogroups B, A, C, Y, and W-135. Surveillance data reported by the Centers for Disease Control and Prevention (CDC) suggest that in the United States, serogroup B is by far the most common cause of meningococcal disease in children $\leq$5 years of age, whereas in individuals $\geq$11 years old, most meningococcal disease is caused by serotypes C, Y, and W-135 [49]. Serotype A tends to be associated with travel, for example, to areas of sub-Saharan Africa where it remains endemic. Inactivated quadrivalent meningococcal vaccines cover serotypes A, C, Y, and W-135. They are available as either polysaccharide (MPSV-4) or conjugated (MCV-4) vaccines. Immunization against serotype B occurs via a multicomponent inactivated vaccine (4CMenB). Meningococcal vaccination is a routine component of the childhood vaccination series. Re-immunization is routinely recommended post-HSCT in patients 11–18 years of age [12]. In adult HSCT recipients, as well as in the SOT population, there is no evidence that the risk of meningococcal disease is greater than that of the general population; therefore, meningococcal vaccine is generally only recommended under specific

circumstances. The detailed recommendations pertaining to meningococcal vaccination in the SOT and HSCT populations will be discussed below.

Recommendations in SOT. Meningococcal vaccination is recommended as part of routine immunization schedules as dictated by each country's national guidelines. In the SOT population, there are no additional recommendations for vaccination against *N. meningitidis* [6, 12]. If a patient has already received routine childhood meningococcal vaccination, additional vaccination post-transplant is done only for certain high-risk scenarios. Such scenarios include travel to the meningitis belt of sub-Saharan Africa or to Saudi Arabia during the Hajj or Umrah pilgrimage [50]. Following SOT, patients may be functionally or anatomically asplenic (e.g., if splenectomy occurs as part of the transplant surgery). In these cases, guidelines recommend re-vaccination against meningococcal disease [6]. Receipt of the monoclonal antibody eculizumab presents another high-risk scenario for development of invasive meningococcal disease. Eculizumab has been used for treatment of recurrent atypical hemolytic-uremic syndrome in renal transplant recipients or for prevention of graft rejection in renal and heart transplant recipients. Its mechanism of action includes binding to C5 causing inhibition of the terminal complement pathway, placing the recipient at a one to two thousand-fold increased risk of developing meningococcal disease [51]. The ACIP recommends simultaneous immunization with MCV-4 as well as 4CMenB prior to eculizumab use [52]. The first dose of meningococcal vaccine should be given a minimum of 2 weeks prior to receipt of eculizumab. Development of meningococcal disease after eculizumab treatment, however, has been reported in the SOT population despite prior immunization against *N. meningitidis* [53]. In fact, the literature suggests suboptimal immune responses to both MPSV-4 and MCV-4 in adult SOT recipients [54]. Adequate response rates were reached in 40% of patients with MPSV-4 and 50% of patients with MCV-4. Further research is needed in order to determine optimal strategies for meningococcal vaccination in SOT recipients at high risk of disease.

Recommendations in HSCT. Routine re-vaccination against meningococcal disease is recommended in post-HSCT recipients aged 11–18 years. For adult HSCT recipients, guidelines suggest vaccination in accordance with guidelines for the general population [7, 12, 21]. However, in the scenario where an adult HSCT recipient is deemed to be functionally asplenic, re-vaccination should be considered. Currently there are no comparative studies evaluating the immunogenicity of MPSV-4 and MCV-4 in the HSCT population. However, extrapolating from the data on pneumococcal vaccination as well as the known mechanisms of action of these vaccine formulations, it is assumed that greater immunogenicity is achieved with the conjugated rather than polysaccharide vaccine. The literature suggests that 52% of allogeneic HSCT recipients who received a single dose of MCV-4 either showed no immunogenic response or showed a response to only one of the four antigens in the vaccine [55]. The most common antigen for which there was a response was serogroup A, which is the least common to cause disease in North America (although it is important in sub-Saharan Africa and the Middle East). This limited immunogenicity was seen despite the fact that median time to immunization was 2.34 years post-allogeneic HSCT. Interestingly, response to vaccination was not affected by

age, time from transplant, or history of GvHD. When a second or booster vaccine was given at a median of 8.9 months following the first vaccine, response was significantly better with 81.2% of patients responding to at least three serogroups. Thus, guidelines suggest administration of two doses of MCV-4 beginning at 6 months post-HSCT in patients aged 11–18 years or in those with other specific risk factors for invasive meningococcal disease [12]. A booster dose is recommended at age 16–18 when the first dose was received between the ages of 11 and 15. Duration of antibody response following meningococcal vaccination is unknown in this population; thus, it is unclear whether further booster doses would be of benefit. Vaccination with 4CMenB is recommended in accordance with each individual country's recommendations and in the setting of an outbreak.

Vaccination Against Hepatitis B Virus

Transplant patients are at increased risk of transmission of hepatitis B virus (HBV) via a number of different mechanisms. HBV in the transplant patient can be donor-derived or, rarely, can be transmitted via nosocomial outbreak. The normal modes of transmission including via sexual transmission, via needle sharing, and via shared products such as razors also apply to the transplant population. There are several vaccine formulations and dosage strengths available for immunization against HBV. Single-antigen formulations are available containing either 10 μg, 20 μg, or 40 μg of HBV surface antigen protein/mL. The 40 μg dose is considered high-dose and is recommended in certain populations who do not respond adequately to standard-dose HBV vaccine. Recently another 20 μg formulation of the HBV vaccine using a novel adjuvant (HepB-CpG) was approved for use in adults aged $\geq$18 years; however, it has not yet been studied in the transplant population [56]. Detailed recommendations regarding HBV vaccine in SOT and HSCT recipients will be discussed below.

Recommendations in SOT. Donor-derived HBV can be contracted by a solid organ transplant recipient via receipt of an organ from an HBV surface antigen or core antibody-positive donor [57]. HBV core antibody-positive donors are now being knowingly used as a strategy to increase the donor pool. Building immunity to HBV prior to transplant significantly reduces the risk of transmission post-transplant, most notably in the liver transplant population where transmission risk is high [58]. All SOT candidates should be evaluated for immunity to HBV as part of their pre-transplant work-up, by measurement of HBV surface antibody titers. Ideally, if found to be non-immune, the HBV vaccination series will be administered prior to transplantation [6, 12]. Typically, this is administered over a 6-month schedule at 0, 1, and 6 months. If transplantation occurs in the interim, the third dose may be given starting at 3–6 months post-transplant. If time is quite limited prior to SOT, an accelerated schedule can be followed (e.g., 0, 1, and 2 months or 0, 7, and 21–28 days with a booster dose at 1 year), although there have been no comparative studies evaluating responses in SOT recipients using an accelerated schedule as compared to the standard of care. This strategy, however, is still

preferred to the alternative option of vaccinating post-transplant, given immunogenicity after SOT is known to be quite low [59, 60]. In certain populations, when the HBV vaccine series is to be given prior to transplantation, high-dose HBV vaccine is recommended over standard-dose [12]. Specifically, high-dose HBV vaccine is recommended in patients with end-stage renal disease on hemodialysis and can be considered in patients with end-stage liver disease, as these populations are known to respond particularly poorly to standard-dose HBV vaccine [61, 62]. Further study is needed to determine the true benefit of high-dose HBV vaccine in these populations, as well as whether high-dose vaccine might improve vaccine efficacy in the post-SOT population.

Following completion of the HBV vaccination series, whether pre- or post-transplant, HBV surface antibody titers should be checked to ensure immunity [6, 13]. If immunity has not been achieved following a three-dose standard vaccination series, re-vaccination with a high-dose series can be attempted, although there is no available literature evaluating the benefit of this strategy. If and when immunity is achieved following HBV vaccination, experts recommend serial monitoring of HBV surface antibody titers to ensure immunity does not wane [6, 13]. Although in the immunocompetent population a three-dose HBV series is thought to confer lifelong immunity even if antibody titers wane, it is unclear whether this is the case in the post-SOT patient. Thus, in this population, if titers fall below the protective threshold, re-vaccination is recommended [13]. How often titers should be checked following demonstration of an adequate response to the HBV vaccine series differs depending on a patient's individual risk of HBV exposure, for example, based on their country of residence or subsequent travel plans to an endemic area.

If concurrent vaccination with hepatitis A virus (HAV) is required, vaccination can occur using a combined HAV/HBV vaccine, which contains 20 μg of HBV surface antigen protein/mL (see specific recommendations regarding HAV vaccination in the section entitled "Vaccination Against Hepatitis A Virus").

Recommendations in HSCT. Similar to the SOT population, HBV can be donor-derived in allogeneic HSCT through the use of HBV surface antigen or core antibody-positive grafts [63]. Guidelines recommend vaccination against HBV in the non-immune HSCT candidate if their donor is known to be surface antigen or core antibody-positive [21]. Regardless of immune status prior to HSCT, given the decline of HSCT recipient immunity post-transplant from vaccinations given pre-transplant, re-vaccination against HBV is generally recommended to occur beginning at 6–12 months post-HSCT [7, 12, 21]. Standard doses of vaccine are recommended for administration using a 0-, 1-, and 6-month schedule. Interestingly, there is evidence to suggest that donor vaccination against HBV allows transfer of immunity to HSCT recipients for some time after transplant [64]. However, due to logistical and ethical issues, donor vaccination is not recommended as part of the guidelines [7, 12]. In a review of 292 non-HBV-immune allogeneic HSCT recipients vaccinated against HBV using a standard 3-dose schedule post-transplant, only 59% of adult patients developed protective titers [65]. Risk factors for a decreased response included age >18 years and a history of chronic GvHD. Given the immunogenicity of the vaccine appears to be significantly lower post-HSCT than

in the general population, it is recommended to measure HBV surface antibody titers following completion of the HBV vaccination series to check for immunity [12]. In the event that protective titers have not been attained post-vaccination, strategies to consider might include re-vaccination or vaccination using high-dose HBV vaccine, although there is no literature evaluating the efficacy of high-dose vaccine in this population. If protective titers have been achieved, experts would recommend serial monitoring of HBV surface antibody titers as immunity is known to wane in this population [65].

In the HSCT population, if concurrent HAV vaccination is indicated, a combined HAV/HBV vaccine can be administered (see specific recommendations regarding HAV vaccination in the section entitled "Vaccination Against Hepatitis A Virus").

Vaccination Against Hepatitis A Virus

In the transplant population, vaccination against HAV is most often considered in relation to travel or in other high-risk situations. However, there are a few specific situations in which HAV vaccination is recommended regardless of the risk of exposure, which will be discussed below. The HAV vaccine is available either as a single-antigen formulation administered over a two-dose schedule or as a combined formulation with HBV administered over a three-dose schedule, as the latter contains a lower dose of HAV-specific antigen.

Recommendations in SOT. HAV vaccine is recommended in SOT candidates at high risk of HAV exposure, as determined by future travel plans, country of residence, occupational risk, or other lifestyle risk factors [6]. In liver transplant candidates, however, routine HAV vaccination is recommended for all those who are non-immune. This is largely based on the ACIP recommendation for all patients with chronic liver disease to be vaccinated against HAV given the knowledge that this population is at increased risk of fulminant liver failure and death if infected with HAV [66]. Given that liver transplant patients are at ongoing risk of developing chronic liver disease secondary to rejection, recurrence of pre-transplant disease, or other post-transplant complications, the same recommendations have been applied to this population. Vaccination can be given as a standard two-dose series or a three-dose series if being administered as a combination vaccine with HBV.

A prospective study of adult post-liver transplant patients in which non-immune patients were vaccinated against HAV at $\geq$1 year post-transplant revealed a response rate of 26% 7 months after the initial dose of vaccine [67]. This was compared to 98% of historical controls with chronic liver disease who had responded to the vaccine at 7 months. Thus, greater response rates are likely to be achieved with vaccination prior to transplant whenever possible. Immunity has been shown to wane after transplant; however, current guidelines recommend monitoring of HAV antibody titers only in the setting of ongoing risk of exposure, such as before travel to a high-risk area [6, 68]. Given poor overall response rates in transplant patients and waning immunity to HAV vaccine post-transplant, further studies are needed to determine the optimal strategy for HAV protection in this population.

Recommendations in HSCT. There are no specific recommendations for HAV vaccination in the HSCT population. Guidelines recommend using HAV vaccination in this population the same way one would in the general population, for example, vaccinating in the case of planned travel to an endemic area for HAV, or based on other high-risk exposures [7, 21]. There have been no specific data on the immunogenicity of HAV vaccine in HSCT recipients, but it is likely that immunogenicity post-transplant would be lower than that of a healthy adult, as has been shown in the SOT population.

Vaccination Against Human Papillomavirus

There are over 180 types of human papillomavirus (HPV) that have been identified to date. HPV can cause two main groups of disease: cutaneous warts and lesions of the mucosal or genital surfaces (including genital and anal warts and cancers of the genital and anal tracts). HPV viral types 1, 2, and 4 are the most common types implicated in cutaneous warts, whereas viral types 6 and 11 account for most cases of genital warts. Viral types 16 and 18 cause the majority of HPV-related anogenital cancers and are considered high-risk oncogenic types. Viral types 31, 33, 45, 52, and 58 are also often implicated in cases of anogenital cancer.

Currently there are three formulations of inactivated HPV vaccine available. The bivalent vaccine (HPV-2) protects against types 16 and 18. The quadrivalent vaccine (HPV-4) protects against types 6, 11, 16, and 18. More recently, a nonavalent vaccine (HPV-9) was developed, which protects against types 6, 11, 16, 18, 31, 33, 45, 52, and 58. In the general population, HPV vaccination is recommended up to the age of 45 in females and 26 in males. The HPV-2 vaccine is approved for use in women only, but HPV-4 and HPV-9 are recommended for both men and women. If administered in healthy persons $\geq$ age 15, HPV-4 and HPV-9 are generally recommended using a three-dose schedule at 0, 2, and 6 months. HPV-2 is also recommended on a three-dose schedule when the series is started at age 15 or later, however at 0, 1, and 6 months. In immunocompetent children aged 9–14 years, a two-dose schedule at 0 and 6 months has been approved for all three vaccine formulations. Specific recommendations with respect to HPV vaccination in SOT and HSCT recipients will be discussed below.

Recommendations in SOT. Solid organ transplant recipients have a 2.2–20.3-fold risk of developing an HPV-related cancer as compared to the general population, depending on the site of the cancer [69]. When reviewing 187,649 SOT recipients in the United States, Madeleine et al. found a 2.2-fold increased risk of HPV-related invasive oropharyngeal cancer as compared to the general population, and a 20.3-fold increased risk of in situ vulvar cancer related to HPV. All other HPV-related anogenital cancers were also found to be much higher risk in SOT recipients aside from invasive cervical cancer which was found to occur at an incidence similar to that of the general population. The risk of developing HPV-related cancer increased with time from transplant, as well as with the number of transplants a given patient had received. This suggests the risk may be correlated with cumulative

duration of exposure to immunosuppressive agents. The results of this study highlight the importance of HPV vaccination in all eligible SOT recipients.

The immunogenicity of HPV-4 was examined in a prospective cohort study of 50 adult SOT recipients aged 18–35 [70]. Patients were a minimum of 3 months post-transplant, and all were on stable immunosuppressive regimens. Each patient received three doses of HPV-4 on a 0-, 2-, and 6-month schedule. Immunogenicity at 1 month post-completion of the vaccination series was measured, and the results revealed a response rate of 52.6–68.4% depending on the HPV type being measured. This is significantly lower than what has been demonstrated in healthy vaccine recipients [71]. In this study, vaccine response was greater as the time from transplant increased, and lower in lung as compared to kidney recipients [70]. At 6 months post-vaccine series completion, antibody titers to each viral type waned significantly overall, although they remained within protective levels. In another study, a three-dose series of HPV-4 was administered to liver and kidney transplant recipients aged 9–17 years, starting at 6 months post-transplant [72]. In this study, a 100% seroconversion rate to all four vaccine types was noted, although only eight patients completed the full vaccine series and underwent serological testing. A third study examining HPV-4 response rates in kidney transplant recipients aged 9–21 reported rates similar to those demonstrated by Kumar et al. in those aged 18–35 [70, 73]. Thus, further studies are needed to determine whether the strength of immune response to HPV vaccine is affected by age.

Guidelines recommend vaccination of all eligible SOT recipients between the ages of 9 and 45 against HPV using the HPV-4 or HPV-9 vaccine in a three-dose schedule [6]. This recommendation applies to SOT recipients aged 9–14 as well, despite the approved two-dose schedule in immunocompetent patients of this age group. Thus far there have been no studies regarding the immunogenicity of the HPV-9 vaccine in SOT recipients; however, the ACIP recommends it be added to HPV-2 and HPV-4 as a third option with broader coverage, including in the immunocompromised population [74]. Ideally, given the reduced immunogenicity to HPV vaccination post-transplant, the three-dose series should be given prior to transplant. If, however, the series is not complete or has not been started prior to transplant, vaccination can resume or begin at 3–6 months post-transplant [6]. At this time, it is unclear whether there is any benefit in vaccinating patients above age 45 against HPV in the SOT population.

Recommendations in HSCT. Current guidelines do not suggest any specific recommendations for HPV vaccination post-HSCT aside from those that exist for the general population [7, 12]. There is a clinical trial ongoing in order to examine the immunogenicity of HPV-9 in allogeneic stem cell transplant recipients, but as of yet there has been no published data on the use of any of the available HPV vaccines in this population (NCT03023631). This is an area of needed research, as HSCT recipients are known to have a high rate of HPV-related genital disease [75]. Similar to other vaccines, it is possible that immunity from pre-transplant vaccination against HPV may wane following HSCT; thus, further studies are also needed to determine whether re-vaccination post-HSCT would be beneficial and safe.

Vaccination Against Varicella Zoster Virus

Varicella zoster virus (VZV) is the causative agent of both primary varicella (chicken pox) and herpes zoster (shingles). Chicken pox is the primary manifestation of VZV, usually occurring in childhood. In the immunocompetent host, chicken pox is generally a benign infection, but in the immunocompromised host, it can be disseminated and life-threatening. Shingles typically occurs later in life, as a consequence of reactivation of latent VZV. Again, infection in the immunocompromised host can be much more severe than it is in the healthy patient. Thus, vaccination against VZV is an important consideration in both SOT and HSCT patients. All patients should be assessed for immunity against VZV prior to transplant, which would determine whether or not a patient is immune from either previous varicella infection or previous immunization. Once this information is available, a decision can be made regarding whether the transplant candidate requires vaccination against both varicella and herpes zoster, which are covered using two different vaccines. The specific details regarding vaccination against varicella and herpes zoster in both SOT and HSCT patients will be discussed below.

Vaccination against primary varicella

Varicella vaccine is a live attenuated viral vaccine. Vaccination against varicella protects not only against primary varicella but also against herpes zoster. The risk of developing herpes zoster has shown a trend toward being lower in those immunized with varicella vaccine as compared to those who have been previously infected with wild-type varicella virus [76]. Once the varicella vaccine became licensed in the United States in 1995, it was added as a routine childhood immunization in order to prevent primary varicella infection. In immunocompetent children, vaccine efficacy is reported to be between 80% and 85% against all varicella disease and greater than 95% against severe disease [77]. Varicella in the immunocompromised host can cause serious complications, including central nervous system disease, pneumonia, secondary bacterial infections, or even death. Furthermore, mortality is much higher for adults with varicella than it is for children [78]. Thus, vaccination against varicella is extremely important in the adult transplant population. Transplant candidates might already be immune from primary varicella infection or previous vaccination. It is important to be aware of any transplant candidate's immune status against VZV prior to transplantation.

Recommendations in SOT

Being a live attenuated viral vaccine, immunization with varicella vaccine is generally contraindicated post-SOT. Thus, if an adult SOT candidate is found to be non-immune to VZV, vaccination is recommended with one dose of varicella vaccine prior to transplant [6]. Post-vaccination, SOT candidates should be re-checked for immunity. If seroconversion has not occurred, guidelines would suggest re-immunizing with one more dose of varicella vaccine prior to transplantation, if time permits [6]. Alternatively, provided there is adequate time prior to transplantation, some guidelines would suggest administration of two doses of varicella vaccine in

non-immune transplant candidates, with each vaccine given a minimum of 4 weeks apart [12]. Safe administration of the vaccine requires that the patient is not on any immunosuppressive therapy and the vaccine is given ≥4 weeks prior to transplantation. The rationale for waiting at least 4 weeks after varicella vaccination prior to undergoing intense immunosuppression is to take into account the clearance of vaccine-induced viremia. If transplant is urgent, a decision will have to be made regarding whether or not it is possible to hold transplant for 4 weeks to allow for varicella vaccination. If there is not adequate time for vaccination prior to transplant, current guidelines would suggest using preventative measures other than vaccination, for example, postexposure prophylaxis in the case of an exposure to VZV post-transplant [6].

There are some data in the pediatric population to suggest that varicella vaccination in the post-transplant setting is safe and immunogenic [79, 80]. In one study, VZV-seronegative pediatric liver transplant recipients were immunized against VZV and monitored for development of varicella disease, as well as response to the vaccine [79]. All patients were ≥1 year post-transplant, with no episodes of acute rejection within the prior 2 months, were on low doses of immunosuppressive therapy, and had a total lymphocyte count ≥750 cells/μL at the time of immunization. A small proportion of children developed a vesicular rash within 8 weeks of vaccination, which may have represented attenuated varicella infection, but all lesions remained isolated and resolved spontaneously within 48 hours without antiviral treatment. This is a known adverse effect of the vaccine in immunocompetent recipients as well. Over 4 years of follow-up, there were no identified cases of varicella disease in these vaccinated patients. This suggests that in a very specific subset of pediatric patients, varicella vaccination may be considered post-SOT. However, no such studies have been completed in adult SOT recipients. In fact, cases of disseminated varicella after inadvertent varicella vaccination in adult post-SOT patients have been reported [81, 82]. Thus, until further study can be done to examine the safety of varicella vaccine in the adult SOT population, the recommendation remains to avoid the use of this live vaccine in adult SOT recipients [6, 12].

Recommendations in HSCT

Following HSCT, patients are at high risk of developing disseminated varicella infection if exposed, even in the setting of previous immunity from natural infection [83]. This likely relates to waning immunity post-HSCT. Thus, re-vaccination post-HSCT is an important strategy in mitigating the risk of recurrent varicella infection. Varicella vaccine in the HSCT recipient follows the general guidelines with respect to live vaccine administration in this population; it is considered safe once a patient is ≥2 years post-HSCT, off all immunosuppressive therapies, and without evidence of active GvHD [7, 12]. However, data on the epidemiology of varicella infection post-HSCT suggest that the majority of cases present within 1 year of transplantation and during this time period mortality secondary to varicella is the highest [83]. In this study, those at highest risk of infection were those who had received allogeneic HSCT or those with acute or chronic GvHD. There are no clear data as to whether or

not pre-transplant vaccination or donor vaccination might help reduce this risk of serious varicella infection in the first year post-HSCT.

Varicella vaccine safety and immunogenicity in adult autologous HSCT recipients was examined using a two-dose schedule with 8 weeks between doses, starting within 6 months post-HSCT [84]. Vaccine response was elicited in only 1 of 19 patients examined after the second dose of vaccine, suggesting the immune system was not yet mature enough to mount an appropriate response this soon after HSCT. The vaccine was noted to be relatively safe, however, with only two patients developing a varicella-like rash, which is not unexpected with this vaccine. There are no similar studies in adult allogeneic HSCT recipients.

Thus, with the lack of available immunogenicity and safety data in adult HSCT recipients, varicella vaccine is recommended in accordance with the recommendations for other live vaccines in this population, when all of the following criteria are met: $\geq$2 years since HSCT, no immunosuppressive use, and no evidence of GvHD [7, 12]. Two doses of vaccine are recommended, a minimum of 4 weeks apart, mimicking the schedule suggested in routine childhood immunization [12]. In addition, patients receiving varicella vaccine must not be on prophylactic anti-herpes viral therapy or immune globulin therapy as many post-HSCT recipients are, because these treatments are known to interfere with vaccine efficacy [12]. Guidelines recommend waiting at least 3 months post-administration of immune globulin prior to administering the vaccine [12, 85].

Vaccination against herpes zoster

Shingles or herpes zoster occurs as a result of reactivation of latent VZV, acquired from primary varicella infection. Therefore, VZV-seronegative persons who have never been exposed should not require vaccination against herpes zoster. VZV-seropositive persons who have acquired immunity from previous varicella infection have a risk of developing shingles later in life and thus should be vaccinated. It is unclear if herpes zoster vaccine is required in those who have immunity from previous varicella immunization *without* a history of natural varicella infection.

Herpes zoster can cause disseminated disease and significant postherpetic neuralgia (PHN) post-transplant [83]. Thus, vaccination against herpes zoster in the transplant patient is an important consideration. There are two main vaccine formulations available. Initially, the only option was a live attenuated zoster vaccine (LZV). In 2017, an inactivated adjuvanted recombinant subunit zoster vaccine (RZV) became widely available. Both vaccines are approved for use in immunocompetent adults $\geq$ age 50 to prevent shingles. Only one dose of LZV is required, whereas RZV is given as a two-dose schedule, separated by a minimum of 8 weeks. The major difference between the two vaccines in the immunocompetent population is with respect to efficacy. LZV has been found to reduce the incidence of herpes zoster by 51.3%, whereas RZV has been estimated to be 97% and 91.3% efficacious in adults up to the age of 70 and those above 70 years of age, respectively [86–88]. Similarly, LZV is 67% efficacious in preventing PHN, whereas RZV has been found to prevent PHN in 88.8–91.2% of cases [86–88]. LZV has been contraindicated in both SOT and HSCT recipients given that the vaccine contains ~14-fold greater

plaque-forming units of live virus than varicella vaccine [7, 12]. Therefore, a theoretical benefit of RZV in the transplant population is the fact that it is an inactivated vaccine and thus does not share the same risks of viral replication in the immunocompromised population. The specific recommendations with respect to vaccination against herpes zoster in the SOT and HSCT populations will be discussed below.

Recommendations in SOT

The crude incidence of herpes zoster in the SOT population has been reported to be as high as 5.7–21.3% depending on the organ transplanted, the type of immunosuppression, and the type of antiviral prophylaxis used [89–93]. The risk of herpes zoster infection in SOT recipients is anywhere from three- to tenfold higher than that of the general population [92, 93]. Thus, clear guidance on the optimal vaccination strategy in this patient population is needed.

Until recently, guidelines regarding vaccination against herpes zoster in the SOT population included guidance on the use of LZV only. As mentioned, given LZV is a live vaccine, its use is contraindicated post-SOT, given the risk of disseminated disease due to viral replication [6, 12]. In the SOT candidate, LZV can be administered to VZV-seropositive individuals $\geq$ age 50. There are preliminary data to suggest the use of LZV in renal transplant candidates with ESRD resulted in a boost in VZV titers equal to that of gender- and age-matched controls; however, it is unclear whether this provides any clinical benefit in prevention of herpes zoster post-transplant [91]. Additionally, it is unclear whether or not the vaccine is useful in patients less than 50 years of age. In a recent phase I RCT comparing safety and immunogenicity of LZV compared to placebo in renal transplant candidates $\geq$ age 18, Miller et al. demonstrated antibody responses in this population were similar to those seen in immunocompetent individuals $\geq$ age 50 [94]. The vaccine was safe with no herpes zoster-related adverse effects, even in patients who were transplanted within 30 days post-receipt of LZV. Interestingly, although antibody responses waned by 6 months post-immunization, there was no significant difference in titers between those who had been transplanted in that period and those who were still awaiting transplant. This is the first study to demonstrate immunogenicity of LZV in the pre-transplant population (including in patients $<$50 years of age), with antibody titers remaining in the protective range even post-SOT with up to 4 years of follow-up. Lastly, in the event that an organ transplant candidate has had previous herpes zoster, LZV can be still be given as long as 1 year has passed since the time of the active infection, with the intention of preventing further episodes; however, again there are no data to support the use of LZV beyond prevention of a first episode of shingles [6].

Vink et al. performed an RCT examining the safety and immunogenicity of RZV in adult renal transplant recipients [95]. Study participants were administered a two-dose series of RZV or placebo beginning at 4–18 months post-transplant. Results demonstrated that RZV was immunogenic in this population, with responses persisting through 1 year post-vaccination. The vaccine appeared safe, and there was no significant difference in graft rejection rates between arms. While these data

are promising, efficacy as well as longer-term immunogenicity data are needed. There is also a clinical trial ongoing with the goal of assessing the immunogenicity of RZV post-lung transplantation (NCT03493776). Despite limited data, RZV is being used at some centers for pre- and post-SOT patients who meet the indications for the vaccine in the general population (e.g., age ≥50 years, VZV seropositive). Extrapolating from recommendations regarding other inactivated vaccines in this population, if RZV is to be used post-transplant, immunization should occur a minimum of 3 months after the date of transplantation. As in the general population, RZV should be administered using a two-dose schedule separated by at least 8 weeks. If a patient has previously received LZV, in the general population, it is reasonable to administer RZV as long as at least 1 year has elapsed since LZV was given; this rule could potentially be applied to the SOT population as well. RZV has shown high efficacy against shingles in the immunocompetent population; thus, further safety and immunogenicity data in SOT candidates and recipients are needed.

Recommendations in HSCT

Herpes zoster in the HSCT population is associated with significant morbidity and mortality [83]. Guidelines with respect to vaccination against herpes zoster in this population were published prior to the availability of RZV. As mentioned above, LZV has not been recommended for use in HSCT patients, even if ≥2 years post-transplant, on no immunosuppression, and with no evidence of GvHD [7, 12]. This is in contrast to the recommendations with respect to varicella vaccination in this population. There are data published on the use of LZV in allogeneic and autologous HSCT recipients >2 years post-transplantation, with 98.2% of patients experiencing no adverse events after receiving LZV [96]. Two patients did develop a herpes zoster-like rash; however, there was no determination as to whether the rashes were due to wild-type or vaccine virus. Both patients required treatment with antivirals. Following the first 42 days post-vaccination, there were no reported cases of VZV reactivation, although median follow-up was only 9.5 months. Thus, further study is needed to determine the safety as well as immunogenicity of LZV in the HSCT population.

The safety and immunogenicity of RZV has been studied in autologous HSCT recipients [97]. Stadtmauer et al. performed a phase I/II RCT comparing RZV with placebo in autologous HSCT patients ≥ age 18. The vaccine was well-tolerated when administered starting at days 50–70 post-transplant, whether it was given on a two- or three-dose schedule. Cellular and humoral responses to the vaccine were strong using both schedules and persisted for up to 1 year after the last vaccine dose. These results are encouraging, yet specific guidance with respect to the use of RZV in the HSCT population has not yet been published. Currently, a phase III efficacy trial is underway in the autologous HSCT population (NCT01610414). As of yet, the vaccine has not been studied in the allogeneic HSCT population.

Recently, results of a randomized, placebo-controlled, phase III trial were published examining the efficacy and safety of an investigational herpes zoster vaccine inactivated by gamma irradiation in autologous HSCT recipients ≥ age 18 [98]. Patients received four doses of vaccine or placebo; the first dose was

administered 5–60 days before transplant, and the remaining doses were administered at approximately 30, 60, and 90 days post-transplant. A significantly higher proportion of patients in the placebo group developed herpes zoster. The estimated vaccine efficacy was 63.8%. The vaccine also appeared to reduce the severity of herpes zoster infection and its complications in those who did develop disease. The proportion of patients with serious adverse events was similar across both groups. The results of this trial are important not only in that they demonstrate promising results for a new vaccine against herpes zoster in HSCT recipients, but also because they show that vaccination during the highest period of immunosuppression post-HSCT can be effective. As of now it is unclear what role this vaccine formulation will play in immunization of the post-transplant patient.

Vaccination Against Measles, Mumps, and Rubella

Measles, mumps, and rubella (MMR) are all viral infections generally causing disease in childhood. Rubella also becomes important in pregnancy, as congenital infection can result from viral transmission to the fetus. In the immunosuppressed adult, measles infection can cause significant morbidity and mortality related to complications of pneumonia, acute encephalitis, and/or subacute sclerosing panencephalitis. Vaccination against MMR generally occurs via a combination live attenuated viral vaccine. In immunocompetent children, vaccination is recommended after the age of 12 months, followed by a booster dose several years later. Thus, adult patients can either be immune to MMR via natural infection or from previous vaccination. Given the constraints with respect to administration of live vaccines following transplant, it is important to know a patient's immune status prior to transplant. Specifically, measurement of measles antibody titers is important given outbreaks of measles continue to occur. Knowledge of immunity against rubella becomes more important in female transplant candidates who wish to become pregnant in the future.

Recommendations in SOT. MMR vaccine is contraindicated post-SOT; thus, if a patient is found to be non-immune, vaccination should occur prior to transplantation if possible [6]. In adults, one dose of vaccine is recommended followed by repeat testing to assess for seroconversion. If seroconversion has not occurred, a second dose can be administered, time-permitting. Similar to other live attenuated vaccines, vaccination against MMR must occur $\geq$4 weeks prior to transplantation in order to avoid giving immunosuppression during the period of viral replication. Preliminary data in very small groups of patients suggest that the use of MMR vaccine post-SOT in pediatric patients might be safe [99–101]. More recently, Pittet et al. demonstrated a strong seroresponse after administration of 1–3 doses of MMR vaccine to 44 pediatric liver transplant recipients $\geq$1 year post-transplant [102]. All patients were minimally immunosuppressed. Adverse events did occur post-vaccine that may have been attributable to viral replication; however, the presence of measles RNA was not confirmed microbiologically in any case, and for each patient, symptoms improved within days. This further suggests live vaccine administration to a carefully selected

subset of pediatric SOT recipients may be safe. As of yet there have been no studies regarding the safety of MMR vaccination after SOT in adult patients.

Recommendations in HSCT. Guidelines suggest vaccination against MMR in non-immune HSCT recipients in accordance with recommendations for all other live vaccines in this population: once the patient is ≥2 years post-transplant, off all immunosuppression, and without evidence of GvHD [12]. Seroconversion after MMR vaccine in this setting has been reported at 77%, 64%, and 75% for measles, mumps, and rubella, respectively, when studied in patients across the age spectrum [103]. Pre-transplant immunity to measles has been shown to wane beginning at 1 year post-HSCT, so re-immunization in the setting of previous vaccination or natural infection is important [104]. Early vaccination using one dose of MMR vaccine between 9 and 18 months post-HSCT resulted in no major safety issues despite the majority of patients still being on immunosuppressive therapy [105]. However, given many patients maintain immunity to measles in the first year post-transplant, administration of another dose of vaccine prior to waning of immunity resulted in a suboptimal vaccine response. Thus, early vaccination should be reserved for use in an outbreak setting. Additionally, MMR vaccine should be delayed a minimum of 3 months post-receipt of immune globulin for any indication [12, 85].

Other Routine Vaccines

Evidence is scarce regarding vaccination against *Haemophilus influenzae* serotype B (HiB) as well as for the use of diphtheria-tetanus-pertussis vaccine in adult transplant recipients. In SOT patients the recommendation is simply to vaccinate in accordance with immunization guidelines for the general population (e.g., tetanus booster every 10 years, etc.) [12]. In HSCT recipients, conjugated HiB vaccine is recommended using a three-dose schedule beginning at 6–12 months post-HSCT. Similarly, combination diphtheria-tetanus-pertussis vaccination with the use of vaccine containing full-dose diphtheria toxoid (e.g., DTaP) is recommended using a three-dose schedule beginning at 6–12 months post-transplant [7, 12].

Special Considerations in Vaccination of the Transplant Recipient

Other Specifics Regarding Vaccine Timing

Receipt of intravenous immunoglobulin (IVIg) and other blood products can interfere with the ability to mount an immune response to live vaccines. Thus, in both the SOT and HSCT populations, it is recommended to avoid MMR and varicella vaccines for 3–11 months post-receipt of IVIg or other blood products, depending on the type of blood product or dose of immunoglobulin replacement being administered [12, 85]. Rituximab is an anti-CD20 monoclonal antibody that may be used in the hematology setting depending on the underlying disease, or in the SOT

population for antibody-mediated rejection. Due to its B-cell-depleting properties, rituximab can significantly impair vaccine responses post-HSCT. Thus, guidelines recommend delaying vaccination for at least 6 months after the last dose of rituximab was received [21]. The same likely applies with receipt of other anti-B-cell monoclonal antibodies.

Two live vaccines can be administered on the same day; however, if they are *not* administered on the same day, guidelines suggest waiting 4 weeks in between each vaccine in order to maximize vaccine responsiveness [85]. Additionally, live vaccines can interfere with responses to tuberculin skin testing (TST). A TST can be implanted on the same day as live vaccine administration; however, if *not* done on the same day, guidelines suggest waiting a minimum of 4 weeks after vaccine administration prior to implantation of a TST [85].

Travel Vaccines

Transplant recipients should seek guidance from their physicians prior to travel for specific recommendations regarding travel safety. Depending on the patient's vaccination/immune status and the area of intended travel, the need for vaccination against HBV, HAV, and meningococcal disease should be routinely assessed (see recommendations with respect to administration of each vaccine in previous sections). If vaccination against *Salmonella typhi* is indicated, administration of the intramuscular, rather than oral formulation is recommended, as the oral formulation is a live attenuated vaccine. Rabies vaccine should be considered, especially if travel will include close animal contact. Vaccination against Japanese encephalitis using inactivated vaccine is recommended if travel to an endemic area will exceed 4 weeks' duration. Oral polio vaccine is contraindicated in the transplant recipient given the increased risk of vaccine-associated paralytic poliomyelitis from viral replication. If vaccination against poliovirus is indicated, inactivated vaccine can be given instead. Similarly, vaccination against tuberculosis with live attenuated bacillus Calmette-Guérin (BCG) vaccine is contraindicated in transplant recipients due to the risk of disseminated mycobacterial disease.

Live attenuated yellow fever vaccine is generally contraindicated due to the increased risk of severe vaccine-related complications, including viscerotropic or neurotropic disease. Certain countries require proof of yellow fever vaccine before entry into that country is permitted; in select cases a waiver can be issued to the transplant recipient exempting them from this requirement. However, transplant patients should be discouraged from travel to endemic countries at times of year in which there is a high risk of exposure [6]. Newer retrospective data regarding yellow fever vaccine in allogeneic HSCT recipients has become available, suggesting administration of the vaccine was safe in patients $\geq$2 years post-HSCT [106]. In this study, patients had been off all immunosuppressive therapies for a median of 33 months, and none had active GvHD at the time of vaccination. The vaccine was found to be efficacious overall when administered in this fashion. Patients who had been vaccinated prior to HSCT were also reviewed. In the majority of situations

where both donor and recipient had been vaccinated against yellow fever prior to transplant, immunity persisted at a median of 8.6 years of follow-up. Thus, in very select cases, yellow fever vaccine may be considered in HSCT recipients at ≥2 years post-transplant after withdrawal of immunosuppression in accordance with other recommendations regarding live vaccine administration in this population. A small series of 19 SOT patients who received inadvertent administration of yellow fever vaccine did not report any serious adverse events; however, not enough data are available to safely make a routine recommendation in this population [107].

Immunization of Household Contacts

In addition to immunization of the transplant recipient his or herself, ensuring household and close contacts (including pets) are fully vaccinated is an important strategy when it comes to maintaining the health of a transplant recipient. Specifically, annual influenza vaccine in all close contacts of a transplant patient is recommended [6, 7]. Preferably, a form of IIV should be used in household contacts of a transplant recipient. However, if LAIV must be used, the ACIP would recommend avoidance of contact with the vaccine recipient for up to 7 days [108].

In addition to influenza vaccine, household contacts should have all routine immunizations in accordance with their specific jurisdiction's recommendations. Receipt of all inactivated vaccines and almost all live attenuated viral vaccines in household contacts and pets is safe for the transplant recipient and in fact should be done in order to further protect these patients from exposure to vaccine-preventable illnesses [6, 7]. Exceptions to this include small pox and oral polio vaccines which should be avoided in household contacts of transplant recipients due to the risk of transmission of live virus to the immunocompromised host. If oral polio vaccine is inadvertently administered to a household contact of a transplant recipient, the ACIP recommends avoidance of close contact for 4–6 weeks [109]. If this is not possible, rigorous hand hygiene techniques should be employed after any contact with the vaccine recipient's feces, and contact with saliva should be avoided. This should continue for 4–6 weeks as maximum excretion of vaccine virus occurs within 4 weeks of receipt of oral polio vaccine. Rotavirus vaccine is not contraindicated in household contacts of transplant recipients. Precautions including frequent handwashing, specifically after diaper changes, are recommended following vaccine administration to a household contact, however, as viral antigen can be detected in the stool up to 15–30 days post-vaccination depending on the formulation used [110, 111].

Conclusion

Adult transplant recipients are at high risk of developing serious vaccine-preventable illnesses in light of their heavily immunosuppressed state. Vaccination in the post-transplant recipient is an important strategy in order to mitigate this risk. Timing of

vaccination in this population is critical and must take into account the desire to provide early protection against vaccine-preventable illnesses while maximizing immune response and safety. Given the reduced immunogenicity to vaccines in the post-transplant population, ongoing development of newer vaccination strategies in this highly-vulnerable group of patients should be a continued area of focus in order to promote healthier living and decrease mortality post-transplant.

Key Points

- Vaccination in patients undergoing SOT or HSCT is an important strategy in mitigating the risk of serious infection post-transplant.
- Careful timing of vaccination in the SOT and HSCT candidate or recipient is critical in order to provide early protection against vaccine-preventable illnesses while maximizing the potential for an adequate and safe immune response.
- Research regarding vaccination in the transplant patient has come a long way, but this is still an area of much needed attention in order to determine optimal vaccine strategies in this population.

Cross-References

- ▶ Changing Definition of Immunosuppression: Targeted Therapies and Resulting Emerging Infections and Their Prevention
- ▶ Hepatitis A Infections in Transplant
- ▶ Hepatitis B and Hepatitis D Infections in the Transplant Setting
- ▶ Herpes Simplex and Varicella Zoster in Transplant Candidates and Recipients
- ▶ Influenza Infections in Solid Organ and Stem Cell Transplant Recipients
- ▶ Preventative Health in Solid Organ Transplant: Safe Living Post-transplant
- ▶ Transplant Tourism and the Traveling Transplant Recipient: Infection Mitigation and Treatment Strategies
- ▶ Yellow Fever: Prevention in Transplant Candidates and Emerging Treatment Data for Liver Transplant

References

1. Mawhinney H, Allen IV, Beare JM, et al. Dysgammaglobulinemia complicated by disseminated measles. Br Med J. 1971;2:380–1.
2. Mitus A, Holloway A, Evans AE, Enders JF. Attenuated measles vaccine in children with acute leukemia. Am J Dis Child. 1962;103:413–8.
3. Kobashigawa JA, Warner L, Stevenson BL, et al. Influenza vaccine does not cause rejection after cardiac transplantation. Transplant Proc. 1993;25:2738–9.
4. Kimball P, Verbeke S, Flattery M, Rhodes C, Tolman D. Influenza vaccination does not promote cellular or humoral activation among heart transplant recipients. Transplantation. 2000;69:2449–51.

5. Magnani G, Falchetti E, Pollini G, et al. Safety and efficacy of two types of influenza vaccination in heart transplant recipients: a prospective randomized controlled study. J Heart Lung Transplant. 2005;24:588–92.
6. Danziger-Isakov L, Kumar D. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13563. https://doi.org/10.1111/ctr.13563.
7. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15:1143–238.
8. Kumar D, Ferreria VH, Blumberg E, et al. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67:1322–9.
9. Ljungman P, de la Camara R, Perez-Bercoff L, Infectious Diseases Working Party, European Group for Blood and Marrow Transplantation, Infectious Complications Subcommittee, Spanish Group of Hematopoietic Stem-Cell Transplantation, et al. Outcome of pandemic H1N1 infections in hematopoietic stem cell transplant recipients. Hematologica. 2011;96:1231–5.
10. Kmeid J, Vanichanan J, Shah DP, et al. Outcomes of influenza infections in hematopoietic stem cell transplant recipients: an application of an immunodeficiency scoring index. Biol Blood Marrow Transplant. 2016;22:542–8.
11. Reid G, Huprikar S, Patel G, et al. A multicenter evaluation of pandemic influenza A/H1N1 in hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2013;15:487–92.
12. Rubin LG, Levin MJ, Ljungman P, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58:309–18.
13. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant. 2009;9 (Suppl 3):S1–S157.
14. Baluch A, Humar A, Eurich D, et al. Randomized controlled trial of high-dose intradermal versus standard-dose intramuscular influenza vaccine in organ transplant recipients. Am J Transplant. 2013;13:1026–33.
15. Mulley WR, Dendle C, Ling JEH, Knight SR. Does vaccination in solid-organ transplant recipients result in adverse immunologic sequelae? A systematic review and meta-analysis. J Heart Lung Transplant. 2018;37:844–52.
16. Kumar D, Blumberg EA, Danziger-Isakov L, et al. Influenza vaccination in the organ transplant recipient: review and summary recommendations. Am J Transplant. 2011;11:2020–30.
17. Arnou R, Eavis P, Pardo JR, Ambrozaitis A, Kazek MP, Weber F. Immunogenicity, large scale safety and lot consistency of an intra-dermal influenza vaccine in adults aged 18–60 years: randomized, controlled, phase III trial. Hum Vaccin. 2010;6:346–54.
18. Kumar D, Campbell P, Hoschler K, et al. Randomized controlled trial of adjuvanted versus nonadjuvanted influenza vaccine in kidney transplant recipients. Transplantation. 2016;100:662–9.
19. Cordero E, Roca-Oporto C, Bulnes-Ramos A, et al. Two doses of inactivated influenza vaccine improve immune response in solid organ transplant recipients: results of TRANSGRIPE 1-2, a randomized controlled clinical trial. Clin Infect Dis. 2017;64:829–38.
20. Natori Y, Shiotsuka M, Slomovic J, et al. A double-blind, randomized trial of high-dose vs standard-dose influenza vaccine in adult solid-organ transplant recipients. Clin Infect Dis. 2018;66:1698–704.
21. Cordonnier C, Einarsdottir S, Cesaro S, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European conference of infections in leukemia (ECIL-7). Lancet Infect Dis. 2019; https://doi.org/10.1016/S1472-3099(18)30600-5.
22. Issa NC, Marty FM, Gagne LS, et al. Seroprotective titers against 2009 H1N1 influenza A virus after vaccination in allogeneic hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2011;17:434–8.
23. Engelhard D, Nagler A, Hardan I, et al. Antibody response to a two-dose regimen of influenza vaccine in allogeneic T-cell depleted and autologous BMT recipients. Bone Marrow Transplant. 1993;11:1–5.

24. Natori Y, Humar A, Lipton J, et al. A pilot randomized trial of adjuvanted influenza vaccine in adult allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2017;52:1016–21.
25. Mohty B, Bel M, Vukicevic M, et al. Graft-versus-host disease is the major determinant of humoral responses to the AS03-adjuvanted influenza A/09/H1N1 vaccine in allogeneic hematopoietic stem cell transplant recipients. Haematologica. 2011;96:896–904.
26. Fukatsu Y, Nagata Y, Adachi M, Yagu T, Ono T. Serum IgM levels independently predict immune response to influenza vaccine in long-term survivors vaccinated at > 1 year after undergoing allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2017;105:638–45.
27. Karras NA, Weeres M, Sessions W, et al. A randomized trial of one versus two doses of influenza vaccine after allogeneic transplantation. Biol Blood Marrow Transplant. 2013;19:109–16.
28. Pauksen K, Linde A, Hammarstrom V, et al. Granulocyte-macrophage colony-stimulating factor as immunomodulating factor together with influenza vaccination in stem cell transplant recipients. Clin Infect Dis. 2000;30:342–8.
29. Ambati A, Boas LS, Ljungman P, et al. Evaluation of pretransplant influenza vaccination in hematopoietic SCT: a randomized prospective study. Bone Marrow Transplant. 2015;50:858–64.
30. Halasa NM, Savani BN, Asokan I, et al. Randomized double-blind study of the safety and immunogenicity of standard-dose trivalent inactivated influenza vaccine versus high-dose trivalent inactivated influenza vaccine in hematopoietic stem cell transplant patients. Biol Blood Marrow Transplant. 2016;22:528–35.
31. Kumar D, Humar A, Plevneshi A, et al. Invasive pneumococcal disease in solid organ transplant recipients – 10-year prospective population surveillance. Am J Transplant. 2007;7:1209–14.
32. Kulkarni S, Powles R, Treleaven J, et al. Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants. Blood. 2000;95:3683–6.
33. Dendle C, Stuart RL, Mulley WR, Holdsworth SR, et al. Pneumococcal vaccination in adult solid organ transplant recipients: a review of current evidence. Vaccine. 2018;36:6253–61.
34. Kumar D, Rotstein C, Miyata G, Arlen D, Humar A. Randomized, double-blinded, controlled trial of pneumococcal vaccination in renal transplant recipients. J Infect Dis. 2003;187:1639–45.
35. Kumar D, Welsh B, Siegal D, Chen MH, Humar A. Immunogenicity of pneumococcal vaccine in renal transplant recipients – three year follow-up of a randomized trial. Am J Transplant. 2007;7:633–8.
36. Dendle C, Stuart RL, Polkinghorne KR, et al. Seroresponses and safety of 13-valent pneumococcal conjugate vaccination in kidney transplant recipients. Transpl Infect Dis. 2018;20:e12866. https://doi.org/10.1111/tid.12866.
37. Kumar D, Chen MH, Wong G, et al. A randomized, double-blind, placebo-controlled trial to evaluate the prime-boost strategy for pneumococcal vaccination in adult liver transplant recipients. Clin Infect Dis. 2008;47:885–92.
38. Tobudic S, Plunger V, Sunder-Plassmann G, Riegersperger M, Burgmann H. Randomized, single blind, controlled trial to evaluate the prime-boost strategy for pneumococcal vaccination in renal transplant recipients. PLoS One. 2012;7:e46133. https://doi.org/10.1371/journalpone.0046133.
39. Barton M, Wasfy S, Dipchand AI, et al. Seven-valent pneumococcal conjugate vaccine in pediatric solid organ transplant recipients. Pediatr Infect Dis J. 2009;28:688–92.
40. Kobayashi M, Bennet N, Gierke R, et al. Intervals between PCV13 and PPSV23 vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 2015;64:944–7.
41. Kumar D, Humar A, Plevneshi A, et al. Invasive pneumococcal disease in adult hematopoietic stem cell transplant recipients: a decade of prospective population-based surveillance. Bone Marrow Transplant. 2008;41:743–7.

42. Engelhard D, Cordonnier C, Shaw PJ, et al. Early and late invasive pneumococcal infection following stem cell transplantation: a European Bone Marrow Transplantation survey. Br J Haematol. 2002;117:444–50.
43. Debbache K, Varon E, Hicheri Y, et al. The epidemiology of invasive *Streptococcus pneumoniae* infections in onco-haematology and haematopoietic stem cell transplant patients in France. Are the serotypes covered by the available anti-pneumococcal vaccines? Clin Microbiol Infect. 2009;15:865–8.
44. Guinan EC, Molrine DC, Antin JH, et al. Polysaccharide conjugate vaccine responses in bone marrow transplant patients. Transplantation. 1994;57:677–84.
45. Kumar D, Chen MH, Welsh B, et al. A randomized, double-blind trial of pneumococcal vaccination in adult allogeneic stem cell transplant donors and recipients. Clin Infect Dis. 2007;45:1576–82.
46. Cordonnier C, Labopin M, Chesnel V, et al. Randomized study of early versus late immunization with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. Clin Infect Dis. 2009;48:1392–401.
47. Cordonnier C, Labopin M, Chesnel V, et al. Immune response to the 23-valent polysaccharide pneumococcal vaccine after the 7-valent conjugate vaccine in allogeneic stem cell transplant recipients: results from the EBMT IDWP01 trial. Vaccine. 2010;28:2730–4.
48. Cordonnier C, Ljungman P, Juergens C, et al. Immunogenicity, safety, and tolerability of 13-valent pneumococcal conjugate vaccine followed by 23-valent pneumococcal polysaccharide vaccine in recipients of allogeneic hematopoietic stem cell transplant aged $\geq$ 2 years: an open-label study. Clin Infect Dis. 2015;61:313–23.
49. CDC. Meningococcal disease. Atlanta: US Department of Health and Human Services. CDC; 2017. https://www.cdc.gov/meningococcal/surveillance/index.html
50. Kotton CN, Hibberd PL, The AST Infectious Diseases Community of Practice. Travel medicine and transplant tourism in solid organ transplantation. Am J Transplant. 2013;13:337–47.
51. Food and Drug Administration. Alexion briefing information for the November 18, 2014, meeting of the Drug Safety and Risk Management Advisory Committee. https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/DrugSDrugSafetyandRiskManagementAdvisoryComm/ucm423029.htm
52. CDC. Meningococcal ACIP recommendations. Atlanta: US Department of Health and Human Services. CDC; 2017. https://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/mening.html
53. Struijk GH, Bouts AHM, Rijkers GT, Kuin EAC, ten Berge IJM, Bemelman FJ. Meningococcal sepsis complicating eculizumab treatment despite prior vaccination. Am J Transplant. 2013;13:819–20.
54. Wyplosz B, Derradji O, Hong E, et al. Low immunogenicity of quadrivalent meningococcal vaccines in solid organ transplant recipients. Transpl Infect Dis. 2015;17:322–7.
55. Mahler MB, Taur Y, Jean R, Kernan NA, Prockop SE, Small TN. Safety and immunogenicity of the tetravalent protein-conjugated meningococcal vaccine (MCV4) in recipients of related and unrelated allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2012;18:141–55.
56. Food and Drug Administration. Product approval information: package insert. Heplisav-B. Silver Spring: US Department of Health and Human Services, Food and Drug Administration; 2018. https://www.fda.gov/BiologicsBloodVaccines/ApprovedProducts/ucm584752.htm
57. Huprikar S, Danziger-Isakov L, Ahn J, et al. Solid organ transplantation from hepatitis B virus-positive donors: consensus guidelines for recipient management. Am J Transplant. 2015;15:1162–72.
58. Skagen CL, Jou JH, Said A. Risk of de novo hepatitis in liver recipients from hepatitis-B core antibody-positive grafts – a systematic analysis. Clin Transpl. 2011;25:E243–9.
59. Lefebure AF, Verpooten GA, Couttenye MM, De Broe ME. Immunogenicity of a recombinant DNA hepatitis B vaccine in renal transplant recipients. Vaccine. 1993;11:397–9.
60. Loinaz C, de Juanes JR, Gonzalez EM, et al. Hepatitis B vaccination results in 140 liver transplant recipients. Hepato-Gastroenterology. 1997;44:235–8.

61. Stevens CE, Alter HJ, Taylor PE, Zang EA, Harley EJ, Szmuness W. Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. N Engl J Med. 1984;311:496–501.
62. Horlander JC, Boyle N, Manam R, et al. Vaccination against hepatitis B in patients with chronic liver disease awaiting liver transplantation. Am J Med Sci. 1999;318:304–7.
63. Mallet V, van Bommel F, Doerig C, et al. Management of viral hepatitis in patients with haematological malignancy and in patients undergoing haemopoietic stem cell transplantation: recommendations of the 5th European Conference on Infections in Leukaemia (ECIL-5). Lancet Infect Dis. 2016;16:606–17.
64. Shouval D, Ilan Y. Transplantation of hepatitis B immune lymphocytes as means for adoptive transfer of immunity to hepatitis B virus. J Hepatol. 1995;23:98–101.
65. Jaffe D, Papadopoulos EB, Young JW, et al. Immunogenicity of recombinant hepatitis B vaccine (rHBV) in recipients of unrelated or related allogeneic hematopoietic cell (HC) transplants. Blood. 2006;108:2470–5.
66. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 2006;55:1–23.
67. Arslan M, Wiesner RH, Poterucha JJ, Zein NN. Safety and efficacy of hepatitis A vaccination in liver transplant recipients. Transplantation. 2001;72:272–6.
68. Arslan M, Wiesner RH, Poterucha JJ, Gross JB Jr, Zein NN. Hepatitis A antibodies in liver transplant recipients: evidence for loss of immunity posttransplantation. Liver Transpl. 2000;6:191–5.
69. Madeleine MM, Finch JL, Lynch CF, Goodman MT, Engels EA. HPV-related cancers after solid organ transplantation in the US. Am J Transplant. 2013;13:3202–9. https://doi.org/10.1111/ajt.12472.
70. Kumar D, Unger ER, Paonicker G, Medvedev P, Wilson L, Humar A. Immunogenicity of quadrivalent human papillomavirus vaccine in organ transplant recipients. Am J Transplant. 2013;13:2411–7. https://doi.org/10.1111/ajt.12329.
71. Munoz N, Manalastas R Jr, Pitisuttithum P, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. Lancet. 2009;373:1949–57.
72. Gomez-Lobo V, Whyte T, Kaufman S, Torres C, Moudgil A. Immunogenicity of a prophylactic quadrivalent human papillomavirus LI virus-like particle vaccine in male and female adolescent transplant recipients. Pediatr Transplant. 2014;18:310–5.
73. Nelson DR, Neu AM, Abraham A, Amaral S, Batisky D, Fadrowski JJ. Immunogenicity of human papillomavirus recombinant vaccine in children with CKD. Clin J Am Soc Nephrol. 2016;11:776–84.
74. Petrosky E, Bocchini JA Jr, Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: updated HPV vaccination recommendations of the advisory committee on immunization practices. MMWR Morb Mortal Wkly Rep. 2015;64:300–4.
75. Savani BN, Stratton P, Shenoy A, Kozanas E, Goodman S, Barrett AJ. Increased risk of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation – implications for screening and HPV vaccination. Biol Blood Marrow Transplant. 2008;14:1072–5. https://doi.org/10.1016/j.bbmt.2008.07.005.
76. Lawrence R, Gershon AA, Holzman R, Steinberg SP. The risk of zoster after varicella vaccination in children with leukemia. N Engl J Med. 1988;318:543–8.
77. Heininger U, Seward JF. Varicella. Lancet. 2006;368:1365–76.
78. Rawson H, Crampin A, Noah N. Deaths from chickenpox in England and Wales 1997–7: analysis of routine mortality data. BMJ. 2001;323:1091–3.
79. Posfay-Barbe KM, Pittet LF, Sottas C, et al. Varicella-zoster immunization in pediatric liver transplant recipients: safe and immunogenic. Am J Transplant. 2012;12:2974–85.
80. Weinberg A, Horslen SP, Kaufman SS, et al. Safety and immunogenicity of varicella-zoster virus vaccine in pediatric liver and intestine transplant recipients. Am J Transplant. 2006;6:565–8.
81. Kraft JN, Shaw JC. Varicella infection caused by Oka strain vaccine in a heart transplant recipient. Arch Dermatol. 2006;142:943–5.

82. Levitsky J, Te HS, Faust TW, Cohen SM. Varicella infection following varicella vaccination in a liver transplant recipient. Am J Transplant. 2002;2:880–2.
83. Locksley RM, Flournoy N, Sullivan KM, Meyers JD. Infection with varicella-zoster virus after marrow transplantation. J Infect Dis. 1985;152:1172–81.
84. Sasadeusz J, Prince HM, Schwarer A, et al. Immunogenicity and safety of a two-dose live attenuated varicella vaccine given to adults following autologous hematopoietic stem cell transplantation. Transpl Infect Dis. 2014;16:1024–31. https://doi.org/10.1111/tid.12295.
85. Ezeanolue E, Harriman K, Hunter P, Kroger A, Pellegrini C. General best practice guidelines for immunization. Best practices guidance of the Advisory Committee on Immunization Practices (ACIP). http://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/downloads/general-recs.pdf
86. Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med. 2005;352:2271–84.
87. Lal H, Cunningham AL, Godeaux O, et al. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. N Engl J Med. 2015;372:2087–96.
88. Lal H, Cunningham AL, Kovac M, et al. Efficacy of the herpes zoster subunit vaccine in adults 70 years of age or older. N Engl J Med. 2016;375:1019–32.
89. Gourishankar S, McDermind JC, Jhangri GS, Preiksaitis JK. Herpes zoster infection following solid organ transplantation: incidence, risk factors, and outcomes in the current immunosuppressive era. Am J Transplant. 2004;4:108–15.
90. Manuel O, Kumar D, Singer LG, Cobos I, Humar A. Incidence and clinical characteristics of herpes zoster after lung transplantation. J Heart Lung Transpant. 2008;27:11–6.
91. Kho M, Weimar W, Bovee D, Boer-Verschragen M, van der Eijk A, Van Besouw N. Vaccination to prevent the high herpes zoster incidence after renal transplantation [abstract]. Am J Transplant. 2015;15 (Suppl 3). https://atcmeetingabstracts.com/abstract/vaccination-to-prevent-the-high-herpes-zoster-incidence-after-renal-transplantation/
92. Hamaguchi Y, Mori A, Uemura T, et al. Incidence and risk factors for herpes zoster in patients undergoing liver transplantation. Transpl Infect Dis. 2015;17:671–8.
93. Koo S, Gagne LS, Lee P, et al. Incidence and risk factors for herpes zoster following heart transplantation. Transpl Infect Dis. 2014;26:17–25.
94. Miller G, Schaefer H, Yoder S, et al. A randomized, placebo-controlled phase I trial of live, attenuated herpes zoster vaccine in subjects with end-stage renal disease immunized prior to renal transplantation. Transpl Infect Dis. 2018;20:e12874. https://doi.org/10.1111/tid.12874.
95. Vink P, Ramon Torrell JM, Sanchez Fructuoso A, et al. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in chronically immunosuppressed adults following renal transplant: a phase III, randomized clinical trial. Clin Infect Dis. 2019. pii: ciz177. https://doi.org/10.1093/cid/ciz177.
96. Issa NC, Marty FM, Leblebjian H, et al. Live attenuated varicella-zoster vaccine in hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2014;20:279–87.
97. Stadtmauer EA, Sullivan KM, Marty FM, et al. A phase 1/2 study of an adjuvanted varicella-zoster virus subunit vaccine in autologous hematopoietic cell transplant recipients. Blood. 2014;124:2921–9.
98. Winston DJ, Mullane KM, Cornely OA, et al. Inactivated varicella zoster vaccine in autologous haemopoietic stem-cell transplant recipients: an international, multicentre, randomised, double-blind, placebo-controlled trial. Lancet. 2018;391:2116–27.
99. Rand EB, McCarthy CA, Whitington PF. Measles vaccination after orthotopic liver transplantation. J Pediatr. 1993;123:87–9.
100. Kano H, Mizuta K, Sakakihara Y, et al. Efficacy and safety of immunization for pre- and post-liver transplant children. Transplantation. 2002;74:543–50.
101. Shinjoh M, Miyairi I, Hoshino K, Takahashi T, Nakayama T. Effective and safe immunizations with live-attenuated vaccines for children after living donor liver transplantation. Vaccine. 2008;26:6859–63.
102. Pittet LF, Verolet CM, McLin VA, et al. Multimodal safety assessment of measles-mumps-rubella vaccination after pediatric liver transplantation. Am J Transplant. 2019;19:844–54.

103. Ljungman P, Fridell E, Lonngvist B, et al. Efficacy and safety of vaccination of marrow transplant recipients with a live attenuated measles, mumps, and rubella vaccine. J Infect Dis. 1989;159:610–5.
104. Machado CM, Goncalves FB, Pannuti CS, Dulley FL, de Souza VA. Measles in bone marrow transplant recipients during an outbreak in Sao Paulo, Brazil. Blood. 2002;99:83–7.
105. Machado CM, de Souza VA, Sumita LM, da Rocha IF, Dulley FL, Pannuti CS. Early measles vaccination in bone marrow transplant recipients. Bone Marrow Transplant. 2005;35:787–91.
106. Sicre de Fontbrune F, Arnaud C, Cheminant M, et al. Immunogenicity and safety of yellow fever vaccine in allogeneic hematopoietic stem cell transplant recipients after withdrawal of immunosuppressive therapy. J Infect Dis. 2018;217:494–7.
107. Azevedo LS, Lasmar EP, Contieri FL, et al. Yellow fever vaccination in organ transplanted patients: is it safe? A multicenter study. Transpl Infect Dis. 2012;14:237–41.
108. Grohskopf LA, Sokolow LZ, Broder KR, Walter EB, Fry AM, Jernigan DB. Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices – United States, 2018–19 influenza season. MMWR Recomm Rep. 2018;67:1–20.
109. Prevots DR, Burr RK, Sutter RW, Murphy TV. Poliomyelitis prevention in the United States. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2000;49:1–22.
110. Food and Drug Administration. Product approval information-licensing action, package insert: Rotateq (Rotavirus Vaccine, Live, Oral Pentavalent), Merck. Rockville: US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research; 2006.
111. Food and Drug Administration. Rotarix clinical review. Rockville: US Department of Health and Human Services, Food and Drug Administration; 2008. http://www.fda.gov/cber/products/rotarix/rotarix031008.rev.pdf

Part II

Bacterial Infections Introduction

Multidrug-Resistant Organisms: Pre-transplant Evaluation and Management

13

Anoma Nellore and Rachael A. Lee

Contents

Introduction 288
Vancomycin-Resistant *Enterococcus* 288
Disease Epidemiology 289
Solid Organ Transplant 289
Bone Marrow Transplant 290
Clinical Impact in Transplant 290
Disease Diagnostics and Clinical Evaluation 291
Management 292
Prevention 292
Decolonization 293
Treatment 293
Disease Epidemiology 295
Solid Organ Transplantation 295
Stem Cell Transplantation 296
Clinical Implications 296
Solid Organ Transplantation 296
Stem Cell Transplantation 297
Disease Diagnostics 297
Management 297
Prevention 297
Infection Control 298
Decolonization 298
Treatment 298
Carbapenem-Resistant *Enterobacteriaceae* (CRE), *Acinetobacter* Species, and *Pseudomonas aeruginosa* 299
Disease Epidemiology 299
Solid Organ Transplant 301
Bone Marrow Transplant 301
CRE Screening 302

A. Nellore (✉) · R. A. Lee
Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA
e-mail: anellore@uabmc.edu; ralee@uab.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_8

Disease Diagnostics 302
Prevention of CRE Infection 304
Conclusion 306
References 307

Introduction

Infections due to multidrug-resistant organisms (MDROs) after transplantation are associated with high morbidity and mortality despite advances in the transplant field. Multidrug-resistant organisms in the solid organ transplant context may be donor-derived, especially from donors with hepatitis C viremia or from donors who have received narrow-spectrum gram-negative antibiotics [1]. More commonly, multidrug-resistant organisms that lead to invasive infections arise from the recipient in the pre- and post-transplant setting. Perioperative antibiotic regimens that are tailored to cover for donor pathogens and recipient pre-transplant MDROs are often necessary through organ transplantation [2]. In recent years, the top six causative organisms found to have increasing resistance to antibiotics have been grouped together under the acronym ESKAPE: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species [3]. Herein we will describe the epidemiology, pre-transplant evaluation, diagnosis, and treatment of these multidrug-resistant organisms (MDROs) in both solid organ transplant (SOT) and hematopoietic stem cell transplantation (HSCT).

Vancomycin-Resistant *Enterococcus*

Enterococci are facultative gram-positive cocci that are found as commensal organisms of the normal human intestinal tract [4]. However, in the 1970s, likely due to selective antibiotic pressure, enterococci evolved into a frequent cause of hospital-acquired infection via a number of virulence factors, including those that permit adhesion and biofilm formation [5, 6]. Two species of enterococci typically cause infection, *E. faecium* and *E. faecalis* [4]. Enterococci have several intrinsic resistance mechanisms to antibiotics [7]. Vancomycin-resistant enterococci (VRE) were first reported in 1986 in France [8]. Since that time, VRE have been named a serious public health threat by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) [9].

VRE colonization typically precedes infection [8]. Serial examination of the intestinal microbiota of HSCT recipients suggests that intestinal domination of VRE species precedes bacteremia [10]. Host and environmental risk factors are associated with VRE colonization. For example, immunosuppressing conditions like SOT, HSCT, hematologic malignancy, critical illness, renal dysfunction, and diabetes all predispose an individual for VRE colonization [11, 12]. VRE colonization has also been associated with prior receipt of antimicrobials [12]. Mouse models suggest that antibiotic elimination of commensal microbiota downregulates innate

immune-protective mechanisms to directly facilitate VRE colonization [13]. As an intra-abdominal microorganism, VRE infection in the pre-transplant setting often manifests as intra-abdominal infections such as spontaneous bacterial peritonitis or biliary tract infections in patients with end-stage liver disease; urinary tract infections, particularly catheter-associated infections; bloodstream infections; and less frequently endocarditis [7]. Here we review the epidemiology, clinical implications, and management of VRE colonization or infection as they pertain to pre-transplant evaluation of candidates for solid organ or bone marrow transplantation.

Disease Epidemiology

Solid Organ Transplant

A recent meta-analysis of VRE colonization and infection pre- and post-solid organ transplantation demonstrated that 11.9% of patients have pre-transplant VRE colonization [10]. Of note, the majority of the studies that composed this meta-analysis were from patients with end-stage liver disease who went on to receive liver transplantation. One of these studies was a single-center prospective study of liver transplant recipients which documented a 13% frequency of VRE colonization pre-transplant in this population [14]. This study also performed a multivariate analysis to analyze risk factors for VRE acquisition and found that risk factors for pre-transplant VRE colonization included prior hospitalization, prior procedures like paracentesis or ERCP, and receipt of an anti-anaerobic antibiotic.

VRE colonization has also been studied in patients with end-stage renal disease (ESRD) on dialysis [15]. A meta-analysis underscored the limitations in these studies with poor patient enrollment from dialysis centers in the majority of studies, variable follow-up of patients after VRE colonization, and variable reporting of antibiotic receipt in the dialysis patients under study. Nevertheless the meta-analysis found a pooled prevalence of 6.2% dialysis patients were colonized with VRE. The meta-analysis also reported associations between receipt of antibiotics, particularly vancomycin, and recent hospitalization as significant risk factors for the acquisition of VRE in the dialysis patient population.

The epidemiology of VRE colonization in patients with end-stage lung or heart disease has not been systematically studied. VRE colonization leading to VRE infection and bacteremia has been documented in individuals on left ventricular assist devices as a bridge to heart transplantation [7]. Additionally, VRE acquisition rate for patients who have been hospitalized in intensive care units has been documented to be 8.8% (95% CI 6.9–11.0) [16]. Thus, we can extrapolate that a significant proportion of patients with end-stage lung or heart disease who are frequently hospitalized in acute care settings are at risk for VRE colonization.

In the context of SOT, VRE colonization of the recipient prior to transplant is typically of greatest concern. Although there is a theoretical risk of transmission of

donor microbiota, including multidrug-resistant organisms to the recipient, this kind of transmission has not been systematically studied. However, a recent report does document the presence of a VRE strain from donor to recipient with a high degree of microbiologic similarity suggesting that donor-derived VRE transmission to recipient is possible [17]. These data underscore the importance of timely communication of donor culture results to transplant centers and warrant further investigation into the microbiota shifts after organ transplantation.

Bone Marrow Transplant

Bone marrow transplantation for hematologic malignancy usually occurs after a period of induction and consolidation chemotherapy for an acute hematologic malignant process. Thus, the incidence of VRE colonization among patients with an acute hematologic malignancy reflects the pre-bone marrow transplant burden. VRE colonization among patients with a hematologic malignancy has been reported to be as high as 24% (95% CI, 16%–34%) [16]. Risk factors for VRE colonization in this patient population include prior vancomycin and prior hospitalization in the past 3 months [16].

Clinical Impact in Transplant

Pre-transplant VRE colonization has been associated with the development of VRE bacteremia in the context of both SOT and HSCT. Here we review the clinical implications of VRE colonization pre-transplant on post-transplant outcomes.

Solid Organ Transplant

As VRE is an intra-abdominal commensal, the clinical consequences of pre-transplant VRE colonization have been studied best in the context of intra-abdominal SOT. For example, single-center prospective evaluation of VRE colonization in liver transplant patients identified 13% rate of VRE colonization in waitlist candidates [14]. Of these, 44% underwent liver transplantation and 32% developed VRE infection after transplantation. In contrast, among the 87% of individuals who were not VRE colonized prior to transplant, only 18% developed VRE infection after liver transplantation [14]. Of note, this study did not assess whether the genome of the pre-transplant VRE-colonizing strain was identical to the post-transplant infecting VRE strain. A study of VRE-colonized liver transplant candidates and recipients reported a significantly increased risk of death within 1 year of VRE colonization, and although this analysis was not restricted to the pre-transplant candidate specifically, half of the VRE colonization subjects in this study were identified and followed in the pre-transplant setting [18]. Collectively, these data underscore the negative consequences of pre-transplant VRE colonization on post-transplant outcomes.

Bone Marrow Transplant

Several studies have examined the relationship between pre-HSCT VRE colonization and post-HSCT VRE bacteremia and mortality [19–23]. In general, these studies associated VRE bacteremia after HSCT with high mortality. However, these studies had conflicting results regarding the relationship between pre-transplant VRE colonization and VRE bacteremia because they differed in the classification of pre-transplant VRE colonization either at time of stem cell transplant or prior to stem cell transplant as well as in the classification of VRE bacteremia pre-stem cell engraftment or post-stem cell engraftment. A more recent examination of the relationship between VRE colonization and hematopoietic stem cell transplant outcomes was designed to distinguish between subjects with VRE colonization at various timepoints prior to stem cell transplant and subjects who went on to develop VRE bacteremia at various timepoints after stem cell transplant [24]. This single-center study prospectively examined 161 patients with acute leukemia, 109 of whom underwent subsequent stem cell transplantation. The study documented that VRE colonization during treatment for acute leukemia was predictive of VRE colonization at the time of stem cell transplant. Molecular analysis performed on a subgroup of patients showed that 10/22 subjects had the same VRE-colonizing strain pre-transplant and at the time of transplant. VRE colonization prior to stem cell transplant was associated with pre-engraftment and post-engraftment VRE bacteremia as well as greater healthcare costs. Among subjects who were VRE colonized prior to transplant but did not have a positive VRE rectal swab culture at the time of transplant, none developed VRE colonization after stem cell transplantation. Thus, these recent data suggest that pre-transplant VRE colonization, particularly on the day of transplant, is predictive of subsequent VRE bacteremia after transplant.

Disease Diagnostics and Clinical Evaluation

Vancomycin binds to the D-alanine-D-alanine (D-ala-D-ala) residues of the enterococcal peptidoglcycan cell wall to exert its antimicrobial activity [25]. Various gene clusters termed *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN* alter the D-ala-D-ala residues of the enterococcal cell wall to prevent binding of vancomycin and thereby mediate resistance [25]. These gene clusters differ from one another according to constitutive versus inducible expression as well as structural arrangement and amino acid sequence [25].

The Clinical and Laboratory Standards Institute (CLSI) defines vancomycin-resistant enterococci as exhibiting a minimum inhibitory concentration or MIC to vancomycin of $\geq$ 32 ug/mL [26]. Tests to determine vancomycin resistance from infected sites require growth of the isolate and subsequent detection of vancomycin resistance using agar screens, disk diffusion screens, or broth microdilution screens. The latter can be automated using a VITEK system [26]. These methods have drawbacks including the required organism growth, user interpretation of results,

and inability to detect isolates with low-level resistance or inducible resistance to vancomycin.

Screening for VRE colonization is performed on sample obtained from rectal swabs. This material is typically plated onto chromogenic agar plates, which have a high sensitivity and specificity for VRE and can differentiate between *E. faecalis* and *E. faecium* species according to colony pigment [27, 28]. However, a rectal swab culture-based approach to determine VRE colonization has limitations, chiefly because other intestinal flora are also cultured from these swabs and may cause false-negative assays.

To overcome the limitations of culture-based VRE detection, molecular-based assays for VRE identification have been developed [29, 30]. These methods use PCR-based testing of the enterococcal genome to detect vancomycin resistance genes described above. Such molecular-based testing has the advantage of providing faster results than culture-based assays. However, false-positive results may occur because vancomycin resistance genes, vanA and vanB, may be present in anaerobic commensals [31]. To minimize false-positive molecular-based assays, two-tier approaches that utilize initial molecular-based testing followed by confirmatory culture results have been suggested [30]. Finally, serial 16s RNA sequencing of the intestinal microbiota to identify VRE strain in the overall microbiota composition may play a role in future early diagnosis of VRE colonization [32].

Management

Prevention

As discussed above, VRE colonization is often preceded by hospitalization. Transmission of VRE in the healthcare setting may occur from contaminated environmental sources as VRE can exist as a fomite, from direct contact with colonized healthcare workers, or from contaminated medical equipment [33]. These transmission events suggest VRE infection control measures to prevent VRE transmission may be important. Such measures include antimicrobial stewardship, rectal VRE surveillance swab cultures, strict hand-washing, staff education, intensive environmental cleaning, and contact isolation [7]. Although these measures have been recommended routinely in various national guidelines, the efficacy of these measures to prevent VRE transmission has not yet been convincingly demonstrated in randomized trials or cohort analyses [34, 35]. Contact precautions have been linked to certain adverse patient outcomes like fewer bedside visits and physical examinations [36]. Certain tertiary care centers have documented successful discontinuation of contact precautions for VRE with associated cost savings [37]. Expert guidance for the discontinuation of contact precautions for VRE is available; however, these guidelines do not pertain to individuals in the pre-transplant or post-transplant setting [38].

Currently, there are no approved vaccines that prevent the colonization of VRE. There are immunogenic antigens on the enterococcal cell wall that could be the basis

of for future vaccine design. One of these antigens, glycosylated lipoteichoic acid (LTA), provides passive immunity in a rabbit model of VRE infection [39, 40].

Decolonization

Screening for VRE in the pre-transplant setting is not routinely recommended unless there is an ongoing outbreak [41]. VRE colonization is often persistent and patients can become spontaneously decolonized and recolonized [42]. Although several antibiotic regimens have been tried to effect VRE decolonization, these have been variably successful. Fecal transplantation is a modality used to treat *Clostridioides difficile* and has been shown to eradicate VRE colonization in a case series [43]. Selective commensal species have been shown to eradicate VRE intestinal carriage in mouse models [44]. Thus, fecal transplantation may be a promising mechanism to eliminate VRE carriage, but this needs to be systematically studied across a variety of patient populations.

Treatment

Outside of infective endocarditis [45], published guidelines for the treatment duration or selection of antimicrobial agents for VRE infection do not exist. Expert opinion indicates that antibiotic selection should be dictated by patient tolerance, drug interactions, as well as site of infection [7]. Source control is a key component of VRE treatment and includes removal of suspected infected central lines, urinary catheters, or drainage of abscesses. A subset of VRE infections are from ampicillin-susceptible isolates, and in these cases, ampicillin is the preferred agent. Other antimicrobials commonly used to treat VRE include daptomycin and linezolid. Linezolid acts by binding the 23S prokaryotic ribosomal subunit preventing protein synthesis [46]. It has high oral bioavailability and tissue penetration and is bacteriostatic against VRE. Side effects of linezolid include reversible myelosuppression as well as neuropathies from mitochondrial toxicities. Linezolid interacts with serotonergic or adrenergic agent to cause serotonin syndrome. It is recommended that serotonergic or adrenergic agents be discontinued prior to receipt of linezolid [47]. In contrast to linezolid, daptomycin has concentration-dependent bactericidal activity against VRE. Daptomycin is renally cleared and must be dosed for renal function. Side effects include myalgias and rhabdomyolysis; therefore weekly creatine kinase monitoring is recommended while on this agent. Although the FDA-approved dosing recommendation of daptomycin is 6 mg/kg, subsequent retrospective cohort studies indicate that higher-dose treatment results in faster microbiologic clearance and lower 30-day mortality rates without adverse event [48]. Thus, high-dose daptomycin 8–10 mg/kg is likely superior for complicated VRE infections like bloodstream infection. Several studies have compared the efficacy of linezolid to daptomycin [5, 49–53]. While most of these studies concluded that linezolid was superior to daptomycin, these studies also acknowledged

significant study limitations, including, importantly, suboptimal daptomycin dosing. A subsequent multicenter study of veterans with VRE bacteremia concluded that daptomycin even dosed at 6 mg/kg is superior therapy to linezolid [54]. Thus, in the absence of a randomized control trial of linezolid versus optimally dosed daptomycin, it remains unclear which of these agents is optimal for the treatment of VRE infection.

Quinupristin/dalfopristin is a streptogramin antibiotic that has in vitro activity against VRE, but its side effect profile and lack of proven efficacy against VRE argue against its use as a first-line agent to treat VRE infection [53]. Tigecycline is structurally similar to tetracyclines but has expanded activity to include activity against VRE. It has higher tissue penetration than blood penetration and is not recommended for VRE bacteremia [55]. Currently, tigecycline carries an FDA black box warning for increased mortality particularly in its use to treat ventilator-associated pneumonia [56]. Thus, this agent should be used as a salvage agent only for VRE infection. Oral agents that have activity against VRE infections of the bladder include fosfomycin and nitrofurantoin. Fosfomycin has a low barrier for the development of resistance [57, 58]. Limited retrospective studies suggest that nitrofurantoin may have reduced efficacy in elderly individuals with compromised renal function and may result in pulmonary toxicities in these populations. Oritavancin and dalbavancin are analogs of vancomycin. While oritavancin has clinical activity against VRE, especially isolates with vanA gene resistance, dalbavancin has in vitro activity only against VRE with vanB gene-mediated resistance [56].

Resistance to daptomycin and linezolid has emerged [59]. Daptomycin-non-susceptible VRE may have thicker cell walls with reduced penetration of daptomycin. Although initially classified as having an MIC of >4 ug/mL by the CLSI, daptomycin treatment of isolates with an MIC 3–4 also appears to be ineffective [60]. Linezolid-resistant enterococci may form from a point mutation in a ribosomal subunit or from the mobile *cfr* gene element that can transfer between bacterial species [61]. Due to the development of these resistance patterns, combination therapies have been pursued to treat these isolates. For example, daptomycin and beta-lactams have been shown to have synergistic activity in vitro putatively because beta-lactam agents alter enterococcal cell membrane potential to allow enhanced bactericidal activity of daptomycin. Case reports have documented successful microbiologic clearance of highly resistant enterococcus using combination daptomycin and beta-lactam therapies [54, 60], but more rigorous studies need to be performed before this synergistic combination is accepted practice.

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus (*S. aureus*) is a gram-positive cocci and a frequent cause of skin/soft tissue infections, bacteremia, infective endocarditis, pneumonia, and osteomyelitis [62]. Methicillin was first used in 1959 to treat infection by penicillin-resistant *S. aureus*. As early as 1961, the first reports of methicillin-resistant *Staphylococcus aureus* or MRSA were reported in the United Kingdom [63]. Currently, MRSA is a global infection in both the hospital and community setting.

Table 1 Mechanism of action of resistance genes in MRSA and VRE and antibiotics identified with in vitro activity

Organism	Resistance gene	Resistance mechanism	Antibiotic affected	Antibiotic considerations (in vitro activity)
MRSA	*MecA*	Encodes for penicillin-binding protein 2a (PBP2a), which has lower affinity for β-lactams	Methicillin	Vancomycin Daptomycin Linezolid Ceftaroline Delafloxacin Doxycycline Tigecycline Omadacycline Eravacycline Sulfamethoxazole-trimethoprim Clindamycin Oritavancin Dalbavancin
VRE	*VanA*, *VanB*, *VanC*, *VanD*, *VanE*, *VanG*	Alteration of cell wall precursors from D-alanyl-D-alanine to D-alanyl-D-lactate	Vancomycin	Daptomycin Linezolid Dalfopristin-quinupristin Oritavancin Tigecycline

Resistance is typically mediated by methicillin-resistant penicillin-binding protein encoded by the *mecA* gene. This variant penicillin-binding protein 2 lowers the avidity of penicillin binding to the staphylococcal cell wall. The mecA gene is found on a mobile genetic element called the staphylococcal cassette chromosome *mec* (*SCCmec*). There are several types of *SCCmec* that correspond to different strains of MRSA and may distinguish community- from hospital-based strains [64]. More recently, MRSA has been isolated with a novel gene, *mecC*, mediating resistance. These cases of *mecC* MRSA appear restricted to animals with sporadic cases in humans documented in Europe [65]. In the context of solid organ transplantation, MRSA has been associated with perioperative surgical site infection and increased mortality, underscoring the importance of identification and eradication of MRSA in the pre-transplant setting (Table 1).

Disease Epidemiology

Solid Organ Transplantation

A meta-analysis of nine studies of MRSA colonization in the adult SOT population identified a 0.1–27.1% pooled prevalence of MRSA colonization in the pre-transplant setting. Immediately prior to transplantation, the prevalence of

MRSA colonization is 8.5% (95% CI 3.2–15.8%) [12]. These data are largely drawn from studies of liver transplant candidate ($N = 7$) studies and the remaining two studies reporting data on kidney, pancreas, and lung transplant candidates. A single-center retrospective study of pediatric SOT patients found the prevalence of MRSA colonization to be 13%, with lung, cardiac, and abdominal visceral organ transplant recipients most likely be colonized [66].

Studies of MRSA colonization with end organ disease who are potential candidates for organ transplantation have been performed. For example, a meta-analysis of MRSA colonization in dialysis patients documented an estimated prevalence of MRSA colonization in this patient to be 6.2% (95% CI 4.2–8.5%) [67]. Patients with MRSA colonization were more often on hemodialysis than on peritoneal dialysis [67]. Left ventricular assist devices (LVADs) are often bridges to heart transplantation. Rates of infection of these devices are as high 60–70% with MRSA infections accounting for 30–44% of infection [68]. These infections may manifest as driveline exit site infections, pocket infection, or pump infections [68]. Although these data indicate that MRSA is prevalent in the pre-transplant subject, additional studies with larger pre-transplant patient populations are needed to truly understand the epidemiology of MRSA in the pre-transplant setting.

Stem Cell Transplantation

Data regarding the epidemiology of MRSA colonization in stem cell transplant candidates is sparse [69]. A recent single-center retrospective study was performed in a tertiary care cancer center that mandates MRSA screening 2 weeks prior to transplantation. This study evaluated the prevalence of MRSA colonization among $N = 1895$ stem transplant candidates from 2008 to 2012. This study found that only 1.1% of this patient population was colonized with MRSA. Given the gaps in the literature regarding MRSA colonization in the patient listed for stem cell transplant, it is difficult to draw firm conclusions about the epidemiology of this pathogen in this patient population.

Clinical Implications

Solid Organ Transplantation

Only three out of nine studies followed adult patients from the time of identification of MRSA colonization pre-transplant into the post-transplant setting. These three studies reported data from liver transplant recipients alone. Data from these three studies indicate that pre-transplant carriage of MRSA results in a 5.5 times increased risk of post-transplant MRSA infection [12]. In a single-center retrospective study of pediatric organ transplant recipients, pre-transplant MRSA colonization resulted in infection in 3.9% of recipients [66]. Among patients with end-stage renal disease

on dialysis who represent a candidate pool of renal transplant recipients, those who were MRSA colonized were more likely to develop MRSA bacteremia with a relative risk of 11.5% (95% CI 4.7 to 28.0) [67]. These studies suggest that MRSA colonization places transplant candidates at risk for subsequent invasive MRSA infection.

Stem Cell Transplantation

Available data does not indicate the pre-transplant MRSA colonization portends post-transplant MRSA infection. However, these data are from single-center retrospective studies and were not performed during MRSA outbreaks, which have been reported to cause high rates of mortality in the stem cell transplant setting [69].

Disease Diagnostics

MRSA colonization may be identified as a colonizing agent via swab of mucosal surfaces, typically the nares, although there is evidence that swabbing multiple services for MRSA may result in increased sensitivity of testing [70]. MRSA infection is typically identified after culturing the relevant infectious source. The CLSI recommends both broth microdilution testing and cefoxitin disk diffusion testing to identify MRSA isolates. Cefoxitin disks are preferred over oxacillin disks because cefoxitin is a more potent inducer of the *mecA* gene. Any given staphylococcal culture may comprise methicillin-resistant and methicillin-sensitive strains, and this is termed hetero. Heteroresistance may be missed by cefoxitin disk diffusion testing unless cells are cultured at 33–35 °C and read at 16–18 h after plating. Newer chromogenic agar-based testing permits detection of MRSA isolates by pigment change. In addition to the aforementioned cell culture-based testing, there is also FDA-approved molecular PCR-based testing for *mecA* gene expression. There are also latex agglutination assays to assess for the expression of the protein product of the *mecA*, the penicillin-binding protein 2. (Please see ▶ Chap. 62, "Emerging Diagnostics for Transplant Infectious Diseases.")

Management

Prevention

-Although prior infection with MRSA does not induce durable immunity to prevent future infection, there is evidence that individuals colonized with MRSA have less severe MRSA infection [71]. These data suggest that prior immunity to MRSA may be partially immune-protective. MRSA prevention via active immunization from vaccines or passive immunization from monoclonal antibodies has been studied. One vaccine, StaphVAX, that included antigens against *S. aureus* polysaccharide

capsule failed to show efficacy in phase III clinical trials in the hemodialysis patient population. Another vaccine candidate, V710, which includes the antigenic MRSA scavenger protein IsdB, was studied in a phase III study of cardiac surgery patients. This study was halted due to increase in mortality from *S. aureus* infection. Current vaccine strategies under study include regimens that target several *S. aureus* antigens [71].

Infection Control

MRSA may be spread in the hospital and community setting. Within the healthcare environment, routine hand hygiene, environmental cleaning, and antimicrobial stewardship are the backbone of infection control measures to prevent the spread of MRSA. The value of patient isolation and contact precautions for MRSA colonization has recently been questioned, and several centers no longer require these measures to prevent the spread of MRSA [72].

Decolonization

In areas with high prevalence of MRSA, pre-transplant nasal swab to identify nasal colonization is recommended [73]. The efficacy and timing of nasal decolonization in the context of the transplant host as a means to prevent invasive infection are unclear [74]. However, MRSA decolonization procedures have been shown to be effective in individuals with end-stage renal disease on hemodialysis [75].

Treatment

Pre-transplant MRSA infection should ideally be treated to cure prior to transplantation [73]. MRSA treatment regimens by clinical manifestation have been reviewed extensively elsewhere [45, 62]. Source control is key to eradication of infection, and this may include device or line removal as MRSA has been known to create biofilms on prosthetic joints, catheters, and heart valves. Glycopeptide antibiotics like vancomycin are commonly used to treat MRSA infections, including uncomplicated and complicated bacteremia. Vancomycin inhibits bacterial cell wall synthesis and the permeability of the bacterial cell membrane [76]. MRSA isolates with decreased susceptibility to vancomycin were reported in Japan as early as 1997. CLSI minimum inhibitory concentration (MIC) for vancomycin intermediate sensitivity (VISA) is 4–8 ug/mL and for vancomycin resistance >16 ug/mL (VRSA). Resistance to vancomycin may be mediated by molecular changes to the staphylococcal cell wall.

Daptomycin, a cyclic lipopeptide antibiotic, is bactericidal against MRSA and disrupts cell membrane function and protein and nucleic acid synthesis [77]. Daptomycin-resistant MRSA may occur in the context of prolonged

infection without appropriate source control or inadequate vancomycin dosing. Typically, daptomycin resistance occurs in the context of prior vancomycin and/or daptomycin receipt; however cases of spontaneous resistance have been documented. Available data suggests that daptomycin may be non-inferior to vancomycin in the treatment of MRSA bacteremia and may actually be superior to vancomycin in isolates with a vancomycin MIC of 1 [78]. In addition, daptomycin may have a better side effect profile than vancomycin, resulting in fewer cases of renal insufficiency.

For uncomplicated skin and soft tissue infections, oral antibiotic therapy may be reasonable; oral options include trimethoprim-sulfamethoxazole, clindamycin, and doxycycline. Another oral antibiotic, bacteriostatic oxazolidinone, linezolid, inhibits the staphylococcal ribosome function and achieves excellent levels in the skin/soft tissues as well as the lung. There is evidence that linezolid may be superior to vancomycin for the treatment of MRSA pneumonia. Currently, the Infectious Diseases Society of America recommends linezolid and vancomycin as agents for the treatment of MRSA pneumonia [79].

The FDA has recently approved new antimicrobials for the treatment of MRSA. For example, the fifth-generation cephalosporin, ceftaroline, is approved to treat skin and soft tissue as well as MRSA pneumonia [80]. Oritavancin and dalbavancin are antibiotics that are chemically similar to vancomycin because they all prevent cell wall formation by binding to the peptidoglycan cell wall residues [80]. However oritavancin and dalbavancin have a unique lipid side change which confers a long half-life, permitting single-dose therapy. Of note, optimal dosing for renal insufficiency still needs to be established for dalbavancin.

For highly resistant MRSA isolates, synergistic therapies have been attempted and reported as case series and case reports [62]. There is a currently a rich pipeline of investigational agents with antimicrobials for MRSA that may provide improved outcomes for the treatment of MRSA infections. For example, a new class of anti-infectives, synthetic retinoids, have in vitro and in vivo activity against dormant MRSA subpopulations that persist after initial infection to cause relapsed disease [81].

Carbapenem-Resistant *Enterobacteriaceae* (CRE), *Acinetobacter* Species, and *Pseudomonas aeruginosa*

Disease Epidemiology

Multidrug-resistant gram-negative bacteria are on the rise, with high associated mortality, particularly in transplantation. The 2011 Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) identified that nearly 52% of all *K. pneumoniae* were resistant to ceftriaxone and produced ESBL and nearly 10% of all *E. coli* were resistant to ceftriaxone with over 75% producing ESBL [82]. In addition to ESBL-producing bacteria, carbapenem-resistant gram-

negative bacteria, in particular *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*, are escalating global public health threats.

Over the past two decades, *Enterobacteriaceae*, a family of gram-negative bacteria part of the commensal gut flora, have increasingly become more resistant to antibiotics. In the United States, carbapenem-resistant *Enterobacteriaceae* (CRE) has increased from 1.2% in 2001 to 4.2% in 2011 and is now present in all states [83]. Infection with CRE has been associated with mortality rates as high as 70% [83]. The major mechanism of resistance is the presence of β-lactamases, encoded by the gene *bla* [84]. In addition to this, many of the bacteria in this family can also carry plasmid-borne genes active against a variety of antibiotics, thus forcing providers to rely upon antibiotics considered the last line of defense [84]. Phenotypic resistance to carbapenems is typically caused by either β-lactamase activity combined with structural mutations or production of carbapenemases [84]. Carbapenemases are classified by their structure and belong to three classes of β-lactamases: classes A, B, and D; classes A and D require serine at their active site, whereas class B requires zinc for β-lactam hydrolysis [84]. One of the most common carbapenemase genes includes the *Klebsiella pneumoniae* carbapenemases (KPCs), which are capable of hydrolyzing all β-lactams and often have acquired resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole [84]. Clonal expansion of KPC-producing *Enterobacteriaceae* initially caused global spread of carbapenem resistance. The class D OXA β-lactamases or oxacillinases are typically found in *Acinetobacter* species and increasingly in *Enterobacteriaceae* [84]. The class B metallo-β-lactamases (MBLs) are a complex group of enzymes that hydrolyze all β-lactams except monobactams. Interestingly, the spread of New Delhi metallo-β-lactamase (NDM)-producing *Enterobacteriaceae* is not associated with clonal strains, but rather due to inter-regional spread of multiple clones associated with outbreaks [85].

Among the increasingly reported multidrug-resistant pathogens are the lactose non-fermenting gram-negative bacteria, including carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) and carbapenem-resistant *Acinetobacter baumannii* (CRAB). These are typically associated with hospital-acquired infections and can lead to large outbreaks with high associated mortality rates, ranging from 16% to 49% [86]. CRAB has a limited number of virulence factors, which is why infections due to this organism classically occur in those who are critically ill [87]. Risk factors for CRAB include immune suppression, burn injuries, mechanical ventilation, and prior colonization [87]. CRAB is able to survive for a prolonged period of time and spread throughout the healthcare institutions. Carbapenem resistance in *A. baumannii* is mediated classically by class D beta-lactamases (OXA-23) or oxacillinases, some of which are located chromosomally and some which are plasmid-mediated. Reports of CRPA strains are increasing, with rates as high as 50% in some institutions. Carbapenem resistance in *P. aeruginosa* has been mediated by outer membrane protein inactivation as well as through carbapenemases, such as metallo-β-lactamases (MBLs) and Verona integrin-mediated (VIM) B-lactamases [88].

Solid Organ Transplant

The prevalence of infections due to multidrug-resistant gram-negative bacteria following SOT is increasing, with an estimated 10–20% of recipients becoming infected with these organisms [89]. Transplantation has been identified as an independent risk factor for carbapenem-resistant *K. pneumoniae* [90]. Colonization with KPC-producing CRE in liver transplant recipients has led to high infection rates with high associated mortality, particularly when acquired in the hospital setting [91]. In a CRE outbreak at a tertiary care center, KPC *K. pneumoniae* disproportionately affected SOT recipients with a 30-day mortality rate as high 42% [92].

The incidence of SOT carbapenem-resistant infections varies considerably by type of organ. Many studies have reported early infection post-transplantation, with a median time of typically <50 days [83]. These infections are associated with a tenfold risk of death, although it is important to note that many of the epidemiologic studies in SOT occurred prior to development of anti-CRE antibiotics [83]. Kidney transplant recipients tend to have lower rates of CRE infection than liver transplant recipients, who have higher rates of surgical site infections and intra-abdominal infections. In addition to infections, there have been 7 cases of donor-derived infections reported with CRE and 1 large outbreak of 14 recipients exposed to carbapenem-resistant *Acinetobacter* [93–95]. No resistant infections occurred in patients who received immediate treatment post-transplant in response to culture results, whereas 50% developed infection without postexposure antibiotics.

P. aeruginosa is common in lung transplantation and typically colonizes up to 80% of patients with cystic fibrosis and bronchiectatic lung disease. Patients with colonization prior to transplant have a 4.7 times higher risk of infection compared to noncolonized patients [96]. CRPA is increasing in patients with CF, particularly with patients exposed to carbapenems [96].

Bone Marrow Transplant

Patients with hematologic malignancies are at increased risk for CRE infections due to multiple reasons, including chemotherapy-induced neutropenia with subsequent gastrointestinal mucositis, prolonged hospitalization, and exposure to broad-spectrum antibiotics [83]. ESBL bacteremia prevalence rates in patients with hematologic malignancy have ranged from 17% to 37% [32]. In areas with higher rates of CRE, such as Italy and Israel, patients with hematologic malignancies represent up to 24% of all patients with CRE bacteremia [83]. CRE are also becoming increasingly common in HSCT recipients; Italian transplant centers have reported an increase in post-allogeneic transplantation CRE infection rates from 0.4% in 2010 to 2.9% in 2013 [97]. CRE bacteremia has been reported at a rate of 2% in HSCT recipients within 12 months of transplantation [32]. Given the delay in diagnosis and that typical recommendations for neutropenic fever do not cover CRE pathogens, patients with hematologic malignancies who develop CRE bacteremia have high

associated 30-day mortality rates ranging from 52% to 63% [32, 97]. A likely explanation for high mortality rates is due to the fact that the recommended empiric therapy for neutropenic fever does not treat CRE, and thus appropriate CRE-active therapy is delayed.

Patients undergoing HSCT are especially at risk for acquiring *P. aeruginosa*. Outbreaks of both CRAB and CRPA have been associated with hematological-oncological wards with high associated mortality rates [98]. Environmental reservoirs, including sanitary and water supply systems, have been implicated in outbreaks, mainly due to biofilm formation [98].

CRE Screening

The incidence of colonization and infection with extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* is increasing and is high in patients with cancer, HSCT, and SOT recipients [82]. A number of studies have reported screening high-risk patients for ESBL-E, with higher rates of subsequent infections in patients who were positive for colonization, yet there is no consensus on the implementation of screening consistently for patients [82]. Colonization by CRE has been described as a significant risk factor for infection by the same bacteria, with Gianella et al. reporting that liver transplant recipients colonized with CRE were 13.9 times more likely to develop a CRE infection than those not colonized [99]. A multicenter study evaluating 57 SOT recipients with CRE colonization found 1-year post-transplant survival approaching 80% [100]. Further studies are needed in regard to identifying if pre-transplant CRE screening is necessary, especially since there are discrepant results determining risk of subsequent infection patients colonized with CRE prior to liver transplantation [89, 99].

The sensitivity of surveillance cultures varies widely among MDR-GNB species, and the natural history of colonization has not been clearly described. A prospective cohort study from Brazil that collected weekly samples immediately before liver transplantation and weekly thereafter identified CRAB as the most common GNB in 46% of patients, followed by CRE in 40% of patients [101]. The CDC have recommended screening for CRE in patients receiving healthcare in areas with endemic CRE; while earlier detection may be helpful to prevent cross-transmission through appropriate contact precautions, further data is needed to identify risk of development of infection.

Disease Diagnostics

In the United States, the CRE epidemic is mediated by rapid expansion of CP-*K. pneumoniae* sequence type 258, which expresses class A KPC carbapenemase [102]. One of the key challenges in controlling the spread of CRE is that not all carbapenem resistance equates to the production of carbapenemases. Current testing requires clinical laboratories to perform susceptibility testing

on isolates recovered in cultures, which can delay diagnosis by 2–5 days. There are several commercial tests for rapid identification of carbapenem resistance and can be divided into two categories: molecular tests that detect the presence of a carbapenemase and phenotypic tests that detect the in vitro activity of carbapenemase enzymes [102]. Nucleic acid amplification tests have been developed to identify carbapenemases and have some advantages, including rapid turnaround time, identification of specific carbapenemase genes which may help in antimicrobial choice, and, in some cases, the ability to detect directly from clinical specimens [102]. The latter advantage may complicate some specimens as occasionally isolates may harbor a carbapenemase gene but are susceptible in vitro to carbapenems; the significance of these findings is unclear. There are currently two tests available that can detect carbapenemase genes present from bacteria in the blood; the culture step is critical because it allows for biological amplification of the organisms and subsequent high sensitivity for resistance genes [102].

In addition to nucleic acid amplification, there are non-nucleic acid-based tests for the detection of CRE that are performed on bacteria grown in pure culture. The RAPIDEC® CARBA NP (bioMérieux) is a rapid phenotypic carbapenemase detection test performed on bacterial colonies. The test is based on a pH change indicated when imipenem is hydrolyzed by bacteria [102]. In addition to this, some laboratories have adapted the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) to detect carbapenemase activity by incubating the organism with a carbapenem and using mass-based detection of the carbapenem and its degradation production [102].

Treatment of CRE

In the recent years, there have been a variety of new antibiotics that have come to market or are in phase II–III trials of development. Prior to these newer agents, antimicrobial therapy relied heavily upon older agents including polymyxins, aminoglycosides, fosfomycin, and tigecycline [83]. Polymyxins such as colistin and polymyxin B work by disrupting the gram-negative cell membrane through electrostatic interaction with lipopolysaccharide, causing leakage of the intracellular components and subsequent cell death [87]. Polymyxins have been shown to have efficacy against KPC-producing CRE and CRAB, but associated nephrotoxicity has been documented in renal SOT, which may limit its use in renal transplantation [103]. Additionally, polymyxins, when administered parenterally, poorly penetrate lung tissue [83].

In terms of aminoglycosides, gentamicin is the most active aminoglycoside against CRE, with reported susceptibility rates ranging from 13% to 90% [83]. Gentamicin works by interfering with bacterial protein synthesis by binding to the 30S ribosomal subunit. Susceptibility rates have been lower with amikacin, and similar to polymyxins, aminoglycosides have high rates of nephrotoxicity. Fosfomycin, a parenteral agent only available in Europe at this time, has been shown to be beneficial in patients with CRE UTIs, given most CRE have been found to be susceptible to it. Unfortunately, clinical data to support the oral formulation in the setting of CRE UTI has not been well evaluated, with 1 retrospective

study showing only 6 out of 13 patients achieving microbiologic cure [104, 105]. Recently, a new semisynthetic aminoglycoside was improved for use in MDR gram-negative urinary tract infections, plazomicin, and was found to be non-inferior in comparison to meropenem [106].

Tigecycline is a glycylcycline antibiotic that inhibits bacterial protein synthesis by binding to the 30s ribosomal subunit [87]. It exhibits a broad range of activity against gram-positive agents and gram-negative bacteria. It has been approved for intra-abdominal and skin and soft tissue infections; however it is suboptimal in the treatment of bacteremia [87]. Two new tetracyclines have recently been approved by the FDA: eravacycline for complicated intra-abdominal infections and omadacycline for both acute bacterial skin and skin structure infections and community acquired pneumonia [107].

In 2015, the FDA approved ceftazidime/avibactam for the treatment of complicated intra-abdominal and urinary tract infections. In vitro evidence has shown >97% of isolates tested were susceptible, with the exception of NDM-producing isolates [108]. Of importance, this agent has only been approved for complicated intra-abdominal infections and complicated urinary tract infections.

In 2014, a novel antipseudomonal cephalosporin combined with B-lactamase inhibitor was introduced on the market (ceftolozane/tazobactam). It is stable against AmpC- and ESBL-producing bacteria as well as CRPA, but is inhibited by carbapenemases and is inactive against CRAB. Similar to ceftolozane/tazobactam, meropenem/vaborbactam is an existing carbapenem combined with a novel B-lactamase inhibitor that has been approved for UTI. However, its spectrum of activity covers both ESBL- and KPC-producing isolates, but not CRPA, MBL, or CRAB.

In terms of antibiotics in the pipeline, there are four agents aimed at treating MDROs: aztreonam/avibactam, cefiderocol, imipenem/relabactam, and tebipenem. Aztreonam/avibactam has been shown to have in vitro activity against Ambler class A, C, and D carbapenemases. Cefiderocol is a novel siderophore cephalosporin, structurally similar to ceftazidime and cefepime, which utilizes iron transport mechanisms to increase periplasmic concentrations in the bacteria. It has been found to have in vitro activity against KPC, NDM, OXA, and VIM carbapenemases [109]. Imipenem/relabactam is an existing antipseudomonal carbapenem attached to a novel B-lactamase inhibitor in phase II trials for UTI and IAI and may have activity against MBLs. Finally, tebipenem is an oral carbapenem stable against ESBL and AMPc, but is inactivated by KPC, OXA, and MBL carbapenemases (Table 2).

Prevention of CRE Infection

Although there are new antimicrobial agents in the pipeline, the high associated morbidity and mortality in SOT and HSCT necessitate development of prevention strategies. The 2015 CDC Facility Guidance for Control of CRE recognizes optimizing compliance with hand hygiene and contact precautions, increased

Table 2 Antibiotics affected by Ambler class beta-lactamases and antibiotics with in vitro activity

Ambler class	Beta-lactamase type	Activity	Enzyme examples	Common bacteria	Antibiotic considerations
A	Penicillinases	Hydrolyzes penicillins and cephalosporins	CTX-M, KPC, SHV, TEM	*E. coli*, *Klebsiella*, *Enterobacteriaceae*, *Pseudomonas*	Ceftazidime/avibactam Meropenem/vaborbactam Aztreonam/avibactam Cefiderocol Ceftolozane/tazobactam
B	Metallo-beta-lactamases	Uses zinc for hydrolysis of the B-lactam ring	IMP, NDM, VIM	*Pseudomonas*, *Acinetobacter*	Aztreonam/avibactam Cefiderocol
C	Cephalosporinases	Hydrolyzes broad- and extended-spectrum cephalosporins	Amp C	*Serratia*, *Pseudomonas*, *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Morganella* (SPACE-M)	Carbapenems Cefepime Ceftazidime/avibactam Meropenem/vaborbactam Aztreonam/avibactam Plazomicin Ceftolozane/tazobactam
D	Oxacillinases	B-lactamase production and reduced permeability or increased efflux	OXA	*Acinetobacter*, *E. coli*, *Pseudomonas*	Ceftazidime/avibactam Ceftolozane/tazobactam

environmental cleaning, increasing personnel education in regard to CRE, minimizing the use of indwelling devices, and most importantly active antimicrobial stewardship program [83]. Active surveillance has been recommended in areas of high endemic CRE rates, as well as during times of controlling outbreaks. Furthermore, infection prevention programs should follow guidelines for endoscope reprocessing given outbreaks associated with contaminated duodenoscopy, including one associated with the death of a kidney-pancreas transplant recipient [110].

In a prospective study of liver transplantation (LT) recipients who developed CRE infection, the need for renal replacement therapy after LT, mechanical ventilation >48 h, hepatitis C recurrence, and CRE colonization were independent risk factors for invasive infections; the authors subsequently developed a risk score with a high negative predictive value [99]. Use of a score system may help clinicians identify the majority of colonized patients after LT with a low

probability for developing CRE infection; further research is necessary to identify if the scoring system can be validated in other facilities. In terms of lung transplantation, many centers will treat recipients with a history of *P. aeruginosa* with antipseudomonal therapy for 2–3 weeks postoperatively to reduce the risk of pneumonia and allograft colonization; additionally, surgical debridement of the sinuses after transplant has been associated with reduced incidence of bacterial pneumonia, although further studies are needed for CRPA [96].

A key factor in the prevention of carbapenem-producing organisms is antimicrobial stewardship. Selective antimicrobial pressure can select for an antibiotic-resistant strain of *Acinetobacter*, which can subsequently spread within an institution [87]. Carbapenem use has been linked to the development of CRE infections as well as the receipt of other antimicrobials, including fluoroquinolones, and B-lactams have been independently associated with CRE infection [83].

Environmental disinfection is important in the control and spread of CRAB. Environmental cleaning with phenol, sodium dichloroisocyanurate, and vaporized hydrogen peroxide has all been studied and is efficacious [87]. *P. aeruginosa* is common in environmental reservoirs, and different strategies have been effective in identifying and eliminating sources of this pathogen, including hand hygiene, contact precautions, screening patients, and point-of-use filters [98]. Hospital construction measures aimed at controlling environmental contamination by and spread of CRPA have been effective at minimizing the risk of infection in immunocompromised hosts [98].

Currently there are no formal recommendations regarding perioperative antibiotic prophylaxis outside of the 2013 clinical practice guidelines for antimicrobial prophylaxis in surgery, which gives general guidelines for prophylaxis but is not specific to MDROs [111]. Given colonization with MDRO has been found to be an independent risk factor for development of infection in SOT and HSCT, it may be imperative to consider a regimen that targets those pathogens [2]. Furthermore, a recent retrospective cohort study identified MDROs in 15% of donor cultures, including those with extended-spectrum cephalosporin-resistant *Enterobacteriaceae* and CRE [1]. Colonization with CRE may limit antimicrobial choice, and thus pre-transplant consultation with transplant infectious disease specialists is recommended.

Conclusion

Pre-transplant colonization with multidrug-resistant organisms is increasingly prevalent and is often associated with post-transplant infection. These data underscore the importance of pre-transplant screening and decolonization procedures. There is a need for further research to understand the epidemiology of pre-transplant MDRO colonization, appropriate screening modalities and timing of screening, as well as effective decolonization procedures.

References

1. Anesi JA, Blumberg EA, Han JH, Lee DH, Clauss H, Climaco A, et al. Risk factors for multidrug-resistant organisms among deceased organ donors. Am J Transplant. 2019;19(9):2468–78.
2. Anesi JA, Blumberg EA, Abbo LM. Perioperative antibiotic prophylaxis to prevent surgical site infections in solid organ transplantation. Transplantation. 2018;102(1):21–34.
3. Song SH, Li XX, Wan QQ, Ye QF. Risk factors for mortality in liver transplant recipients with ESKAPE infection. Transplant Proc. 2014;46(10):3560–3.
4. Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol. 2012;10(4):266–78.
5. McKinnell JA, Kunz DF, Chamot E, Patel M, Shirley RM, Moser SA, et al. Association between vancomycin-resistant Enterococci bacteremia and ceftriaxone usage. Infect Control Hosp Epidemiol. 2012;33(7):718–24.
6. Van Tyne D, Gilmore MS. Friend turned foe: evolution of enterococcal virulence and antibiotic resistance. Annu Rev Microbiol. 2014;68:337–56.
7. Nellore A, Huprikar S, AST ID Community of Practice. Vancomycin-resistant Enterococcus in solid organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13549.
8. Hayden MK. Insights into the epidemiology and control of infection with vancomycin-resistant enterococci. Clin Infect Dis. 2000;31(4):1058–65.
9. Okano A, Isley NA, Boger DL. Peripheral modifications of [Psi[CH2NH]Tpg(4)]vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics. Proc Natl Acad Sci U S A. 2017;114(26):E5052–E61.
10. Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, et al. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. J Clin Invest. 2010;120(12):4332–41.
11. Bonten MJ, Slaughter S, Ambergen AW, Hayden MK, van Voorhis J, Nathan C, et al. The role of "colonization pressure" in the spread of vancomycin-resistant enterococci: an important infection control variable. Arch Intern Med. 1998;158(10):1127–32.
12. Ziakas PD, Pliakos EE, Zervou FN, Knoll BM, Rice LB, Mylonakis E. MRSA and VRE colonization in solid organ transplantation: a meta-analysis of published studies. Am J Transplant. 2014;14(8):1887–94.
13. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008;455(7214):804–7.
14. McNeil SA, Malani PN, Chenoweth CE, Fontana RJ, Magee JC, Punch JD, et al. Vancomycin-resistant enterococcal colonization and infection in liver transplant candidates and recipients: a prospective surveillance study. Clin Infect Dis. 2006;42(2):195–203.
15. Zacharioudakis IM, Zervou FN, Ziakas PD, Rice LB, Mylonakis E. Vancomycin-resistant enterococci colonization among dialysis patients: a meta-analysis of prevalence, risk factors, and significance. Am J Kidney Dis. 2015;65(1):88–97.
16. Ziakas PD, Thapa R, Rice LB, Mylonakis E. Trends and significance of VRE colonization in the ICU: a meta-analysis of published studies. PLoS One. 2013;8(9):e75658.
17. Bashir A, Attie O, Sullivan M, Sebra R, Singh KV, Altman D, et al. Genomic confirmation of vancomycin-resistant Enterococcus transmission from deceased donor to liver transplant recipient. PLoS One. 2017;12(3):e0170449.
18. Russell DL, Flood A, Zaroda TE, Acosta C, Riley MM, Busuttil RW, et al. Outcomes of colonization with MRSA and VRE among liver transplant candidates and recipients. Am J Transplant. 2008;8(8):1737–43.
19. Weinstock DM, Conlon M, Iovino C, Aubrey T, Gudiol C, Riedel E, et al. Colonization, bloodstream infection, and mortality caused by vancomycin-resistant Enterococcus early after

allogeneic hematopoietic stem cell transplant. Biol Blood Marrow Transplant. 2007;13(5):615–21.
20. Vydra J, Shanley RM, George I, Ustun C, Smith AR, Weisdorf DJ, et al. Enterococcal bacteremia is associated with increased risk of mortality in recipients of allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012;55(6):764–70.
21. Kang Y, Vicente M, Parsad S, Brielmeier B, Pisano J, Landon E, et al. Evaluation of risk factors for vancomycin-resistant Enterococcus bacteremia among previously colonized hematopoietic stem cell transplant patients. Transpl Infect Dis. 2013;15(5):466–73.
22. Avery R, Kalaycio M, Pohlman B, Sobecks R, Kuczkowski E, Andresen S, et al. Early vancomycin-resistant Enterococcus (VRE) bacteremia after allogeneic bone marrow transplantation is associated with a rapidly deteriorating clinical course. Bone Marrow Transplant. 2005;35(5):497–9.
23. Tavadze M, Rybicki L, Mossad S, Avery R, Yurch M, Pohlman B, et al. Risk factors for vancomycin-resistant Enterococcus bacteremia and its influence on survival after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2014;49(10):1310–6.
24. Ford CD, Gazdik MA, Lopansri BK, Webb B, Mitchell B, Coombs J, et al. Vancomycin-resistant Enterococcus colonization and bacteremia and hematopoietic stem cell transplantation outcomes. Biol Blood Marrow Transplant. 2017;23(2):340–6.
25. Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in enterococci. Expert Rev Anti-Infect Ther. 2014;12(10):1221–36.
26. Tenover FC, Tokars J, Swenson J, Paul S, Spitalny K, Jarvis W. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. J Clin Microbiol. 1993;31(7):1695–9.
27. Jenkins SG, Raskoshina L, Schuetz AN. Comparison of performance of the novel chromogenic spectra VRE agar to that of bile esculin azide and Campylobacter agars for detection of vancomycin-resistant enterococci in fecal samples. J Clin Microbiol. 2011;49(11):3947–9.
28. Anderson NW, Buchan BW, Young CL, Newton DW, Brenke C, Lapsley L, et al. Multicenter clinical evaluation of VRESelect agar for identification of vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J Clin Microbiol. 2013;51(8):2758–60.
29. Tan TY, Jiang B, Ng LSY. Faster and economical screening for vancomycin-resistant enterococci by sequential use of chromogenic agar and real-time polymerase chain reaction. J Microbiol Immunol Infect. 2017;50(4):448–53.
30. Both A, Franke GC, Mirwald N, Lutgehetmann M, Christner M, Klupp EM, et al. Two-tier approach combining molecular and culture-based techniques for optimized detection of vancomycin-resistant enterococci. Diagn Microbiol Infect Dis. 2017;89(4):253–7.
31. Mak A, Miller MA, Chong G, Monczak Y. Comparison of PCR and culture for screening of vancomycin-resistant Enterococci: highly disparate results for vanA and vanB. J Clin Microbiol. 2009;47(12):4136–7.
32. Satlin MJ, Walsh TJ. Multidrug-resistant *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and vancomycin-resistant Enterococcus: three major threats to hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2017;19(6):e12762.
33. Zhou Q, Moore C, Eden S, Tong A, McGeer A, Mount Sinai Hospital Infection Control Team. Factors associated with acquisition of vancomycin-resistant enterococci (VRE) in roommate contacts of patients colonized or infected with VRE in a tertiary care hospital. Infect Control Hosp Epidemiol. 2008;29(5):398–403.
34. Harris AD, Pineles L, Belton B, Johnson JK, Shardell M, Loeb M, et al. Universal glove and gown use and acquisition of antibiotic-resistant bacteria in the ICU: a randomized trial. JAMA. 2013;310(15):1571–80.
35. De Angelis G, Cataldo MA, De Waure C, Venturiello S, La Torre G, Cauda R, et al. Infection control and prevention measures to reduce the spread of vancomycin-resistant enterococci in hospitalized patients: a systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(5):1185–92.

36. Bardossy AC, Alsafadi MY, Starr P, Chami E, Pietsch J, Moreno D, et al. Evaluation of contact precautions for methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococcus. Am J Infect Control. 2017;45(12):1369–71.
37. Martin EM, Russell D, Rubin Z, Humphries R, Grogan TR, Elashoff D, et al. Elimination of routine contact precautions for endemic methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococcus: a retrospective quasi-experimental study. Infect Control Hosp Epidemiol. 2016;37(11):1323–30.
38. Isenman H, Fisher D. Advances in prevention and treatment of vancomycin-resistant Enterococcus infection. Curr Opin Infect Dis. 2016;29(6):577–82.
39. Kodali S, Vinogradov E, Lin F, Khoury N, Hao L, Pavliak V, et al. A vaccine approach for the prevention of infections by multidrug-resistant *Enterococcus faecium*. J Biol Chem. 2015;290(32):19512–26.
40. Laverde D, Wobser D, Romero-Saavedra F, Hogendorf W, van der Marel G, Berthold M, et al. Synthetic teichoic acid conjugate vaccine against nosocomial Gram-positive bacteria. PLoS One. 2014;9(10):e110953.
41. Gearhart M, Martin J, Rudich S, Thomas M, Wetzel D, Solomkin J, et al. Consequences of vancomycin-resistant Enterococcus in liver transplant recipients: a matched control study. Clin Transpl. 2005;19(6):711–6.
42. Cervera C, van Delden C, Gavalda J, Welte T, Akova M, Carratala J, et al. Multidrug-resistant bacteria in solid organ transplant recipients. Clin Microbiol Infect. 2014;20(Suppl 7):49–73.
43. Stripling J, Kumar R, Baddley JW, Nellore A, Dixon P, Howard D, et al. Loss of vancomycin-resistant Enterococcus fecal dominance in an organ transplant patient with clostridium difficile colitis after fecal microbiota transplant. Open Forum Infect Dis. 2015;2(2):ofv078.
44. Ubeda C, Bucci V, Caballero S, Djukovic A, Toussaint NC, Equinda M, et al. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant *Enterococcus faecium* colonization. Infect Immun. 2013;81(3):965–73.
45. Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Tleyjeh IM, Rybak MJ, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation. 2015;132(15):1435–86.
46. Zahedi Bialvaei A, Rahbar M, Yousefi M, Asgharzadeh M, Samadi Kafil H. Linezolid: a promising option in the treatment of Gram-positives. J Antimicrob Chemother. 2017;72(2):354–64.
47. Taylor JJ, Wilson JW, Estes LL. Linezolid and serotonergic drug interactions: a retrospective survey. Clin Infect Dis. 2006;43(2):180–7.
48. Chuang YC, Lin HY, Chen PY, Lin CY, Wang JT, Chen YC, et al. Effect of daptomycin dose on the outcome of vancomycin-resistant, daptomycin-susceptible *Enterococcus faecium* bacteremia. Clin Infect Dis. 2017;64(8):1026–34.
49. Balli EP, Venetis CA, Miyakis S. Systematic review and meta-analysis of linezolid versus daptomycin for treatment of vancomycin-resistant enterococcal bacteremia. Antimicrob Agents Chemother. 2014;58(2):734–9.
50. Kraft S, Mackler E, Schlickman P, Welch K, DePestel DD. Outcomes of therapy: vancomycin-resistant enterococcal bacteremia in hematology and bone marrow transplant patients. Support Care Cancer. 2011;19(12):1969–74.
51. Whang DW, Miller LG, Partain NM, McKinnell JA. Systematic review and meta-analysis of linezolid and daptomycin for treatment of vancomycin-resistant enterococcal bloodstream infections. Antimicrob Agents Chemother. 2013;57(10):5013–8.
52. Twilla JD, Finch CK, Usery JB, Gelfand MS, Hudson JQ, Broyles JE. Vancomycin-resistant Enterococcus bacteremia: an evaluation of treatment with linezolid or daptomycin. J Hosp Med. 2012;7(3):243–8.
53. Crank CW, Scheetz MH, Brielmaier B, Rose WE, Patel GP, Ritchie DJ, et al. Comparison of outcomes from daptomycin or linezolid treatment for vancomycin-resistant

enterococcal bloodstream infection: a retrospective, multicenter, cohort study. Clin Ther. 2010;32(10):1713–9.
54. Britt NS, Potter EM, Patel N, Steed ME. Comparison of the effectiveness and safety of linezolid and daptomycin in vancomycin-resistant enterococcal bloodstream infection: a national cohort study of Veterans Affairs patients. Clin Infect Dis. 2015;61(6):871–8.
55. Mercuro NJ, Davis SL, Zervos MJ, Herc ES. Combatting resistant enterococcal infections: a pharmacotherapy review. Expert Opin Pharmacother. 2018;19(9):1–14.
56. Perez F, Salata RA, Bonomo RA. Current and novel antibiotics against resistant Gram-positive bacteria. Infect Drug Resist. 2008;1:27–44.
57. Perri MB, Hershberger E, Ionescu M, Lauter C, Zervos MJ. In vitro susceptibility of vancomycin-resistant enterococci (VRE) to fosfomycin. Diagn Microbiol Infect Dis. 2002;42(4):269–71.
58. Allerberger F, Klare I. In-vitro activity of fosfomycin against vancomycin-resistant enterococci. J Antimicrob Chemother. 1999;43(2):211–7.
59. Lewis JD, Enfield KB, Cox HL, Mathers AJ, Sifri CD. A single-center experience with infections due to daptomycin-nonsusceptible *Enterococcus faecium* in liver transplant recipients. Transpl Infect Dis. 2016;18(3):341–53.
60. Moise PA, Sakoulas G, McKinnell JA, Lamp KC, DePestel DD, Yoon MJ, et al. Clinical outcomes of daptomycin for vancomycin-resistant Enterococcus bacteremia. Clin Ther. 2015;37(7):1443–53 e2.
61. Bi R, Qin T, Fan W, Ma P, Gu B. The emerging problem of linezolid-resistant enterococci. J Glob Antimicrob Resist. 2017;13:11–9.
62. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–61.
63. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A. 2002;99(11):7687–92.
64. Huang YH, Tseng SP, Hu JM, Tsai JC, Hsueh PR, Teng LJ. Clonal spread of SCCmec type IV methicillin-resistant *Staphylococcus aureus* between community and hospital. Clin Microbiol Infect. 2007;13(7):717–24.
65. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 2014;22(1):42–7.
66. Paulsen G, Blum S, Danziger-Isakov L. Epidemiology and outcomes of pretransplant methicillin-resistant *Staphylococcus Aureus* screening in pediatric solid organ transplant candidates. Pediatr Transplant. 2018;22:e13246.
67. Zacharioudakis IM, Zervou FN, Ziakas PD, Mylonakis E. Meta-analysis of methicillin-resistant *Staphylococcus aureus* colonization and risk of infection in dialysis patients. J Am Soc Nephrol. 2014;25(9):2131–41.
68. Koval CE, Rakita R, ASTIDCo P. Ventricular assist device related infections and solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):348–54.
69. Miles-Jay A, Podczervinski S, Stednick ZJ, Pergam SA. Evaluation of routine pre-transplantation screening for methicillin-resistant *Staphylococcus aureus* in hematopoietic cell transplant recipients. Am J Infect Control. 2015;43(1):89–91.
70. Senn L, Basset P, Nahimana I, Zanetti G, Blanc DS. Which anatomical sites should be sampled for screening of methicillin-resistant *Staphylococcus aureus* carriage by culture or by rapid PCR test? Clin Microbiol Infect. 2012;18(2):E31–3.
71. Giersing BK, Dastgheyb SS, Modjarrad K, Moorthy V. Status of vaccine research and development of vaccines for *Staphylococcus aureus*. Vaccine. 2016;34(26):2962–6.
72. Kullar R, Vassallo A, Turkel S, Chopra T, Kaye KS, Dhar S. Degowning the controversies of contact precautions for methicillin-resistant *Staphylococcus aureus*: a review. Am J Infect Control. 2016;44(1):97–103.
73. Avery RK. Recipient screening prior to solid-organ transplantation. Clin Infect Dis. 2002;35 (12):1513–9.

74. Pereira MR, Rana MM, AICo P. Methicillin-resistant *Staphylococcus aureus* in solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13611.
75. Gebreselassie HM, Lo Priore E, Marschall J. Effectiveness of meticillin-resistant *Staphylococcus aureus* decolonization in long-term haemodialysis patients: a systematic review and meta-analysis. J Hosp Infect. 2015;91(3):250–6.
76. Watanakunakorn C. Mode of action and in-vitro activity of vancomycin. J Antimicrob Chemother. 1984;14(Suppl D):7–18.
77. Heidary M, Khosravi AD, Khoshnood S, Nasiri MJ, Soleimani S, Goudarzi M. Daptomycin. J Antimicrob Chemother. 2018;73(1):1–11.
78. Claeys KC, Zasowski EJ, Casapao AM, Lagnf AM, Nagel JL, Nguyen CT, et al. Daptomycin improves outcomes regardless of vancomycin MIC in a propensity-matched analysis of methicillin-resistant *Staphylococcus aureus* bloodstream infections. Antimicrob Agents Chemother. 2016;60(10):5841–8.
79. Rodvold KA, McConeghy KW. Methicillin-resistant *Staphylococcus aureus* therapy: past, present, and future. Clin Infect Dis. 2014;58(Suppl 1):S20–7.
80. Abbas M, Paul M, Huttner A. New and improved? A review of novel antibiotics for Gram-positive bacteria. Clin Microbiol Infect. 2017;23(10):697–703.
81. Kim W, Zhu W, Hendricks GL, Van Tyne D, Steele AD, Keohane CE, et al. A new class of synthetic retinoid antibiotics effective against bacterial persisters. Nature. 2018;556(7699):103–7.
82. Biehl LM, Schmidt-Hieber M, Liss B, Cornely OA, Vehreschild MJ. Colonization and infection with extended spectrum beta-lactamase producing *Enterobacteriaceae* in high-risk patients – review of the literature from a clinical perspective. Crit Rev Microbiol. 2016;42(1):1–16.
83. Pouch SM, Satlin MJ. Carbapenem-resistant *Enterobacteriaceae* in special populations: solid organ transplant recipients, stem cell transplant recipients, and patients with hematologic malignancies. Virulence. 2017;8(4):391–402.
84. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. J Infect Dis. 2017;215(suppl_1):S28–36.
85. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence. 2017;8(4):460–9.
86. Reddy P, Zembower TR, Ison MG, Baker TA, Stosor V. Carbapenem-resistant *Acinetobacter baumannii* infections after organ transplantation. Transpl Infect Dis. 2010;12(1):87–93.
87. Pogue JM, Mann T, Barber KE, Kaye KS. Carbapenem-resistant *Acinetobacter baumannii*: epidemiology, surveillance and management. Expert Rev Anti-Infect Ther. 2013;11(4):383–93.
88. Karampatakis T, Antachopoulos C, Tsakris A, Roilides E. Molecular epidemiology of carbapenem-resistant *Pseudomonas aeruginosa* in an endemic area: comparison with global data. Eur J Clin Microbiol Infect Dis. 2018;37(7):1211–20.
89. Massa E, Michailidou E, Agapakis D, Papadopoulos S, Tholioti T, Aleuroudis I, et al. Colonization and infection with extensively drug resistant gram-negative bacteria in liver transplant recipients. Transplant Proc. 2019;51(2):454–6.
90. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol. 2008;29(12):1099–106.
91. Lubbert C, Becker-Rux D, Rodloff AC, Laudi S, Busch T, Bartels M, et al. Colonization of liver transplant recipients with KPC-producing *Klebsiella pneumoniae* is associated with high infection rates and excess mortality: a case-control analysis. Infection. 2014;42(2):309–16.
92. Bergamasco MD, Barroso Barbosa M, de Oliveira Garcia D, Cipullo R, Moreira JC, Baia C, et al. Infection with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* in solid organ transplantation. Transpl Infect Dis. 2012;14(2):198–205.
93. Ariza-Heredia EJ, Patel R, Blumberg EA, Walker RC, Lewis R, Evans J, et al. Outcomes of transplantation using organs from a donor infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*. Transpl Infect Dis. 2012;14(3):229–36.

94. Goldberg E, Bishara J, Lev S, Singer P, Cohen J. Organ transplantation from a donor colonized with a multidrug-resistant organism: a case report. Transpl Infect Dis. 2012;14(3):296–9.
95. Mularoni A, Bertani A, Vizzini G, Gona F, Campanella M, Spada M, et al. Outcome of transplantation using organs from donors infected or colonized with carbapenem-resistant gram-negative bacteria. Am J Transplant. 2015;15(10):2674–82.
96. Shoham S, Shah PD. Impact of multidrug-resistant organisms on patients considered for lung transplantation. Infect Dis Clin N Am. 2013;27(2):343–58.
97. Girmenia C, Rossolini GM, Piciocchi A, Bertaina A, Pisapia G, Pastore D, et al. Infections by carbapenem-resistant *Klebsiella pneumoniae* in SCT recipients: a nationwide retrospective survey from Italy. Bone Marrow Transplant. 2015;50(2):282–8.
98. Kossow A, Kampmeier S, Willems S, Berdel WE, Groll AH, Burckhardt B, et al. Control of multidrug-resistant *Pseudomonas aeruginosa* in allogeneic hematopoietic stem cell transplant recipients by a novel bundle including remodeling of sanitary and water supply systems. Clin Infect Dis. 2017;65(6):935–42.
99. Giannella M, Bartoletti M, Morelli MC, Tedeschi S, Cristini F, Tumietto F, et al. Risk factors for infection with carbapenem-resistant *Klebsiella pneumoniae* after liver transplantation: the importance of pre- and posttransplant colonization. Am J Transplant. 2015;15(6):1708–15.
100. Huprikar SCL, Pierrotti LC, Nellore A, Madan R, Garcia-Diaz J, Jacobs S, Lee D, Trindade Clemente W, Alangaden G, et al. Outcomes associated with carbapenem-resistant *Enterobacteriaceae* infection after solid organ transplantation in a multicenter study. Program and abstracts of the 16th American Transplant congress, American Society of Transplant Surgeons and the American Society of Transplantation. Boston. 2016.
101. Freire MP, Villela Soares Oshiro IC, Bonazzi PR, Pierrotti LC, de Oliveira LM, Machado AS, et al. Surveillance culture for multidrug-resistant gram-negative bacteria: performance in liver transplant recipients. Am J Infect Control. 2017;45(3):e40–e4.
102. Banerjee R, Humphries R. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant *Enterobacteriaceae*. Virulence. 2017;8(4):427–39.
103. Rodrigues Dos Santos BG, Amaral ES Jr, Fernandes PF, Oliveira CM, Rodrigues JL, Perdigao Neto LV, et al. Urinary tract infections and surgical site infections due to carbapenem-resistant *Enterobacteriaceae* in renal transplant. Transplant Proc. 2016;48(6):2050–5.
104. Endimiani A, Patel G, Hujer KM, Swaminathan M, Perez F, Rice LB, et al. In vitro activity of fosfomycin against blaKPC-containing *Klebsiella pneumoniae* isolates, including those non-susceptible to tigecycline and/or colistin. Antimicrob Agents Chemother. 2010;54(1):526–9.
105. Neuner EA, Sekeres J, Hall GS, van Duin D. Experience with fosfomycin for treatment of urinary tract infections due to multidrug-resistant organisms. Antimicrob Agents Chemother. 2012;56(11):5744–8.
106. Wagenlehner FME, Cloutier DJ, Komirenko AS, Cebrik DS, Krause KM, Keepers TR, et al. Once-daily plazomicin for complicated urinary tract infections. N Engl J Med. 2019;380 (8):729–40.
107. Zhang Y, Lin X, Bush K. In vitro susceptibility of beta-lactamase-producing carbapenem-resistant *Enterobacteriaceae* (CRE) to eravacycline. J Antibiot (Tokyo). 2016;69(8):600–4.
108. de Jonge BL, Karlowsky JA, Kazmierczak KM, Biedenbach DJ, Sahm DF, Nichols WW. In vitro susceptibility to ceftazidime-avibactam of carbapenem-nonsusceptible *Enterobacteriaceae* isolates collected during the INFORM global surveillance study (2012 to 2014). Antimicrob Agents Chemother. 2016;60(5):3163–9.
109. Zhanel GG, Golden AR, Zelenitsky S, Wiebe K, Lawrence CK, Adam HJ, et al. Cefiderocol: a siderophore cephalosporin with activity against carbapenem-resistant and multidrug-resistant gram-negative bacilli. Drugs. 2019;79(3):271–89.
110. Epstein L, Hunter JC, Arwady MA, Tsai V, Stein L, Gribogiannis M, et al. New Delhi metallo-beta-lactamase-producing carbapenem-resistant *Escherichia coli* associated with exposure to duodenoscopes. JAMA. 2014;312(14):1447–55.
111. Bratzler DW, Dellinger EP, Olsen KM, Perl TM, Auwaerter PG, Bolon MK, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. Am J Health Syst Pharm. 2013;70(3):195–283.

14 Multidrug-Resistant Organisms: Posttransplant Management for Extended-Spectrum Beta-Lactamase Producers and Carbapenem-Resistant Enterobacterales

Jessica Howard-Anderson and Stephanie M. Pouch

Contents

Introduction 313
Disease Epidemiology 314
ESBL-Producing Enterobacterales 314
Carbapenem-Resistant Enterobacterales 317
Clinical Impact in Transplant 323
ESBL-Producing Enterobacterales 323
Carbapenem-Resistant Enterobacterales 324
Disease Diagnostics and Clinical Evaluation 325
ESBL-Producing Enterobacterales 325
Carbapenem-Resistant Enterobacterales 326
Management 328
ESBL-Producing Enterobacterales 328
Carbapenem-Resistant Enterobacterales 330
Conclusion 334
Key Points 334
References 334

Introduction

The incidence of multidrug-resistant Gram-negative bacterial infections, in particular those due to extended-spectrum β-lactamase (ESBL)-producing Enterobacterales (ESBL-EB) and carbapenem-resistant Enterobacterales (CRE), has been increasing worldwide [1–5]. This is of particular concern to immunocompromised patients, including solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients [6–8], although risk factors vary based on geographical location and organ transplanted. ESBL-EB and CRE infections can cause significant

J. Howard-Anderson (✉) · S. M. Pouch
Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA
e-mail: jessica.renee.howard-anderson@emory.edu; stephanie.pouch@emory.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_14

challenges in a patient's posttransplant course. Here we will review the clinical implications of these infections including recommendations for diagnosis and treatment.

Disease Epidemiology

ESBL-Producing Enterobacterales

ESBL enzymes are a family of diverse and evolving β-lactamases, often encoded by plasmids, which can hydrolyze third-generation cephalosporins and confer resistance to penicillins, first through third-generation cephalosporins and aztreonam. They are inhibited by clavulanic acid and remain susceptible in vitro to cephamycins and carbapenems, although the former antibiotics are not recommended for treatment as ESBL-producing organisms usually develop resistance to cephamycins through other mechanisms. The most common type of ESBL is a CTX-M enzyme (named for its ability to hydrolyze cefotaxime), but SHV(sulfhydryl variable) and TEM (named for the first patient to have this enzyme identified) enzymes are also frequently encountered [3, 9].

In SOT recipients, the proportion of Enterobacterales that harbor ESBL enzymes in clinical blood samples fluctuates by region and local resistance patterns, ranging from 3% in the USA (although this estimate is from 2007) to 60% in China [5, 10–13]. Similarly, the risk of developing an ESBL-EB infection posttransplant varies by geography but ranges between 3.4 and 16.9% in abdominal organ transplant recipients (Table 1) [14–21]. The highest risk has been reported in Brazil, where 16.9% of kidney transplant patients at one hospital developed an ESBL-EB urinary tract infection (UTI) [15]. The risk of ESBL bacteremia is estimated to be closer to 4% [14, 18]. Bacteremia is most commonly secondary to a UTI, especially in kidney transplant patients, but can also be caused by catheter-associated infections and surgical site infections [14]. In one study analyzing Gram-negative bacteremia after SOT, the highest incidence of bacteremia was in the first month [13], although other studies have found that the mean or median time from transplant to ESBL-EB infection can vary significantly from 15 days to 52 months [14, 18, 20–22].

Klebsiella pneumoniae and *Escherichia coli* are the most common ESBL-EB identified in SOT recipients [5, 14–17, 21]. Regional differences, however, do exist. A study in France reported *Enterobacter cloacae* as the most common ESBL-EB isolated in urine within 3 months of kidney transplant [18], and a study in China documented *Klebsiella aerogenes* as the most frequent cause of ESBL-EB bacteremia [11]. In China, the most common ESBLs identified in SOT recipients were in the TEM family, but CTX-M-9 and SHV β-lactamases were also frequently detected [16]. In France, CTX-M-1 was the most common β-lactamase identified in liver transplant patients [21].

The risk factors for developing an ESBL-EB infection following SOT include older age, increased immunosuppression, diabetes, posttransplant infection, reoperation or

Table 1 Studies analyzing the risk of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales infections in transplant patients published in the last 10 years

Transplant type (last name of first author)	Year	Location	Organism	Risk factors for ESBL infection	Risk of infection posttransplant[a]	Median time from transplant to infection	Type of infection (top 2 listed)	Mortality rate	Graft dysfunction
Lung									
Raviv [22]	2012	Israel	KP	NS	5/136 (3.7%)	38 days	Pulmonary, 60% Wound, 40%	100% (overall)	NS
Liver									
Bert [21]	2012	France	EB	Pretransplant ESBL fecal colonization, MELD ≥25, reoperation	39/734 (5.3%)	15 days	Intraabdominal, 49% Urinary, 38%	15% (28 days)	NS
Kidney									
Pinheiro [15][b]	2010	Brazil	EB	Recurrent UTIs, reoperation	14/83 (16.9%)	NS	Urinary, 100%	NS	NS
Ramadas [19][b]	2014	USA	EB	NS	5/147 (3.4%)	NS	Urinary, 100%	NS	1 patient with ESBL had chronic allograft dysfunction
Pilmis [18][b]	2015	France	EB	NS	34/659 (5.2%)	59 days	Urinary, 100% Bacteremia, 12%	NS	NS
Bodro [17][b]	2015	Spain	EB	NS	38/867 (4.4%)	NS	Urinary, 100%	NS	NS
Brakemeier [20][b]	2017	Germany	EB	Older age, higher doses of IS, DM, acute rejection, hospitalization, and antibiotics in the last 6 months	63/1019 (6.2%)	52 months (mean)	Urinary, 100%	22% (5 years)	Graft function was similar between patients with ESBL and controls

(continued)

Table 1 (continued)

Transplant type (last name of first author)	Year	Location	Organism	Risk factors for ESBL infection	Risk of infection posttransplant[a]	Median time from transplant to infection	Type of infection (top 2 listed)	Mortality rate	Graft dysfunction
Multi-organ									
Men [16][c]	2013	China	EB	NS	33/350 (9.4%)	NS	Pulmonary, 33% Cholangitis, 21%	42% (6 months)	NS
Aguiar [14][c,d]	2014	Brazil	EB	NS	39/997 (3.9%)	63 days	Bacteremia, 100%	26% (30 days)	NS
HSCT									
Weisser [25][d,e]	2017	Germany, Switzerland, Austria	EB	NS	64/15181 (0.4%)	NS	Bacteremia, 100%	NS	NS
Kharrat [32]	2018	Tunisia	EB	Prior hospitalization, prior antibiotic use, pretransplant colonization with ESBL-EB were common (> 60%). This was not compared to a control group	34/1018 (3.3%)	141 days	Bacteremia, 40% Urinary, 35%	9% (overall)	NS
Satlin [31][d]	2018	USA	EB	Pretransplant colonization with ESBL-EB	11/312 (3.5%)	NS	Bacteremia, 100%	NS	NS

KP Klebsiella pneumoniae, *EB* Enterobacterales, *NS* not stated, *MELD* model for end-stage liver disease, *UTI* urinary tract infection, *IS* immunosuppression, *DM* diabetes mellitus, *HSCT* hematopoietic stem cell transplant

[a]Number of patients who developed an ESBL-EB infection divided by the number of patients transplanted

[b]The study evaluated urinary infections only

[c]Kidney and liver transplant patients

[d]The study evaluated bacteremia only

[e]All patients had neutropenia

hemodialysis, graft rejection, hospitalization or antibiotic use in the last 6 months, pretransplant ESBL colonization, and a model for end-stage liver disease (MELD) score ≥ 25 in liver transplant patients [11, 15, 20, 23]. Organ transplant itself has also been identified as a risk factor for ESBL *E. coli* bacteremia [24].

Lastly, patients undergoing HSCT are at particularly high risk for bloodstream infections due to prolonged neutropenia and breakdown of the gastrointestinal mucosal border from high-dose conditioning regimens. Recipients of allogeneic HSCT have a higher incidence of bacteremia than patients undergoing autologous HSCT [25]. In patients with hematologic malignancies who develop Enterobacterales bacteremia, approximately 17–37% will harbor an ESBL [8]. For HSCT recipients, the ESBL prevalence is similar and ranges from 9 to 44% of all Enterobacterales infections [25–30]. The risk of developing an ESBL-EB infection after a HSCT ranges from 0.4 to 3.5%, although most of the literature has been limited to bloodstream infections [25, 31, 32]. The majority of ESBL-EB infections are due to *E. coli* or *K. pneumoniae* [26, 31–33] that produce a CTX-M β-lactamase, although production of SHV and TEM β-lactamases has also been identified in this patient population [27, 29, 31, 32]. In one retrospective cohort, the median time from HSCT to ESBL-EB infection was 141 days [32]. Risk factors for developing an ESBL-EB infection in HSCT patients have not been well defined, but likely include prior antibiotic use, prior hospitalizations, and pretransplant colonization with ESBL-EB [31, 32].

Carbapenem-Resistant Enterobacterales

Enterobacterales develop carbapenem resistance through a heterogenous array of mechanisms, including acquisition of carbapenemases. The plasmid-mediated *K. pneumoniae* carbapenemase (KPC) is the most common means of carbapenem resistance in the USA, South America, Europe, China, and Israel and is easily transferred between bacterial species. Other carbapenemases include oxacillinase (OXA)-48-like β-lactamases and metallo-β-lactamases (MBLs) including Verona integron-encoded MBL (VIM), imipenemase (IMP), and New Delhi MBL (NDM). NDMs are the most common carbapenemases in India, Pakistan, and the UK [7].

The risk of CRE infection following SOT depends on geography, occurrence of local outbreaks, patterns of resistance, and the organ transplanted. The risk is documented to range from 0.4 to 26.3%, but the majority of data is limited to single-center studies analyzing abdominal organ transplant recipients (Table 2) [22, 34–45]. In the USA, the incidence of CRE infection posttransplant is less than 10% [35–37, 42, 43], but in Brazil the risk can be as high as 26% in renal transplant patients [34]. These estimates probably more accurately reflect institutional trends rather than true national risks. However, a national surveillance program in Italy reported that 26.5% of all Gram-negative isolates from SOT recipients were carbapenem resistant [46]. Most CRE infections occur within the first 3 months

Table 2 Studies analyzing the risk of carbapenem-resistant Enterobacterales (CRE) infections in transplant patients published in the last 10 years

Transplant type (last name of first author)	Year	Location	Organism	Risk factors for CRE infection	Risk of infection posttransplant[a]	Median time from transplant to infection	Type of infection (top 2 listed)	Mortality rate	Graft dysfunction
Heart									
Bergamasco [34]	2012	Brazil	KP	NS	2/12 (16.7%)	90 days	Bacteremia, 100% Pneumonia, 50%	50% (30 days)	NS
Lung									
Raviv [22]	2012	Israel	KP	NS	8/136 (5.9%)	27 days	Pneumonia, 38% Urinary, 25% Bacteremia, 25%	88% (overall)	NS
Clancy [35][b]	2013	USA	KP	NS	2/546 (0.4%)	218 days	Bacteremia, 100%	0% (30 days)	NS
Liver									
Bergamasco [34]	2012	Brazil	KP	NS	4/31 (12.9%)	16 days	Bacteremia, 100% Pneumonia, 25%	25% (30 days)	NS
Kalpoe [36]	2012	USA	KP	NS	14/175 (8.0%)	12 days	Bacteremia, 86% Peritonitis, 79%	50% (30 days)	NS
Clancy [35][b]	2013	USA	KP	NS	8/610 (1.3%)	24 days	Bacteremia, 100% Pneumonia, 50%	25% (30 days)	NS
Mouloudi [47][b]	2014	Greece	KP	HCV, HCC	17 cases (denominator NS)	13 days	Bacteremia, 100%	82% (ICU mortality)	NS
Giannella [38]	2015	Italy	KP	Renal replacement therapy and mechanical ventilation >48 hours after transplant, HCV recurrence, rectal colonization with CR-KP	20/237 (8.4%)	42 days	Bacteremia, 90% Pneumonia, 30%	45% (180 days)	Graft dysfunction was associated with CR-KP infection in a univariable but not multivariable model

Pereira [37]	2015	USA	KP	MELD score, HCC, Roux-en-Y biliary choledochojejunostomy, bile leak	20/304 (6.6%)	11 days	SSI, 65% Bacteremia, 55%	55% (1 year)	NS
Freire [39]	2017	Brazil	EB	Pretransplant CRE colonization, MELD score > 32 at transplant, combined transplant, reoperation, >2 posttransplant dialysis sessions, and posttransplant CRE acquisition	59/386 (15.3%)	11 days	Bacteremia:50% SSI:47%	51% (60 days)	NS
Kidney									
Bergamasco [34]	2011	Brazil	KP	NS	5/19 (26.3%)	17 days	Bacteremia, 60% Urinary, 60%	60% (30 days)	NS
Cicora [41]	2013	Argentina	KP	NS	6/45 (13.3%)	36 days	Urinary, 83% Bacteremia,17%	33% (overall)	4 of 6 patients had DGF
Simkins [43]	2014	USA	KP	Recent broad-spectrum antibiotics	13/522 (2.5%)	185 days	Urinary, 69% Bacteremia, 38%	46% (overall)	No difference in DGF or rejection episodes comparing patients with CR-KP to those with CS-KP
Freire [40]	2015	Brazil	KP	Multi-organ transplant, ureteral stents	21/1101 (1.9%)	49 days	SSI, 48% Bacteremia, 39%	42% (30 days)	NS
Pouch [42][c]	2015	USA	KP	Multiple organ transplant, deceased-donor allograft, transplant admission LOS, pretransplant CR-KP, prior antimicrobials other than TMP/SMX	20/1852 (1.1%)	47 days	Bacteriuria, 100%	30% (overall)	No difference in DGF or rejection comparing patients with CR-KP to those with CS-KP

(continued)

Table 2 (continued)

Transplant type (last name of first author)	Year	Location	Organism	Risk factors for CRE infection	Risk of infection posttransplant[a]	Median time from transplant to infection	Type of infection (top 2 listed)	Mortality rate	Graft dysfunction
Rodrigues dos Santos [44][d]	2016	Brazil	EB	NS	33/428 (7.7%)	26 days	Urinary, 74% SSI, 26%	6% (overall)	NS
Varotti [45]	2017	Italy	KP	Lower BMI, higher creatinine posttransplant, hospital LOS, longer cold ischemia time, DGF, and higher Clavien-Dindo score (risk factors in a univariable analysis only)	26/234 (11.1%)	97 days (mean)	Urinary, 65% Bacteremia, 27%	8% (overall)	Patients with CR-KP had worse graft survival than patients without CR-KP
Intestinal									
Clancy [35][b]	2013	USA	KP	NS	6/112 (5.4%)	209 days	Bacteremia, 100%	33% (30 day)	NS
HSCT									
Zuckerman [114]	2011	Israel	KP	NS	5 patients (denominator NS)	NS	Bacteremia, 100%	60% (overall)	NS

Girmenia [51]	2015	Italy	KP	NS	112/10,447 (1.1%)	8 days (auto-HSCT); 15 days (allo-HSCT)	Bacteremia, 88% Pneumonia, 21%	58% (90 days)	NS
Satlin [49][b]	2016	USA	EB	NS	18 patients (denominator NS)	NS	NS	NS	NS
Trecarichi [48][b]	2016	Italy	KP	NS	26/3560 (0.7%)	NS	NS	NS	NS

KP Klebsiella pneumoniae, *NS* not stated, *UTI* urinary tract infection, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *MELD* model for end-stage liver disease, *CR-KP* carbapenem-resistant *K. pneumoniae*, *EB* Enterobacterales, *SSI* surgical site infection, *DGF* delayed graft function; *LOS* length of stay, *TMP/SMX* trimethoprim/sulfamethoxazole, *CS-KP* carbapenem-susceptible *K. pneumoniae*, *HSCT* hematopoietic stem cell transplant

[a]Number of patients who developed a CRE infection divided by the number of patients transplanted

[b]The study evaluated bacteremia only

[c]The study evaluated urinary infections only

[d]The study evaluated UTIs and SSI only

Table 3 Risk factors for carbapenem-resistant Enterobacterales (CRE) infections in solid organ and hematopoietic stem cell transplant recipients

Central venous catheter
Multi-organ transplantation
Pretransplant colonization with CRE
Prior receipt of broad-spectrum antibiotics
Hepatitis C virus[a]
Bile leak[a]
Renal replacement therapy[a]
Mechanical ventilation after transplant[a]
Reoperation after transplant[a]
Ureteral stents[b]
Deceased donor allografts[b]
Longer transplant admission[b]
Being in the intensive care unit[c]

[a]Specifically reported in liver transplant recipients
[b]Specifically reported in renal transplant recipients
[c]Specifically reported in hematopoietic stem cell transplant recipients

after transplant, but the median or mean time from transplant to infection ranges from 11 to 218 days [22, 34–45, 47].

In liver transplant patients with CRE infections, bacteremia and intra-abdominal infections, including surgical site infections, are the most common sites of infection and frequently occur in tandem [34–39, 47]. Risk factors include hepatitis C virus, hepatocellular carcinoma, higher MELD score, Roux-en-Y choledochojejunostomy, bile leak, renal replacement therapy and mechanical ventilation for greater than 48 h after transplant, pretransplant colonization with CRE, combined organ transplant, and need for reoperation after transplant (Table 3) [37–39, 47]. In renal transplant patients, UTIs are the most common source of CRE infection, with secondary bacteremia frequently observed [34, 40–45]. Central venous catheters have also been implicated in CRE infections, and in one study including heart, liver, and kidney transplant recipients, 75% of the primary bloodstream infections were catheter-related [34]. Risk factors for CRE infections in renal transplant patients include multi-organ transplantation, ureteral stents, deceased donor allografts, transplant admission length of stay, pretransplant CRE colonization, and prior broad-spectrum antibiotics [40, 42, 43].

Most studies have focused on carbapenem-resistant *K. pneumoniae*, the most common type of CRE infection in SOT recipients [39, 44]. However, one study evaluating CRE infections in renal transplant patients identified *Enterobacter cloacae* in 4 patients (11.8%) and *Escherichia coli* in 3 (8.8%) [44]. The most common carbapenemase described in SOT recipients is KPC [35, 39], but there have been reports of infections due to NDM- and OXA-48-producing Enterobacterales [7].

Patients with hematologic malignancies are at high risk for Gram-negative bacteremia although few studies have specifically analyzed the epidemiology of

CRE in HSCT recipients. Geography can contribute significantly, and in 13 centers in Italy, where CRE is an endemic nosocomial pathogen, 58% of the *K. pneumoniae* isolated from patients with hematologic malignancies were carbapenem resistant [48]. This was associated with a worrisome increase in resistance from 2010 to 2013, and the 21-day mortality in this group was 52%. In contrast, in two New York City oncology centers, 6.5% of Enterobacterales blood isolates among neutropenic patients with hematologic malignancies were carbapenem resistant [49]. Specific to HSCT recipients, one large study of 65 HSCT centers in Europe, Australia, and Asia found that 8.4% of Enterobacterales isolates were carbapenem resistant in patients with Gram-negative bacteremia [50]. Additionally, the incidence of CRE has been increasing in HSCT patients [7, 51]. In one Italian study, the incidence of CRE in allogenic HSCT patients increased from 0.4% in 2010 to 2.9% in 2013. The authors hypothesize that this was due to select transplant centers having large outbreaks of KPC-producing CRE in a country where CRE was already endemic during that time [51, 52]. For neutropenic patients with hematologic malignancies, risk factors for CRE bacteremia include prior β-lactam/β-lactamase inhibitor (BLBLI) or carbapenem use, current trimethoprim-sulfamethoxazole or glucocorticoid use, and prior CRE [49]. In HSCT recipients who develop Gram-negative bacteremia after transplant, carbapenem resistance was associated with breakthrough bacteremia on a carbapenem, longer hospitalization, being in the intensive care unit, and prior antibiotic use (Table 3) [50].

Clinical Impact in Transplant

ESBL-Producing Enterobacterales

Infections due to ESBL-EB have been associated with increased morbidity and mortality among SOT recipients. The 30-day mortality rate for abdominal SOT recipients with ESBL-EB infections can be up to 26%, with the highest mortality reported in bacteremic patients (Table 1) [14, 16, 20, 21]. In the context of lung transplantation, one small study in Israel identified five patients with definitive ESBL-producing *K. pneumoniae* infections, and all five died within 14 months of follow-up; the proportion of deaths attributable to the ESBL infection is unknown [22]. In general, patients with ESBL-EB infections posttransplant have a higher mortality than patients who do not develop ESBL-EB infections [20, 21]. However, this may be confounded by underlying comorbidities and individual risk factors contributing to the acquisition of an ESBL-EB infection. When comparing patients with resistant versus susceptible Enterobacterales infections, outcome results are conflicting [11, 20, 53].

ESBL-EB UTIs in renal transplant patients are associated with high rates of recurrence. One study demonstrated an increase in the incidence of ESBL-EB UTIs from 13% for patients with their first UTI to 45% for patients with their third UTI [15, 17]. In addition, acute organ rejection has been associated with ESBL-EB

infections [11, 16, 20], but any impact on long-term graft function has not been definitely demonstrated [19, 20].

Donor-derived infections are a unique and emerging concern for transplant recipients, especially as the prevalence of multidrug-resistant organisms increases. There have been at least five probable cases of ESBL-EB infections transmitted from donors to organ transplant recipients. In the first and second cases, the donor had an ESBL-EB UTI, and one kidney recipient developed a complicated UTI and pseudoaneurysm at the arterial anastomosis, and the other kidney transplant recipient developed ESBL-EB pyelonephritis and perinephric abscess. Both recipients survived but required allograft nephrectomies. In the remaining three probable cases of transmission, the source of donors' infection was unknown. Two liver transplant recipients developed intra-abdominal infections, and one liver transplant patient developed ESBL-EB bacteremia. One of the patients with the intra-abdominal infection died within 3 months of transplant. In all five cases, the donor's culture results were either not available or not adequately communicated to the recipient's healthcare team prior to the development of the recipient's infection [54]. It is also possible that donor-derived infections have occurred where the link between donor infection and recipient disease went unrecognized or unreported. Clinical presentation may also be different than for recipient-derived infections. Timely inter-facility communication is imperative in both preventing and treating patients with donor-derived infections.

Among HSCT patients, the mortality associated with ESBL-EB infections is similarly high, with one study estimating a 1-year mortality of 52% for patients with multidrug-resistant infections, of which 87% of the multidrug-resistant isolates were ESBL-EB [33]. In neutropenic cancer patients, including those with HSCTs, the 30-day mortality rate associated with ESBL-EB bacteremia was reported to be 38% [27]. Cancer patients with ESBL-EB bacteremia are suspected to have an increased mortality compared to patients without resistant organisms [27, 29], although conflicting reports exist [30, 33].

Carbapenem-Resistant Enterobacterales

CRE infections portend an increased risk of morbidity and mortality among SOT recipients [22, 36, 37, 42, 45, 47]. The 30-day mortality rate among SOT recipients with CRE infections ranges from 0 to 50% and can approach 75% at 1-year (Table 2) [22, 34–45, 47]. Mortality rates are typically higher in bacteremic patients compared to those with urinary infections, and in one study, the 30-day mortality rate increased from 25% in patients with a UTI to 44% in patients with bacteremia [34]. SOT recipients with CRE infections are more likely to have microbiologic failure [55] and develop recurrent infections [42, 45] than those with infections due to susceptible bacteria. Further, the relatively limited antimicrobial armamentarium, frequent need for nephrotoxic agents, and often delayed time to initiation of adequate antibiotic therapy [55] also impact morbidity and mortality.

In one of the largest studies addressing donor-derived infections due to multidrug-resistant Gram-negative organisms, 30 patients received an organ from a deceased

donor found to harbor carbapenem-resistant Gram-negative bacteria. Four (29%) of 14 patients who received an organ from a high-risk donor who was bacteremic or harbored an infection of the transplanted organ had proven transmission of the carbapenem-resistant bacteria, all due to carbapenem-resistant *K. pneumoniae*. However, none of the organ recipients who received appropriate antibiotic therapy, defined as targeted therapy initiated within 7 days of transplantation and continued for at least 7 days, developed an infection with the resistant organism [56]. In the literature, there have been at least nine cases of reported CRE transmission from donor to organ recipient [54, 57]. Rapid diagnosis and communication with the recipient's healthcare team can significantly impact empiric antibiotic regimens and mortality [54].

Among patients with a hematologic malignancy and CRE infection, the mortality rate can be greater than 50% at 30 days, with one study demonstrating a median time from infection to death of only 4 days [48, 49]. This is likely similar in patients who have undergone HSCT, and in Italy the 90-day mortality of this group was 58% [51]. Allogenic HSCT recipients with CRE infections have a higher mortality than autologous HSCT recipients [51]. As with SOT, delay in administration of appropriate antibiotic therapy is associated with high bacteremia-associated mortality rates in patients with hematologic malignancies [49].

Disease Diagnostics and Clinical Evaluation

ESBL-Producing Enterobacterales

Historically, the Clinical and Laboratory Standards Institute (CLSI) recommended confirming ESBL production by phenotypic testing, typically with the addition of clavulanic acid [58]. However, in 2009 and 2010, respectively, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CSLSI lowered the breakpoints for cephalosporins and aztreonam and no longer recommend phenotypic ESBL confirmation for Enterobacterales [56, 58–61]. This was based on data demonstrating that in vitro and in vivo efficacy correlated better with the minimum inhibitory concentration (MIC) and pharmacokinetic data rather than with the presence of a β-lactamase [59]. Nevertheless, these organizations acknowledge that ESBL phenotypic testing may still be useful for epidemiologic and infection control purposes [59, 61]. With this update, most labs are no longer screening for ESBLs, and the presence of an ESBL-producing isolate is often assumed when resistance to ceftriaxone, cefotaxime, or ceftazidime is documented [58, 62]. Because of this, differences in the EUCAST and CLSI breakpoints for ceftazidime and cefpodoxime may affect the reported prevalence of ESBL-EB [63]. In the recently published MERINO trial, 86% of *E. coli* and *K. pneumoniae* bloodstream isolates which were non-susceptible to ceftriaxone or cefotaxime tested positive on an ESBL phenotypic screen, and the presence of an ESBL gene was confirmed in 85% through whole-genome sequencing [62].

Molecular testing for ESBL production has the potential to rapidly diagnose both the organ donor and recipient, allowing for quicker initiation of appropriate

antibiotics. The Verigene Gram-negative blood culture test (Luminex, US) is one of the commercially available molecular testing methods for ESBLs. It uses microarray hybridization and signal amplification to test for CTX-M β-lactamases as well as many carbapenemases within 2 h [64]. This microarray system has been shown to reduce the time to bacterial identification compared to conventional phenotypic analysis as well as the time to initiate optimal antimicrobial therapy [65, 66]. The sensitivity ranges from 77 to 100% for all tested antibiotic resistance genes and the specificity from 99 to 100%. False-negative results can occur, especially in polymicrobial infections, and this cannot replace traditional susceptibility testing [64]. Additional non-Food and Drug Administration (FDA)-approved molecular diagnostic kits using real-time PCR are available and include the Antibiotic Resistance Genes Microbial DNA qPCR Array (Qiagen, Netherlands) and Antibiotic Resistance TaqMan Assays (ThermoFisher Scientific, USA) [67]. Additional methods are also being studied to rapidly identify ESBL genes including loop-mediated isothermal amplification (LAMP) [68], liquid chromatography and tandem mass spectrometry proteomic analysis [69], and whole-genome sequencing [67]. A notable limitation of molecular testing is that it can only detect known antibiotic resistance genes or mutations and novel resistance mechanisms will be missed [67]. Molecular testing may fail to detect less common ESBLs, and most of the current molecular tests only focus on identifying the CTX-M family of β-lactamases.

Carbapenem-Resistant Enterobacterales

Similar to ESBL testing, CLSI lowered the carbapenem breakpoints for Enterobacterales in 2010 and no longer recommends routine testing for carbapenemase production. However, phenotypic screening for carbapenemases is recommended by CLSI when the imipenem or meropenem MIC is 2–4 μg/mL or when the ertapenem MIC is >2 μg/mL [60]. EUCAST has higher MIC breakpoints for meropenem and imipenem than CLSI (≤2 μg/mL vs. ≤1 μg/mL) but a lower threshold to perform carbapenemase screening (MIC >0.125 μg/mL) [61]. This may lead to differences in detection of carbapenemases. In one study, application of the CLSI screening breakpoint did not detect 14% of carbapenemase-producing Enterobacterales isolates; in contrast, implementation of the EUCAST breakpoints failed to detect <2% of isolates. CLSI guidelines based on phenotypic resistance patterns and traditional antimicrobial susceptibility testing are easier for laboratories to implement and maintain. However, they will likely continue to fail to identify a small proportion of patients with carbapenemase-producing organism. Epidemiologically, this may make CRE outbreaks more difficult to trace as not all carbapenemase-producing isolates will be identified; however, at the individual patient level, it is not known whether identification of carbapenemases should effect treatment decisions if the MIC value remains within the susceptible range [70].

In 2015, the Centers for Disease Control and Prevention (CDC) simplified the surveillance definition of CRE to Enterobacterales resistant to imipenem, meropenem, doripenem, or ertapenem or that demonstrates carbapenemase production. Previously,

the definition did not include ertapenem and required the isolate to be non-susceptible to imipenem, meropenem, or doripenem and resistant to a third-generation cephalosporin [71, 72]. By modifying this definition, most carbapenemase-producing CRE are now captured, including those producing OXA-48-like carbapenemases which could have previously gone undetected. However, this definition now captures more CRE isolates which are not carbapenemase producers but may carry other resistance determinants, including overproduction of AmpC or ESBL in combination with other mutations such as porin modifications or efflux pumps [71].

Testing for carbapenemases is often still necessary for infection prevention and epidemiologic purposes, especially in the setting of an outbreak. The most common phenotypic tests include the modified Hodge test (MHT), the Carba NP test, and carbapenem inactivation method (CIM) test. None of these tests is able to differentiate between specific carbapenemases. The MHT is easy to perform, and in the USA, it has a sensitivity between 93% and 98%. Unfortunately, its ability to detect NDMs is poor, and it has a relatively low specificity. This limits its ability to be used in low resource settings outside of the USA, despite its low cost, where KPCs are not the most common carbapenemases [71]. The Carba NP test detects hydrolysis of imipenem by a carbapenemase and is quicker to perform than the MHT. The sensitivity is between 73 and 100% for most carbapenemases except OXA-48-like carbapenemases [71, 73]. Different variations of this assay have been produced including Rapid CARB test (Rosco Diagnostics, Denmark) and Rapidec Carba NP (bioMérieux, France) [71]. The CIM and modified CIM (mCIM) tests asses for the hydrolysis of meropenem. The mCIM has a high sensitivity, ranging from 93 to 100% for all major types of carbapenemases [71, 74]. More specific assays such as using EDTA to test for the presence of an MBL and boronic acid to assess for a KPC have been used [71, 73]. A new rapid phenotypic carbapenemase test, BD Phoenix CPO detect (BD Diagnostic Systems, US), was recently approved by the FDA. This test is unique because it can classify types of carbapenemases and is completely automated [74]. Lastly, matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry (MS) is being developed to detect carbapenem breakdown products but has a low sensitivity for detecting OXA-48-like carbapenemases [71].

Molecular testing for carbapenemases can be helpful in rapidly determining which carbapenemases and resistance mechanisms are present. Several commercial PCR assays are available, including, but not limited to, Xpert Carba-R (Cephid, US), eazyplex SuperBug panels (Amplex, Germany), and Check-Direct CPE assay (Check-Points, Netherlands); however, not all assays detect all carbapenemases [71]. Xpert Carba-R is FDA approved in the USA for testing on rectal surveillance swabs and pure bacterial colonies and is validated in the European Union for use on rectal swabs. It takes less than 1 h to run and can detect KPC, NDM, IMP, VIM, and OXA-48-like carbapenemases [75]. The sensitivity has been reported to be 93.5–100% with a specificity of 98.6–100%, although there have been concerns regarding its ability to detect the VIM carbapenemase [67]. The FilmArray blood culture identification panel (bioMérieux, France) is a multiplex PCR test that is FDA approved for the diagnosis of bacteremia. It can detect KPCs (as well as other antimicrobial resistance genes) directly from a positive blood culture bottle within 1 h [67, 76]. Additionally,

many hospitals develop "in-house" DNA detection assays that can have high sensitivity and specificity [64].

The Verigene Gram-negative blood culture test (Luminex, US), a microarray test, can also detect KPC, NDM, VIM, IMP, and OXA carbapenemases in 2 h with a sensitivity close to 100% [66, 71]. However, a positive blood culture and Gram stain are needed before the appropriate panel can be selected [66]. As whole-genome sequencing becomes more affordable and widely adapted, it also has the potential to assist with rapid detection of carbapenemases [67]. As with all molecular-based assays, only known carbapenemases genes can be tested, making it imperative to perform conventional antimicrobial susceptibility testing alongside these new tests.

Management

ESBL-Producing Enterobacterales

Carbapenems demonstrate the most reliable activity for ESBL-EB infections and should be the first-line therapy for transplant recipients [9, 77, 78]. Carbapenems are typically not affected by ESBL hydrolysis or large bacterial inoculums [77].

First-generation β-lactam/β-lactamase inhibitor (BLBLIs), namely, piperacillin/tazobactam (PTZ), should not be the first choice of therapy for ESBL-EB infections in transplant recipients. ESBL-EB are usually susceptible in vitro to PTZ, but treatment failures have been described, possibly due to high inoculum effects [77]. One post hoc analysis of six prospective cohorts found no difference in the 30-day mortality between those treated with a BLBLI versus those treated with a carbapenem [79]. However, the MERINO trial, published in 2018, was the first randomized, non-inferiority clinical trial to compare PTZ to meropenem in patients with ceftriaxone-resistant *E. coli* or *K. pneumoniae* bacteremia. PTZ did not reach the non-inferiority endpoint, suggesting worse outcomes in the PTZ group and raising serious concerns about using BLBLIs in patients with ESBL-EB infections. 24% of the subjects in the trial were immunocompromised, but details regarding organ transplantation were not delineated [62].

Cefepime may also exhibit in vitro activity against ESBL-EB. However, treatment of ESBL-EB infections with cefepime has been associated with increased mortality in several studies. Cefepime is generally not recommended for patient with ESBL-EB infections, especially in those patients with MICs $\geq$4 μg/mL [77, 80].

Fosfomycin is only available in the oral formulation in the USA but has the potential to treat UTIs due to susceptible ESBL-EB [77]. One study of ESBL uropathogens found that while only 4% of *E. coli* isolates were resistant to fosfomycin, 46% of the *Klebsiella* species isolates were resistant [81]. Additionally, retrospective data show that microbiologic cure rates with fosfomycin for ESBL-EB UTIs may only reach 57% and solid organ transplant patients could be more likely to fail therapy [82]. Therefore, the use of fosfomycin in the management of UTIs in transplant recipients should be guided by antimicrobial susceptibility testing, and

additional studies addressing the use of this agent in the transplant population are warranted.

Second-generation BLBLI antibiotics such a ceftazidime/avibactam (CAZ/AVI), ceftolozane/tazobactam (TOL/TAZ), meropenem/vaborbactam (MER/VAB), and imipenem/relebactam (IMI/REL) all have activity against ESBL-EB. Each respective beta-lactamase inhibitor (avibactam, tazobactam, vaborbactam, and relebactam) can inhibit ESBLs including those in the CTX-M, TEM, and SHV family [83]. Up to 99.6% and 87.1% of ESBL-EB isolates are susceptible in vitro to CAZ/AVI and TOL/TAZ, respectively [84, 85]. CAZ/AVI was originally studied in patients with complicated intra-abdominal infections (RECLAIM Trial), complicated UTIs (RECAPTURE Trial), and nosocomial pneumonias (REPROVE Trial) and was non-inferior to carbapenem therapy in each study [86–88]. All three studies showed that patients with ceftazidime-resistant Enterobacterales had similar cure rates irrespective of whether they were treated with CAZ/AVI versus a carbapenem [86–88]. Additionally, the REPRISE study was a randomized, phase III trial limited to patients with complicated UTIs or intra-abdominal infections due to ceftazidime-resistant Enterobacterales or *Pseudomonas aeruginosa*. Rates of clinical cure were similar for those treated with CAZ/AVI compared to those treated with best available therapy, 97% of whom received a carbapenem [89]. TOL/TAZ was shown to be non-inferior to meropenem in patients with complicated intra-abdominal infections (ASPECT-cIAI trial), including a subset of patients with ESBL-EB infections. The clinical cure rate among these patients was 95.8% [90]. MER/VAB and IMI/REL have not been extensively studied for the management of infections due to ESBL-EB, but these agents should maintain adequate activity against ESBL-EB infections [83]. Due to their broad spectrum of activity, however, these second-generation BLBLIs are best suited for the management of CRE and other multidrug-resistant Gram-negative pathogens and should be used in a stewardship-concordant manner. For more information on the stewardship implications of drug resistance, please refer to ► Chaps. 7, "Antimicrobial Stewardship for Transplant Candidates and Recipients," and ► 8, "Antimicrobial Prophylaxis in the Age of Emerging Transplant Infections" strategies.

The ideal preventative strategies for ESBL-EB infections in transplant recipients remain unknown. Depending on local epidemiology, as many as 31% of pre-transplant liver and kidney patients have been reported to be colonized with ESBL-EB, a factor which has been associated with posttransplant infection [21, 54]. One single-center US study described a 10% rate of fecal ESBL-EB carriage among HSCT candidates, and this was also associated with a risk of ESBL-EB bacteremia following transplant [31]. Guidelines from the American Society of Transplantation (AST) do not recommend screening for ESBL-producing bacteria in asymptomatic patients and instead place emphasis on improving infection control practices, minimizing unnecessary device use, and reducing unnecessary antibiotic exposure before and after transplantation [78]. Intestinal decolonization in patients with ESBL-EB colonization is not recommended and has been associated with the development of colistin- and tobramycin-resistant *K. pneumoniae* isolates [78, 91]. As described above, ESBL-EB donor-derived infections can also occur, and clear

and timely communication between hospitals and providers is critical for success in these scenarios [54].

Carbapenem-Resistant Enterobacterales

CAZ/AVI and MER/VAB are emerging as the first-line therapy for patients with infections due to susceptible CRE isolates [92]. While both display in vitro activity against KPC-producing CRE, neither is active against MBL-producing Enterobacterales. In contrast to MER/VAB, CAZ/AVI is active against OXA-48-producing Enterobacterales (Table 4). In retrospective, observational studies, CAZ/AVI has been associated with improved clinical outcomes compared to colistin- or aminoglycoside-based regimens [92].

CAZ/AVI has not been systematically evaluated in SOT patients. However, in one observational study of 109 patients with carbapenem-resistant *K. pneumoniae* bacteremia, 37% of whom were SOT recipients, higher clinical success rates were reported among patients who received CAZ/AVI-based therapy compared to those who received other regimens (85% vs. 41%, $p = 0.006$). While organ transplant was not a risk factor for clinical failure, 55% of the SOT patients demonstrated clinical failure at 3 days [93]. In another study inclusive of SOT and HSCT recipients treated with CAZ/AVI for invasive CRE infections, the 30-day mortality rate was 24%; 40% of patients with microbiologic failure were SOT recipients [94]. Three patients in this study developed resistance while on CAZ/AVI, and concerns about the development of CAZ/AVI resistance due to porin mutations or alterations in the omega loop of the KPC enzyme are growing [92]. One study also reported bla_{KPC-3} mutations which developed on therapy, conferring resistance to CAZ/AVI but restoring susceptibility to meropenem [95]. Treatment failures may also be caused by CAZ/AVI heteroresistance [96].

MER/VAB was recently approved by the US FDA for the treatment of complicated UTIs. In the TANGO II Trial, which evaluated the safety and efficacy of MER/VAB versus best available therapy (defined as mono- or combination therapy with polymyxins, carbapenems, aminoglycosides, tigecycline, or CAZ/AVI alone) for CRE infections, MER/VAB was associated with a higher rate of clinical cure than best available therapy. While there was no significant difference in 28-day mortality, MER/VAB was associated with less nephrotoxicity than best available therapy (4% vs. 24%) [97]. In one case report of a liver transplant patient with a KPC-producing *K. pneumoniae* bacteremia and a subphrenic abscess, MER/VAB was successfully used as salvage therapy after the patient developed resistance to CAZ/AVI on therapy [98]. It is unknown whether MER/VAB will prove to be superior to CAZ/AVI due to a possible higher barrier to resistance among KPC producers, and empiric therapy should be guided by local epidemiology [92]. IMI/REL has a similar spectrum of activity as MER/VAB and may have a comparable role in treating patients with infections due to KPC-producing Enterobacterales [83]. However, while the phase III RESTORE-IMI 1 trial reported similar clinical response rates between patients receiving IMI/REL and colistin plus imipenem/cilastatin for

Table 4 Summary of novel antibiotics with activity against extended-spectrum β-lactamase-producing Enterobacterales and carbapenem-resistant Enterobacterales [8, 77, 92, 100, 108, 115]

Drug	Class	Active against ESBLs	Active against KPCs	Active against MBLs	Active against OXA-48-like β-lactamases	Dose for CrCl >50
Ceftolozane/tazobactam	BLBLI	Yes	No	No	No	1 g/0.5 g q 8 h
Ceftazidime/avibactam	BLBLI	Yes	Yes	No	Yes	2 g/0.5 g q 8 h[a]
Meropenem/vaborbactam	BLBLI	Yes	Yes	No	No	2 g/2 g q 8 h
Imipenem/relebactam	BLBLI	Yes	Yes	No	No	0.5 g/0.25 g q 6 h
Aztreonam/avibactam	BLBLI	Yes	Yes	Yes	Yes	In development
Plazomicin	Aminoglycoside	Yes	Yes	Some[b]	Likely	15 mg/kg q 24 h
Cefiderocol	Siderophore cephalosporin	Yes	Yes	Yes	Yes	2 g q 8 h
Eravacycline	Tetracycline	Yes	Yes	Yes	Yes	1 mg/kg q 12 h

ESBL extended-spectrum β-lactamase, *KPC K. pneumoniae* carbapenemase, *MBL* metallo-β-lactamase, *OXA* oxacillinase, *CrCl* creatinine clearance, *BLBLI* β-lactam/β-lactamase inhibitor, *g* grams

[a]Has also been studied with a dose of 2 g/0.125 g q 6 h

[b]Higher MICs reported in the presence of the New Delhi metallo-β-lactamase

imipenem-resistant Gram-negative infections, only a minority of clinical isolates were KPC-producing CRE [99].

None of the second-generation BLBLIs have activity against MBL-producing Enterobacterales, but aztreonam (ATM) may be effective in the management of Enterobacterales infections when used in conjunction with avibactam. While ATM is stable against MBLs, avibactam maintains activity against other β-lactamases, including ESBLs and KPCs, which are commonly co-produced by MBL-producing Enterobacterales [100]. A few case reports have described successful use of CAZ/AVI with ATM in patients with infections due to MBL-producing Enterobacterales, including one in a multivisceral transplant recipient and one in a child who was a HSCT recipient [100–102]. This combination is also effective against multidrug-resistant *Stenotrophomonas maltophilia* but unfortunately, due to different mechanisms of resistance, is not reliably active against *P. aeruginosa or Acinetobacter baumannii* [103–105]. Aztreonam/avibactam is currently being evaluated in a phase II clinical trial for complicated intra-abdominal infections [100].

Plazomicin was also recently approved by the FDA for the treatment of complicated UTIs. It is the most potent aminoglycoside against KPC-producing Enterobacterales but displays diminished activity against NDM-producing Enterobacterales [100]. The recently published phase III, open-label CARE trial evaluated 28-day mortality among patients with CRE bacteremia or nosocomial pneumonia receiving plazomicin or colistin, each combined with meropenem or tigecycline. Both clinically significant disease-related complications and 28-day mortality were lower in the plazomicin arm (24% vs. 50%), and rates of nephrotoxicity were lower among patients treated with plazomicin than colistin (16.7% vs. 50%) [106]. Additional studies addressing the role of plazomicin in solid organ and hematopoietic stem cell transplant recipients with invasive CRE infections are warranted.

Eravacycline, a tetracycline derivative with a chemical structure similar to tigecycline, has demonstrated potent in vitro activity against CRE, including KPC-, OXA-, and NDM-producing Enterobacterales. It has been approved by the FDA for use in complicated intra-abdominal infections but did not meet the non-inferiority outcome for complicated UTIs (https://clinicaltrials.gov/ct2/show/study/NCT01978938). Omadacycline, another tetracycline derivative with an oral formulation, may have activity against CRE although it has primarily been investigated for use in community-acquired pneumonia and skin and skin structure infections without a specific focus on drug resistance [107]. Additional studies evaluating the efficacy of eravacycline and omadacycline in patients with invasive CRE infections and its use in solid organ and hematopoietic stem cell transplant recipients are needed [100].

Cefiderocol is a new siderophore cephalosporin that has activity against CRE as well as carbapenem-resistant *P. aeruginosa* and *A. baumannii* in vitro, including activity against KPCs, oxacillin carbapenemases, and many MBLs [100, 108, 109]. In a phase II trial, cefiderocol was found to be non-inferior to imipenem/cilastatin for the treatment of complicated UTIs or uncomplicated pyelonephritis and has now been approved by the FDA for use in these conditions [108]. The

proportion of enrolled patients with uncomplicated pyelonephritis was limited to 30%, and individuals with CRE infections were excluded. While kidney transplant recipients were included in the study, a subgroup analysis of this population was not performed. A trial comparing cefiderocol to best available therapy for severe CRE infections, including bacteremia, nosocomial pneumonia, and complicated urinary tract infection, was recently completed (https://clinicaltrials.gov/ct2/show/NCT02714595). The final results are forthcoming, but documentation provided to the FDA suggested there was a higher infection-related mortality in patients treated with cefiderocol compared to the control group (15.8% vs. 8.2%). The use of cefiderocol for CRE infections in SOT and HSCT recipients warrants further evaluation.

Prevention of CRE infections, including rapid identification and control of outbreaks, is crucial to the well-being of transplant recipients given the associated morbidity and mortality due to these infections, as well as the limited therapeutic armamentarium. Implementation of the CDC CRE toolkit, which emphasizes the use of contact precautions, including proper hand hygiene, gowns, and gloves, as well as patient and staff cohorting wherever possible, is recommended by the American Society of Transplantation Infectious Diseases Community of Practice for the prevention of CRE transmission [78, 110].

Pretransplant colonization with CRE has been cited as a risk factor for post-SOT transplant CRE infections [38, 39, 42]. However, one prospective study described spontaneous CRE clearance in 83% of colonized patients and highlighted the complex and fluid nature of CRE colonization dynamics [111]. While screening for rectal colonization of CRE may assist in identifying SOT candidates and recipients at risk for subsequent CRE infection, the optimal approach to doing so remains unclear, and a history of CRE infection or colonization is not a contraindication to transplantation [78]. In one study of 232 patients undergoing HSCT, 6% were colonized with carbapenem-resistant *K. pneumoniae* at the time of transplant, and intestinal colonization with multidrug-resistant bacteria (vancomycin-resistant *Enterococci* and carbapenem-resistant Gram-negative bacteria) increased the risk of developing a multidrug-resistant bloodstream infection in the early posttransplant period. The authors of this study posit that gut decolonization could help reduce posttransplant multidrug-resistant bacteremia in HSCT recipients [112]. However, for SOT recipients, selective gut decontamination is not recommended and has been associated with the development of resistance to the antibiotic being used for decontamination [78, 113].

Donor-derived CRE infections have been reported, raising the issue of whether patients infected or colonized with CRE are acceptable organ donors. In one series inclusive of 14 patients who received organs from donors with CRE or carbapenem-resistant *A. baumannii* bacteremia or infection of the transplanted organ, transmission did not occur when recipients received at least 7 days of appropriate antibiotic therapy initiated within the first 6 days of transplant [56]. Current guidelines recommend at least 1 week of targeted antimicrobials for recipients whose donors are either bacteremic with CRE or if there is evidence of CRE colonization or infection of the transplanted organ [78].

Conclusion

SOT and HSCT recipients are at high risk for ESBL-EB and CRE infections given their frequent healthcare exposures and need for ongoing immunosuppression. A patient's risk depends on both individual factors, including which organ was transplanted, prior courses of antibiotics, previous colonization with ESBL-EB and CRE, and need for medical devices, as well as institutional risk factors, including recent outbreaks of multidrug-resistant infections. Donor-derived infections can also occur, and inter-facility communication is pivotal in ensuring appropriate management. Given the high morbidity and mortality associated with these infections, novel diagnostic techniques and treatment regimens continue to emerge. However, targeted research in SOT and HSCT recipients, particularly as it pertains to management strategies, is needed.

Key Points

- The risk of developing an ESBL-EB or CRE infection after transplant depends on individual health factors, local resistance patterns, and the organ transplanted. Donor-derived infections can also occur.
- ESBL and CRE infections are associated with significant morbidity and mortality in transplant recipients.
- CLSI and EUCAST cephalosporin breakpoints can be used to diagnose ESBL-EB and CRE, although molecular testing for resistance genes is emerging as a rapid diagnostic technique.
- Carbapenems should be used as a first-line therapy for transplant recipients with ESBL-EB infections.
- Treatment of CRE is challenging. Many novel therapeutic agents are either being used or are in development, but options for MBL-producing Enterobacterales infections remain limited.

References

1. Jacob JT, Klein E, Laxminarayan R, Beldavs Z, Lynfield R, Kallen AJ, et al. Vital signs: carbapenem-resistant Enterobacterales. MMWR Morb Mortal Wkly Rep. 2013;62(9):165–70.
2. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacterales: the impact and evolution of a global menace. J Infect Dis. 2017;215(Suppl_1):S28–36.
3. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother. 2017;72(8):2145–55.
4. McDanel J, Schweizer M, Crabb V, Nelson R, Samore M, Khader K, et al. Incidence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and Klebsiella infections in the United States: a systematic literature review. Infect Control Hosp Epidemiol. 2017; 38(10):1209–15.
5. Oriol I, Sabé N, Simonetti AF, Lladó L, Manonelles A, González J, et al. Changing trends in the aetiology, treatment and outcomes of bloodstream infection occurring in the first year after solid organ transplantation: a single-centre prospective cohort study. Transpl Int. 2017; 30(9):903–13.

6. Satlin MJ, Jenkins SG, Walsh TJ. The global challenge of carbapenem-resistant Enterobacterales in transplant recipients and patients with hematologic malignancies. Clin Infect Dis. 2014;58(9):1274–83.
7. Pouch SM, Satlin MJ. Carbapenem-resistant Enterobacterales in special populations: solid organ transplant recipients, stem cell transplant recipients, and patients with hematologic malignancies. Virulence. 2017;8(4):391–402.
8. Satlin MJ, Walsh TJ. Multidrug-resistant Enterobacterales, *Pseudomonas aeruginosa*, and vancomycin-resistant Enterococcus: three major threats to hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2017;19(6):e12762.
9. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005;18(4):657–86.
10. Bert F, Larroque B, Paugam-Burtz C, Janny S, Durand F, Dondero F, et al. Microbial epidemiology and outcome of bloodstream infections in liver transplant recipients: an analysis of 259 episodes. Liver Transpl. 2010;16(3):393–401.
11. Shi SH, Kong HS, Xu J, Zhang WJ, Jia CK, Wang WL, et al. Multidrug resistant gram-negative bacilli as predominant bacteremic pathogens in liver transplant recipients. Transpl Infect Dis. 2009;11(5):405–12.
12. Linares L, García-Goez JF, Cervera C, Almela M, Sanclemente G, Cofán F, et al. Early bacteremia after solid organ transplantation. Transplant Proc. 2009;41(6):2262–4.
13. Al-Hasan MN, Razonable RR, Eckel-Passow JE, Baddour LM. Incidence rate and outcome of Gram-negative bloodstream infection in solid organ transplant recipients. Am J Transplant. 2009;9(4):835–43.
14. Aguiar EB, Maciel LC, Halpern M, de Lemos AS, Ferreira ALP, Basto ST, et al. Outcome of bacteremia caused by extended-spectrum β-lactamase–producing Enterobacterales after solid organ transplantation. Transplant Proc. 2014;46(6):1753–6.
15. Pinheiro HS, Mituiassu AM, Carminatti M, Braga AM, Bastos MG. Urinary tract infection caused by extended-spectrum beta-lactamase–producing bacteria in kidney transplant patients. Transplant Proc. 2010;42(2):486–7.
16. Men T-Y, Wang J-N, Li H, Gu Y, Xing T-H, Peng Z-H, et al. Prevalence of multidrug-resistant gram-negative bacilli producing extended-spectrum β-lactamases (ESBLs) and ESBL genes in solid organ transplant recipients. Transpl Infect Dis. 2013;15(1):14–21.
17. Bodro M, Sanclemente G, Lipperheide I, Allali M, Marco F, Bosch J, et al. Impact of antibiotic resistance on the development of recurrent and relapsing symptomatic urinary tract infection in kidney recipients. Am J Transplant. 2015;15(4):1021–7.
18. Pilmis B, Scemla A, Join-Lambert O, Mamzer M-F, Lortholary O, Legendre C, et al. ESBL-producing Enterobacterales-related urinary tract infections in kidney transplant recipients: incidence and risk factors for recurrence. Infect Dis. 2015;47(10):714–8.
19. Ramadas P, Rajendran PP, Krishnan P, Alex A, Siskind E, Kadiyala A, et al. Extended-spectrum-beta-lactamase producing bacteria related urinary tract infection in renal transplant recipients and effect on allograft function. PLoS One. 2014;9(3):e91289.
20. Brakemeier S, Taxeidi SI, Zukunft B, Schmidt D, Gaedeke J, Dürr M, et al. Extended-spectrum beta-lactamase–producing Enterobacterales–related urinary tract infection in kidney transplant recipients: risk factors, treatment, and long-term outcome. Transplant Proc. 2017; 49(8):1757–65.
21. Bert F, Larroque B, Paugam-Burtz C, Dondero F, Durand F, Marcon E, et al. Pretransplant fecal carriage of extended-spectrum β-lactamase–producing Enterobacterales and infection after liver transplant, France. Emerg Infect Dis. 2012;18(6):908–16.
22. Raviv Y, Shitrit D, Amital A, Fox B, Bakal I, Tauber R, et al. Multidrug-resistant *Klebsiella pneumoniae* acquisition in lung transplant recipients. Clin Transpl. 2012;26(4):E388–94.
23. Linares L, Cervera C, Hoyo I, Sanclemente G, Marco F, Cofán F, et al. *Klebsiella pneumoniae* infection in solid organ transplant recipients: epidemiology and antibiotic resistance. Transplant Proc. 2010;42(8):2941–3.
24. Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Cisneros JM, Peña C, et al. Risk factors and prognosis of nosocomial bloodstream infections caused by extended-spectrum-β-lactamase-producing *Escherichia coli*. J Clin Microbiol. 2010;48(5):1726–31.

25. Weisser M, Theilacker C, Sutter ST, Babikir R, Bertz H, Götting T, et al. Secular trends of bloodstream infections during neutropenia in 15 181 haematopoietic stem cell transplants: 13-year results from a European multicentre surveillance study (ONKO-KISS). Clin Microbiol Infect. 2017;23(11):854–9.
26. Yemişen M, Balkan İİ, Salihoğlu A, Eşkazan AE, Mete B, Ar MC, et al. The changing epidemiology of bloodstream infections and resistance in hematopoietic stem cell transplantation recipients. Turk J Haematol. 2016;33(3):216–22.
27. Gudiol C, Calatayud L, Garcia-Vidal C, Lora-Tamayo J, Cisnal M, Duarte R, et al. Bacteraemia due to extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) in cancer patients: clinical features, risk factors, molecular epidemiology and outcome. J Antimicrob Chemother. 2010;65(2):333–41.
28. Mikulska M, Del Bono V, Raiola AM, Bruno B, Gualandi F, Occhini D, et al. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance. Biol Blood Marrow Transplant. 2009;15(1): 47–53.
29. Ha YE, Kang C-I, Cha MK, Park SY, Wi YM, Chung DR, et al. Epidemiology and clinical outcomes of bloodstream infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* in patients with cancer. Int J Antimicrob Agents. 2013;42(5):403–9.
30. Kim S-H, Kwon J-C, Choi S-M, Lee D-G, Park SH, Choi J-H, et al. Escherichia coli and *Klebsiella pneumoniae* bacteremia in patients with neutropenic fever: factors associated with extended-spectrum β-lactamase production and its impact on outcome. Ann Hematol. 2013; 92(4):533–41.
31. Satlin MJ, Chavda KD, Baker TM, Chen L, Shashkina E, Soave R, et al. Colonization with levofloxacin-resistant extended-spectrum β-lactamase-producing Enterobacterales and risk of bacteremia in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2018;67(11): 1720–8.
32. Kharrat M, Chebbi Y, Ben Tanfous F, Lakhal A, Ladeb S, Othmen TB, et al. Extended spectrum beta-lactamase-producing Enterobacterales infections in hematopoietic stem cell transplant recipients: epidemiology and molecular characterization. Int J Antimicrob Agents. 2018;52(6):886–92.
33. César-Arce A, Volkow-Fernández P, Valero-Saldaña LM, Acosta-Maldonado B, Vilar-Compte D, Cornejo-Juárez P. Infectious complications and multidrug-resistant bacteria in patients with hematopoietic stem cell transplantation in the first 12 months after transplant. Transplant Proc. 2017;49(6):1444–8.
34. Bergamasco MD, Barbosa MB, de Oliveira Garcia D, Cipullo R, JCM M, Baia C, et al. Infection with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* in solid organ transplantation. Transpl Infect Dis. 2012;14(2):198–205.
35. Clancy CJ, Chen L, Shields RK, Zhao Y, Cheng S, Chavda KD, et al. Epidemiology and molecular characterization of bacteremia due to carbapenem-resistant *Klebsiella pneumoniae* in transplant recipients. Am J Transplant. 2013;13(10):2619–33.
36. Kalpoe JS, Sonnenberg E, Factor SH, del Rio Martin J, Schiano T, Patel G, et al. Mortality associated with carbapenem-resistant *Klebsiella pneumoniae* infections in liver transplant recipients. Liver Transpl. 2012;18(4):468–74.
37. Pereira MR, Scully BF, Pouch SM, Uhlemann A-C, Goudie S, Emond JE, et al. Risk factors and outcomes of carbapenem-resistant *Klebsiella pneumoniae* infections in liver transplant recipients. Liver Transpl. 2015;21(12):1511–9.
38. Giannella M, Bartoletti M, Morelli MC, Tedeschi S, Cristini F, Tumietto F, et al. Risk factors for infection with carbapenem-resistant *Klebsiella pneumoniae* after liver transplantation: the importance of pre- and posttransplant colonization. Am J Transplant. 2015; 15(6):1708–15.
39. Freire MP, Oshiro ICVS, Pierrotti LC, Bonazzi PR, de Oliveira LM, Song ATW, et al. Carbapenem-resistant Enterobacterales acquired before liver transplantation: impact on recipient outcomes. Transplantation. 2017;101(4):811–20.

40. Freire MP, Abdala E, Moura ML, de Paula FJ, Spadão F, Caiaffa-Filho HH, et al. Risk factors and outcome of infections with *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* in kidney transplant recipients. Infection. 2015;43(3):315–23.
41. Cicora F, Mos F, Paz M, Allende NG, Roberti J. Infections with blaKPC-2-producing *Klebsiella pneumoniae* in renal transplant patients: a retrospective study. Transplant Proc. 2013;45(9):3389–93.
42. Pouch SM, Kubin CJ, Satlin MJ, Tsapepas DS, Lee JR, Dube G, et al. Epidemiology and outcomes of carbapenem-resistant *Klebsiella pneumoniae* bacteriuria in kidney transplant recipients. Transpl Infect Dis. 2015;17(6):800–9.
43. Simkins J, Muggia V, Cohen HW, Minamoto GY. Carbapenem-resistant *Klebsiella pneumoniae* infections in kidney transplant recipients: a case–control study. Transpl Infect Dis. 2014;16(5):775–82.
44. Rodrigues dos Santos BG, Amaral ES, Fernandes PFCBC, Oliveira CMC, Rodrigues JLN, Perdigão Neto LV, et al. Urinary tract infections and surgical site infections due to carbapenem-resistant Enterobacterales in renal transplant. Transplant Proc. 2016;48(6):2050–5.
45. Varotti G, Dodi F, Terulla A, Santori G, Mariottini G, Bertocchi M, et al. Impact of carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) infections in kidney transplantation. Transpl Infect Dis. 2017;19(6):e12757.
46. Lanini S, Costa AN, Puro V, Procaccio F, Grossi PA, Vespasiano F, et al. Incidence of carbapenem-resistant gram negatives in Italian transplant recipients: a nationwide surveillance study. PLoS One. 2015;10(4):e0123706.
47. Mouloudi E, Massa E, Papadopoulos S, Iosifidis E, Roilides I, Theodoridou T, et al. Bloodstream infections caused by carbapenemase-producing *Klebsiella pneumoniae* among intensive care unit patients after orthotopic liver transplantation: risk factors for infection and impact of resistance on outcomes. Transplant Proc. 2014; 46(9):3216–8.
48. Trecarichi EM, Pagano L, Martino B, Candoni A, Blasi RD, Nadali G, et al. Bloodstream infections caused by *Klebsiella pneumoniae* in onco-hematological patients: clinical impact of carbapenem resistance in a multicentre prospective survey. Am J Hematol. 2016;91(11):1076–81.
49. Satlin MJ, Cohen N, Ma KC, Gedrimaite Z, Soave R, Askin G, et al. Bacteremia due to carbapenem-resistant Enterobacterales in neutropenic patients with hematologic malignancies. J Infect. 2016;73(4):336–45.
50. Averbuch D, Tridello G, Hoek J, Mikulska M, Akan H, Yanez San Segundo L, et al. Antimicrobial resistance in Gram-negative rods causing bacteremia in hematopoietic stem cell transplant recipients: intercontinental prospective study of the Infectious Diseases Working Party of the European Bone Marrow Transplantation Group. Clin Infect Dis. 2017;65(11): 1819–28.
51. Girmenia C, Rossolini GM, Piciocchi A, Bertaina A, Pisapia G, Pastore D, et al. Infections by carbapenem-resistant *Klebsiella pneumoniae* in SCT recipients: a nationwide retrospective survey from Italy. Bone Marrow Transplant. 2015;50(2):282–8.
52. Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, et al. Worsening epidemiological situation of carbapenemase-producing Enterobacterales in Europe, assessment by national experts from 37 countries, July 2018. Euro Surveill [Internet]. 2019 [cited 2020 Mar 12];24(9). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6402177/
53. Moreno A, Cervera C, Gavaldá J, Rovira M, Cámara RDL, Jarque I, et al. Bloodstream infections among transplant recipients: results of a nationwide surveillance in Spain. Am J Transplant. 2007;7(11):2579–86.
54. Bartoletti M, Giannella M, Tedeschi S, Viale P. Multidrug-resistant bacterial infections in solid organ transplant candidates and recipients. Infect Dis Clin N Am. 2018;32(3):551–80.
55. Brizendine KD, Richter SS, Cober ED, van Duin D. Carbapenem-resistant *Klebsiella pneumoniae* urinary tract infection following solid organ transplantation. Antimicrob Agents Chemother. 2015;59(1):553–7.

56. Mularoni A, Bertani A, Vizzini G, Gona F, Campanella M, Spada M, et al. Outcome of transplantation using organs from donors infected or colonized with carbapenem-resistant Gram-negative bacteria. Am J Transplant. 2015;15(10):2674–82.
57. Galvão LM, de Oliveira APR, Ibanês AS, Monteiro J, Inoue F, Dantas DC, et al. Fatal case of donor-derived colistin-resistant carbapenemase-producing *Klebsiella pneumoniae* transmission in cardiac transplantation. Braz J Infect Dis. 2018;22(3):235–8.
58. Nguyen HM, Shier KL, Graber CJ. Determining a clinical framework for use of cefepime and β-lactam/β-lactamase inhibitors in the treatment of infections caused by extended-spectrum-β-lactamase-producing Enterobacterales. J Antimicrob Chemother. 2014;69(4):871–80.
59. Dudley MN, Ambrose PG, Bhavnani SM, Craig WA, Ferraro MJ, Jones RN. Background and rationale for revised Clinical and Laboratory Standards Institute interpretive criteria (breakpoints) for Enterobacterales and *Pseudomonas aeruginosa*: I. Cephalosporins and Aztreonam. Clin Infect Dis. 2013;56(9):1301–9.
60. EM100 Connect – CLSI M100 ED29:2019 [Internet]. [cited 2019 Mar 8]. Available from: http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSI%20M100%20ED29:2019&sbsso k=CLSI%20M100%20ED29:2019%20TABLE%203A&format=HTML&hl=ESBL
61. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0, 2019. http://www.eucast.org
62. Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients with *E coli* or *Klebsiella pneumoniae* bloodstream infection and ceftriaxone resistance: a randomized clinical trial. JAMA. 2018;320(10):984–94.
63. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Hombach M. Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum β-lactamase production in clinical Enterobacterales isolates. J Antimicrob Chemother. 2012;67(1):159–66.
64. Rood IGH, Li Q. Review: molecular detection of extended spectrum-β-lactamase- and carbapenemase-producing Enterobacterales in a clinical setting. Diagn Microbiol Infect Dis. 2017;89(3):245–50.
65. Sothoron C, Ferreira J, Guzman N, Aldridge P, McCarter YS, Jankowski CA. A stewardship approach to optimize antimicrobial therapy through use of a rapid microarray assay on blood cultures positive for Gram-negative bacteria. J Clin Microbiol. 2015;53(11):3627–9.
66. Dodémont M, De Mendonça R, Nonhoff C, Roisin S, Denis O. Performance of the Verigene Gram-negative blood culture assay for rapid detection of bacteria and resistance determinants. J Clin Microbiol. 2014;52(8):3085–7.
67. Fleece ME, Pholwat S, Mathers AJ, Houpt ER. Molecular diagnosis of antimicrobial resistance in *Escherichia coli*. Expert Rev Mol Diagn. 2018;18(3):207–17.
68. Rivoarilala OL, Garin B, Andriamahery F, Collard JM. Rapid in vitro detection of CTX-M groups 1, 2, 8, 9 resistance genes by LAMP assays. PLoS One. 2018;13(7):e0200421.
69. Fleurbaaij F, Goessens W, van Leeuwen HC, Kraakman MEM, Bernards ST, Hensbergen PJ, et al. Direct detection of extended-spectrum beta-lactamases (CTX-M) from blood cultures by LC-MS/MS bottom-up proteomics. Eur J Clin Microbiol Infect Dis. 2017;36(9):1621–8.
70. Fattouh R, Tijet N, McGeer A, Poutanen SM, Melano RG, Patel SN. What is the appropriate meropenem MIC for screening of carbapenemase-producing Enterobacterales in low-prevalence settings? Antimicrob Agents Chemother. 2016;60(3):1556–9.
71. Lutgring JD, Limbago BM. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacterales detection. J Clin Microbiol. 2016;54(3):529–34.
72. FAQs about choosing and implementing a CRE definition. [Internet]. [cited 2019 Mar 13]. Available from: https://www.cdc.gov/hai/organisms/cre/definition.html
73. Iovleva A, Doi Y. Carbapenem-resistant Enterobacterales. Clin Lab Med. 2017;37(2):303–15.
74. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, et al. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacterales. J Clin Microbiol. 2017;55(8):2321–33.

75. Traczewski MM, Carretto E, Canton R, Moore NM, Team for the C-RS. Multicenter evaluation of the xpert carba-R assay for detection of carbapenemase genes in Gram-negative isolates. J Clin Microbiol [Internet]. 2018 [cited 2019 Dec 7];56(8). Available from: https://jcm.asm.org/content/56/8/e00272-18
76. Salimnia H, Fairfax MR, Lephart PR, Schreckenberger P, DesJarlais SM, Johnson JK, et al. Evaluation of the FilmArray Blood Culture Identification Panel: results of a multicenter controlled trial. J Clin Microbiol. 2016;54(3):687–98.
77. Sheu C-C, Lin S-Y, Chang Y-T, Lee C-Y, Chen Y-H, Hsueh P-R. Management of infections caused by extended-spectrum β–lactamase-producing Enterobacterales: current evidence and future prospects. Expert Rev Anti-Infect Ther. 2018;16(3):205–18.
78. Pouch SM, Patel G, AST Infectious Diseases Community of Practice. Multidrug-resistant Gram-negative bacterial infections in solid organ transplant recipients – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33(9):e13594.
79. Rodríguez-Baño J, Navarro MD, Retamar P, Picón E, Pascual Á. β-Lactam/β-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum β-lactamase–producing *Escherichia coli*: A post hoc analysis of prospective cohorts. Clin Infect Dis. 2012;54(2):167–74.
80. Lee N-Y, Lee C-C, Li C-W, Li M-C, Chen P-L, Chang C-M, et al. Cefepime therapy for monomicrobial *Enterobacter cloacae* bacteremia: unfavorable outcomes in patients infected by cefepime-susceptible dose-dependent isolates. Antimicrob Agents Chemother. 2015;59(12):7558–63.
81. Linsenmeyer K, Strymish J, Weir S, Berg G, Brecher S, Gupta K. Activity of fosfomycin against extended-spectrum-β-lactamase-producing uropathogens in patients in the community and hospitalized patients. Antimicrob Agents Chemother. 2016;60(2):1134–6.
82. Neuner EA, Sekeres J, Hall GS, van Duin D. Experience with fosfomycin for treatment of urinary tract infections due to multidrug-resistant organisms. Antimicrob Agents Chemother. 2012;56(11):5744–8.
83. Zhanel GG, Lawrence CK, Adam H, Schweizer F, Zelenitsky S, Zhanel M, et al. Imipenem–relebactam and meropenem–vaborbactam: two novel carbapenem-β-lactamase inhibitor combinations. Drugs. 2018;78(1):65–98.
84. Karlowsky JA, Kazmierczak KM, Bouchillon SK, de Jonge BLM, Stone GG, Sahm DF. In vitro activity of ceftazidime-avibactam against clinical isolates of Enterobacterales and *Pseudomonas aeruginosa* collected in Asia-Pacific countries: results from the INFORM global surveillance program, 2012 to 2015. Antimicrob Agents Chemother. 2018;62(7):e02569–17.
85. Shortridge D, Pfaller MA, Castanheira M, Flamm RK. Antimicrobial activity of ceftolozane-tazobactam tested against Enterobacterales and *Pseudomonas aeruginosa* collected from patients with bloodstream infections isolated in United States hospitals (2013–2015) as part of the Program to Assess Ceftolozane-Tazobactam Susceptibility (PACTS) surveillance program. Diagn Microbiol Infect Dis. 2018 Oct;92(2):158–63.
86. Mazuski JE, Gasink LB, Armstrong J, Broadhurst H, Stone GG, Rank D, et al. Efficacy and safety of ceftazidime-avibactam plus metronidazole versus meropenem in the treatment of complicated intra-abdominal infection: results from a randomized, controlled, double-blind, phase 3 program. Clin Infect Dis. 2016;62(11):1380–9.
87. Wagenlehner FM, Sobel JD, Newell P, Armstrong J, Huang X, Stone GG, et al. Ceftazidime-avibactam versus doripenem for the treatment of complicated urinary tract infections, including acute pyelonephritis: RECAPTURE, a phase 3 randomized trial program. Clin Infect Dis. 2016;63(6):754–62.
88. Torres A, Zhong N, Pachl J, Timsit J-F, Kollef M, Chen Z, et al. Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomised, double-blind, phase 3 non-inferiority trial. Lancet Infect Dis. 2018;18(3):285–95.
89. Carmeli Y, Armstrong J, Laud PJ, Newell P, Stone G, Wardman A, et al. Ceftazidime-avibactam or best available therapy in patients with ceftazidime-resistant Enterobacterales

and *Pseudomonas aeruginosa* complicated urinary tract infections or complicated intra-abdominal infections (REPRISE): a randomised, pathogen-directed, phase 3 study. Lancet Infect Dis. 2016;16(6):661–73.
90. Solomkin J, Hershberger E, Miller B, Popejoy M, Friedland I, Steenbergen J, et al. Ceftolozane/tazobactam plus metronidazole for complicated intra-abdominal infections in an era of multidrug resistance: results from a randomized, double-blind, phase 3 trial (ASPECT-cIAI). Clin Infect Dis. 2015;60(10):1462–71.
91. Halaby T, Al Naiemi N, Kluytmans J, van der Palen J, Vandenbroucke-Grauls CMJE. Emergence of colistin resistance in Enterobacterales after the introduction of selective digestive tract decontamination in an intensive care unit. Antimicrob Agents Chemother. 2013;57(7):3224–9.
92. Pogue JM, Bonomo RA, Kaye KS. Ceftazidime/avibactam, meropenem/vaborbactam or both? Clinical and formulary considerations. Clin Infect Dis. 2018;68(3):519–524.
93. Shields RK, Nguyen MH, Chen L, Press EG, Potoski BA, Marini RV, et al. Ceftazidime-avibactam is superior to other treatment regimens against carbapenem-resistant *Klebsiella pneumoniae* bacteremia. Antimicrob Agents Chemother. 2017;61(8):e00883–17.
94. Shields RK, Potoski BA, Haidar G, Hao B, Doi Y, Chen L, et al. Clinical outcomes, drug toxicity, and emergence of ceftazidime-avibactam resistance among patients treated for carbapenem-resistant Enterobacterales infections. Clin Infect Dis. 2016;63(12):1615–8.
95. Shields RK, Chen L, Cheng S, Chavda KD, Press EG, Snyder A, et al. Emergence of ceftazidime-avibactam resistance due to plasmid-borne blaKPC-3 mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. Antimicrob Agents Chemother. 2017;61(3):e02097–16.
96. Gaibani P, Campoli C, Lewis RE, Volpe SL, Scaltriti E, Giannella M, et al. In vivo evolution of resistant subpopulations of KPC-producing *Klebsiella pneumoniae* during ceftazidime/avibactam treatment. J Antimicrob Chemother. 2018;73(6):1525–9.
97. Wunderink RG, Giamarellos-Bourboulis EJ, Rahav G, Mathers AJ, Bassetti M, Vazquez J, et al. Effect and safety of meropenem-vaborbactam versus best-available therapy in patients with carbapenem-resistant Enterobacterales infections: the TANGO II randomized clinical trial. Infect Dis Ther. 2018;7(4):439–55.
98. Athans V, Neuner EA, Hassouna H, Richter SS, Keller G, Castanheira M, et al. Meropenem-vaborbactam as salvage therapy for ceftazidime-avibactam-resistant *Klebsiella pneumoniae* bacteremia and abscess in a liver transplant recipient. Antimicrob Agents Chemother. 2019;63(1):e01551–18.
99. Motsch J, De Oliveira C, Stus V, et al. RESTORE-IMI 1: a multicenter, randomized doubleblind, comparator-controlled trial comparing the efficacy and safety of imipenem/relebactam versus colistin plus imipenem in patients with imipenem non-susceptible bacterial infections. European Congress of Clinical Microbiology and Infectious Diseases. Madrid, Spain, 21–24 Apr 2018. (Abstract O0427).
100. Wright H, Bonomo RA, Paterson DL. New agents for the treatment of infections with Gram-negative bacteria: restoring the miracle or false dawn? Clin Microbiol Infect. 2017;23(10):704–12.
101. Rosa R, Rudin SD, Rojas LJ, Perez-Cardona A, Aragon L, Nicolau DP, et al. Application of "precision medicine" through the molecular characterization of extensively drug-resistant *Klebsiella pneumoniae* in a multivisceral transplant patient. Clin Infect Dis. 2017;65(4):701–2.
102. Yasmin M, Fouts DE, Jacobs MR, Haydar H, Marshall SH, White R, et al. Monitoring ceftazidime-avibactam and aztreonam concentrations in the treatment of a bloodstream infection caused by a multidrug-resistant Enterobacter sp. carrying both *Klebsiella pneumoniae* carbapenemase–4 and New Delhi metallo-β-lactamase–1. Clin Infect Dis [Internet]. [cited 2020 Mar 13]. Available from: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciz1155/5658442

103. Biedenbach DJ, Kazmierczak K, Bouchillon SK, Sahm DF, Bradford PA. In vitro activity of aztreonam-avibactam against a global collection of Gram-negative pathogens from 2012 and 2013. Antimicrob Agents Chemother. 2015;59(7):4239–48.
104. Emeraud C, Escaut L, Boucly A, Fortineau N, Bonnin RA, Naas T, et al. Aztreonam plus clavulanate, tazobactam, or avibactam for treatment of infections caused by metallo-β-lactamase-producing Gram-negative bacteria. Antimicrob Agents Chemother [Internet]. 2019 [cited 2020 Mar 13];63(5). Available from: https://aac.asm.org/content/63/5/e00010-19
105. Mojica MF, Papp-Wallace KM, Taracila MA, Barnes MD, Rutter JD, Jacobs MR, et al. Avibactam restores the susceptibility of clinical isolates of Stenotrophomonas maltophilia to Aztreonam. Antimicrob Agents Chemother. 2017;61(10):e00777–17.
106. McKinnell JA, Dwyer JP, Talbot GH, Connolly LE, Friedland I, Smith A, et al. Plazomicin for infections caused by carbapenem-resistant Enterobacterales. N Engl J Med. 2019;380(8):791–3.
107. Barber KE, Bell AM, Wingler MJB, Wagner JL, Stover KR. Omadacycline Enters the Ring: A New Antimicrobial Contender. Pharmacotherapy. 2018;38(12):1194–204.
108. Portsmouth S, van Veenhuyzen D, Echols R, Machida M, Ferreira JCA, Ariyasu M, et al. Cefiderocol versus imipenem-cilastatin for the treatment of complicated urinary tract infections caused by Gram-negative uropathogens: a phase 2, randomised, double-blind, non-inferiority trial. Lancet Infect Dis. 2018;18(12):1319–28.
109. Doi Y. Treatment options for carbapenem-resistant Gram-negative bacterial infections. Clin Infect Dis. 2019;69(Suppl_7):S565–75.
110. Facility Guidance for Control of Carbapenem-resistant Enterobacterales (CRE) [Internet]. Atlanta: Centers for Disease Control and Prevention (US); [cited 2018 Feb 18]. Available from: https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf
111. Macesic N, Gomez-Simmonds A, Sullivan SB, Giddins MJ, Ferguson SA, Korakavi G, et al. Genomic surveillance reveals diversity of multidrug-resistant organism colonization and infection: a prospective cohort study in liver transplant recipients. Clin Infect Dis. 2018;67(6):905–12.
112. Ferreira AM, Moreira F, Guimaraes T, Spadão F, Ramos JF, Batista MV, et al. Epidemiology, risk factors and outcomes of multi-drug-resistant bloodstream infections in haematopoietic stem cell transplant recipients: importance of previous gut colonization. J Hosp Infect. 2018;100(1):83–91.
113. Tascini C, Sbrana F, Flammini S, Tagliaferri E, Arena F, Leonildi A, et al. Oral gentamicin gut decontamination for prevention of KPC-producing *Klebsiella pneumoniae* infections: relevance of concomitant systemic antibiotic therapy. Antimicrob Agents Chemother. 2014;58(4):1972–6.
114. Zuckerman T, Benyamini N, Sprecher H, Fineman R, Finkelstein R, Rowe JM, et al. SCT in patients with carbapenem resistant *Klebsiella pneumoniae*: a single center experience with oral gentamicin for the eradication of carrier state. Bone Marrow Transplant. 2011;46(9):1226.
115. Stewart A, Harris P, Henderson A, Paterson D. Treatment of infections by OXA-48-producing Enterobacterales. Antimicrob Agents Chemother. 2018;62(11):e01195–18.

Pseudomonas aeruginosa Infections in Transplant: Epidemiology and Emerging Treatment Options

15

John I. Hogan, Brandon K. Hill, and Costi D. Sifri

Contents

Epidemiology of *Pseudomonas aeruginosa* Infection in the Transplant Population 344
Solid Organ Transplantation 344
Host Factors in Solid Organ Transplant Recipients 346
Hematopoietic Stem Cell Transplantation 349
Host Factors in Hematopoietic Stem Cell Transplant Recipients 350
Pathogenesis of Infection 352
Microbiology 352
Environmental Factors in Transplant Recipients 353
Recent Diagnostic Advances 354
Resistance to Antimicrobials 354
Emerging Therapeutic Strategies 358
Ceftolozane-Tazobactam 360
Ceftazidime-Avibactam 360
Cefiderocol 361
Meropenem-Vaborbactam 362
Imipenem-Relebactam 362
Plazomicin 362
Investigational Agents: Aztreonam-Avibactam, Intravenous Fosfomycin 362
Conclusion 363
Key Points 363
References 364

J. I. Hogan · C. D. Sifri (✉)
Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA, USA
e-mail: jih8u@hscmail.mcc.virginia.edu; CDS9N@hscmail.mcc.virginia.edu

B. K. Hill
Department of Pharmacy Clinical Services, UVA Health, Charlottesville, VA, USA
e-mail: BKH6RN@virginia.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_20

Epidemiology of *Pseudomonas aeruginosa* Infection in the Transplant Population

Recognized as the sixth most common bacterial species contributing to healthcare-associated-infections (HAIs), PsA represents a major nosocomial pathogen in the general population. Further solidifying its rank among the most important nosocomial pathogens, PsA represents the second most common cause of ventilator-associated-pneumonia (VAP) in the United States [1]. When resistant to multiple conventional antibiotics, PsA infection is associated with a longer duration of hospitalization and substantially increased healthcare expenditure [2]. The high virulence and potential lethality of this pathogen in the nosocomial environment mandates that all infectious disease providers have a firm understanding of the epidemiology of PsA infection. This becomes particularly true in transplant patients, a population in which unchecked infection can rapidly lead to clinical deterioration.

Solid Organ Transplantation

Despite advances in surgical techniques, immunosuppression protocols, and the ongoing evolution of the field of transplant infectious disease, bacterial infections continue to represent a major source of morbidity and mortality in solid organ transplant (SOT) patients. A recent single-center study from Canada identified PsA as the fifth most common bacterial species and the third most common Gram-negative agent causing infection in graft recipients. Overall, PsA accounted for 5.5% of microbiologically proven bacterial infections in this cohort [3]. As in the general population, PsA most often contributes to bacteremia and respiratory tract infections in the SOT population as a whole [4, 5]. A 2018 study that reviewed 116 episodes of bacteremia in a cohort of renal transplant patients determined that PsA contributed to 8% of these infections [6]. In a broader cohort of patients that included liver, kidney, and heart transplant recipients in Spain, PsA accounted for 15% of 318 total bouts of bacteremia [7]. Like PsA infection in the general hospitalized population, PsA clearly remains one of the most common bacterial pathogens encountered in the SOT population.

There are, however, some critical differences between PsA infection in the general nosocomial population and SOT patients. Most importantly, multiple studies have suggested that antimicrobial resistance is more prevalent in PsA strains isolated from the SOT population. Whereas a recent study demonstrated that the overall rate of PsA bloodstream infection (BSI) in a cohort of SOT patients did not appear to differ significantly from that noted in non-SOT patients, the rate of meropenem resistance was significantly higher in transplant recipients [8]. The authors suggest that excessive antibiotic use, inconsistent infection control practices, and perhaps even climatic factors could account for this observation. Even more concerning is the prevalence of MDR and XDR PsA isolates in organ recipients. Consensus guidelines define MDR pathogens as those exhibiting nonsusceptibility to at least one agent in three or more antimicrobial categories. XDR pathogens are characterized by

nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories [9]. In a single-center US study from 2009 that identified 207 PsA blood isolates obtained from 108 SOT and nine hematopoietic stem cell transplant (HSCT) patients, 43% of these were MDR. This figure was significantly higher than the 18% of PsA blood isolates that exhibited the MDR phenotype in a cohort of non-transplant patients. Previous transplant, prior ICU admission, and nosocomial acquisition all served as independent risk factors for infection related to MDR pathogens in this study [10]. This trend appears to extend to other site-specific PsA infections as well. The MDR phenotype was observed in 46% of patients who experienced PsA infection after abdominal organ transplantation [5]. Considering infection in lung transplant recipients, a recent study suggested that PsA may account for up to 27% of the bacteria isolated from recipients (only 1% with cystic fibrosis) in the immediate postop setting, and that 51% of these isolates may exhibit the MDR phenotype [11]. Duration of ICU stay, duration of ventilation, exposure to broad antimicrobials, bilateral transplant, and tracheostomy all appear to correlate with an increased risk of MDR infection in this population. Similarly, the XDR phenotype is also observed more frequently in SOT patients. A 2015 single-center Spanish study detected this resistance pattern in 63% of PsA strains isolated from the blood of transplant patients [7]. Furthermore, this highly resistant phenotype was associated with a mortality rate of 38%, a significant increase from the 16% mortality rate observed with other more susceptible isolates. While many transplant centers in the United States may not experience rates of MDR/XDR infection that are this high, these figures do not appear to represent isolated experiences. A meta-analysis of studies from North America, Europe, and Asia determined that of the PsA isolates contributing to bacteremia in liver transplant patients, 47% were MDR and 63% were XDR [12]. These alarming figures highlight the shifting climate of PsA infection in the SOT population, a trend that could forecast the need for significant changes in the future management of PsA infection.

Another key characteristic that defines the epidemiology of bacterial infection in the SOT population is a strong correlation between the organ transplanted and the frequency of infection at specific sites. This correlation holds true for PsA infection. The respiratory tract is the single most common site of PsA infection in recipients of lung or heart transplants [3]. Mechanical ventilation prior to transplant and significant transfusion requirements may serve as risk factors in the development of early pneumonia after thoracic transplantation [13]. Urinary tract infection (UTI) rates related to PsA are highest in renal and renal/pancreas transplant patients [3]. Of the 7% of renal transplant patients who experienced urinary tract infection (UTI) in the large RESITRA cohort based in Spain, PsA accounted for 10% of these infections [13]. Likewise, the hepatobiliary system is the most common site of infection in liver transplant patients [3]. Recipients of hepatic grafts procured from living donors may be at particularly high risk for PsA-related intraabdominal complications. In a group of patients experiencing PsA infection after living donor liver transplantation, intraabdominal infection was observed in 52% of patients [14].

The timing of PsA infections in SOT recipients appears to differ from that of hosts who have not undergone transplant. Traditional teaching suggests that infections

caused by healthcare-associated pathogens are highest within the first month of SOT [15]. Bacteremia in the SOT population illustrates this principle well, as more than half of the BSIs that occur within the first 2 years after SOT are documented in the first month after transplant [16]. In the case of PsA infection, however, it is important to note that while the risk of infection related to this pathogen may decrease over time in the SOT population, some patients may remain at risk for PsA-related infection for a period that extends at least several months after transplant. In a 2011 Spanish study of an SOT cohort that documented 276 episodes of bacterial, mycobacterial, viral, and protozoal infection occurring greater than 6 months after transplant, 5% of all infections were related to PsA [17].

Rarely, early post-transplant *Pseudomonas* infections may arise following receipt of a PsA-contaminated graft. PsA infection of a fresh arterial anastomosis may lead to pseudoaneurysm formation, sepsis, and eventual rupture. Complications include massive hemorrhage, graft loss, embolic phenomena, and death. Unfortunately, poor outcomes may occur despite early administration of directed antibiotic treatment, and drug resistant pathogens like MDR/XDR PsA appear to have higher attack rates than organs contaminated by nondrug resistant bacteria attributed to the failure of routine perioperative antibiotic prophylaxis [18, 19]. Optimal management for a potential donor-derived transmission event due to MDR/XDR PsA or other drug-resistant pathogens is not well-defined but includes at a minimum: (1) appropriately targeted antibiotic treatment, (2) close clinical follow-up with early surgical intervention as needed, and (3) rapid, effective communications between transplant centers and organ procurement organizations so that other recipients of potentially PsA-contaminated organs can promptly receive targeted preemptive antibiotic treatment [19, 20].

Host Factors in Solid Organ Transplant Recipients

Multiple host-specific factors contribute to PsA infection in the SOT population. Preexisting comorbidities contributing to the indication for organ transplant can have a major impact on the susceptibility to infection. For instance, cystic fibrosis (CF) prior to lung transplant represents a significant risk factor for PsA pulmonary infection after transplantation. Quantifying this risk, one study observed a 60% rate of PsA respiratory infection in lung transplant recipients with CF compared to 38% in non-CF patients [21]. Compounding this problem, early nosocomial infection including pneumonia in CF patients is a known risk factor for increased mortality in the posttransplant setting. Early risk factors may have implications that extend to the late posttransplant period, as PsA infection late after lung transplant has been associated with the development of the bronchiolitis obliterans syndrome [22]. PsA may evoke the production of certain chemokines that predispose to this process [23]. Persistent sinus carriage in CF patients may predispose to recurrent pulmonary colonization that may progress to infection [24]. Other pretransplant comorbidities contributing to immune dysfunction or structural abnormalities including bronchiectasis may similarly serve as risk factors for recurrent PsA infection after lung transplant. In the renal transplant population, autosomal

dominant polycystic kidney disease, ureteral reflux, diabetes-related bladder dysfunction, and other preexisting structural and functional problems may serve as risk factors for recurrent infections of the urinary tract. At least one study has identified autosomal dominant polycystic kidney disease in renal transplant patients as an independent risk factor for infection with MDR/XDR pathogens, including *Pseudomonas* species, on multivariate analysis [25]. Primary sclerosing cholangitis (PSC) and other antecedent hepatobiliary comorbidities resulting in biliary stasis or recurrent infection may predispose to infection at the time of liver transplant. PSC and autoimmune hepatitis serve as risk factors for nonanastomotic biliary strictures, which can predispose to recurrent infection after transplant [26]. Although diabetic patients may enjoy substantial improvement in glucose control and quality of life after pancreas and/or renal transplant, hyperglycemia in the years leading up to SOT may contribute to chronic vascular, neuropathic, and musculoskeletal changes that predispose to diabetic foot infections after transplant. PsA contributes to diabetic foot infections with a respectable frequency in the transplant population [27]. These common examples highlight a few of the many ways in which preexisting comorbidities in organ recipients can predispose to PsA infection after SOT.

Complications directly related to surgical procedures represent another major class of risk factors predisposing to PsA infection in the SOT population. In lung transplant patients, anastomotic complications including leaks and stenoses are significant concerns. Compared to non-transplant patients requiring sternotomy for other indications, the rate of sternal infection in transplant recipients is substantially higher. In a study of patients undergoing heart transplantation, 6% experienced sternal infection, and PsA contributed to 15% of these infections [28]. Factors including the need for delayed sternal closure, bleeding complications, and repeat sternotomy may all potentially increase the risk of sternal infection. In recipients of abdominal organs, anastomotic stenoses can contribute to processes including pyelonephritis, pancreatitis, and cholangitis. Fluid collections related to anastomotic leaks or the cut edges of hepatic grafts procured from living donors may also predispose to infection in the abdomen. When compared to duct-to-duct anastomosis, Roux-en-Y hepaticojejunostomy may increase the risk of bacterial infection as bacterial species may more easily ascend the biliary tree after this surgical approach [29]. Vascular anastomoses may rarely predispose to intravascular bacterial infection as PsA has been known to contribute to mycotic pseudoaneurysm formation after liver and other solid organ transplants [30, 31]. In all transplanted organs, ischemic injury related to the procurement process itself, shock at the time of procurement, or vascular complications in the operating room can predispose to infection either by inducing organ dysfunction or producing areas of frank necrosis in the graft which can harbor chronically infecting organisms. In contrast to hepatocytes, which receive dual blood flow from both the hepatic portal vein and the hepatic artery, bile ducts only receive blood flow from the hepatic artery. As a result, the biliary tree is more susceptible to ischemic insult, and this factor is thought to contribute to the pathogenesis of nonanastomotic biliary stricture after liver transplant [26]. Biliary tract ischemia can predispose to chronic stasis and recurrent infection. Finally, one must anticipate the expected anatomical consequences of transplant when considering the

risk of PsA infection after SOT. Injury to the vagus nerve during pulmonary transplantation, for example, may impair the cough reflex that normally assists the host in clearing pathogens from the airways [32].

Indwelling prosthetic devices are another host factor predisposing to infection in the SOT population. Devices placed to augment cardiopulmonary support such as cannulae for extracorporeal membrane oxygenation (ECMO), ventricular assist devices (VADs), and balloon pumps may predispose to bacteremia, septic thrombophlebitis, and exit site infections. In a recent retrospective review of 175 initial episode VAD infections at a US hospital, 8% of infections were attributed to PsA [33]. When complicated by extensive biofilm formation, PsA-related VAD infections have the capability to persist after robust courses of antibiotics, potentially lingering into the peri-transplant period. Similarly, tracheostomy, prolonged intubation, bronchial stents, vascular grafts or stents, embolization coils, urinary catheters, ureteral stents, pancreatic duct stents, biliary stents, and venous catheters can also predispose to infection in this population.

Clinicians should consider the quality of the transplanted graft and donor derived risk factors when evaluating a patient with PsA infection. Processes such as aspiration in lung donors, antecedent trauma in the donor, size mismatch, and prolonged hospitalization may predispose to infection. Suboptimal graft function at the time of SOT related to factors including shock around the time of the donor's death, the age of the graft, steatosis, vascular disease, and other processes can contribute to chronic graft dysfunction that may increase the risk of subsequent infection. When considering an organ recipient's risk factors for PsA infection, providers need to evaluate two sets of risks, those in the recipient as well as those of the donor.

Finally, the complex interplay between immunosuppression and opportunistic infection may predispose to PsA infection in the SOT population. Neutrophil function appears to be essential for PsA clearance, as is evident by the predisposition of patients with neutropenia or neutrophil dysfunction to severe disseminated PsA infection (Fig. 1) [34–36]. As a result, any immunocompromising condition or medication that either directly impairs innate immunity or diminishes the ability of the adaptive immune response to augment innate immunity may decrease the ability of the host to clear bacilli. Providers should consider host factors including chronic underlying immunosuppressing conditions, induction immunosuppression, maintenance immunosuppressing medications, and therapy for rejection when considering a patient's risk of infection related to typical bacteria. Opportunistic infections in SOT patients may also increase the risk of bacterial infection. As early as the 1970s, researchers established a link between cytomegalovirus (CMV) infection and progression of bacterial disease in a murine model of PsA infection [37]. By the 1990s, studies had established a link between CMV and severe bacterial infections in SOT recipients [38]. We now know that strategies employing antiviral prophylaxis targeting CMV confer a substantial reduction in the rate of bacterial and fungal infections after transplant, and recent guidelines incorporate CMV prophylaxis into standard practice [39, 40]. Recent data also suggest an association between XDR PsA infection and opportunistic infection related to CMV and fungi [7]. CMV dysregulates the immune response via several mechanisms, increases the risk of

Fig. 1 Ecthyma gangrenosum in a 65-year-old woman with advanced endometrial cancer, chemotherapy-induced neutropenia, *Pseudomonas aeruginosa* bacteremia, and septic shock

allograft rejection, and should always be considered when assessing a patient's risk of infection related to pathogens such as PsA.

Hematopoietic Stem Cell Transplantation

While data suggest that the overall incidence of PsA infection in HSCT patients may be relatively low (in the range of 2%) in the modern era, the severity of these infections is substantial as the diagnosis of PsA infection in this population is associated with a mortality rate of 35% [41]. Importantly, PsA infection in HSCT recipients not only appears to be associated with substantial mortality but also seems to have a proclivity for recurrence. A 2007 study documented recurrence in 16% of patients occurring a median time of 9 days after stopping antibiotics [41]. High-grade graft-versus-host disease (GVHD) appeared to increase the risk of recurrent infection [41]. As in the general nosocomial population and the SOT population, the bloodstream and the respiratory tract appear to be the most common sites of infection in HSCT patients [41]. Addressing bacteremia specifically, relatively recent studies suggest that PsA contributes to 3–26% of BSIs in patients who have undergone HSCT [42–44]. Collectively, these data suggest that while PsA may not be a very common pathogen in the HSCT patients, it contributes to a substantial portion of the severe infections in this compromised population.

As in the SOT population, rates of antimicrobial resistance in PsA appear to be rising in the HSCT population. A 2014 study of an Australian HSCT cohort identified a common trend experienced at other institutions over the past several years [44]. In this work, PsA accounted for 17% of Gram-negative infections at all sites. During the course of a nine-year surveillance period, providers introduced fluoroquinolone prophylaxis into their practice. While the rate of Gram-negative infection remained relatively stable over the course of nearly a decade of monitoring, the rate of antibiotic resistance in these pathogens increased substantially. Specifically, rates of

fluoroquinolone and extended-spectrum beta-lactam resistance rose significantly. Fluoroquinolone resistance rose from 13% to 36% over the study period, and fluoroquinolone-resistant isolates were significantly more likely to exhibit an extended-spectrum beta-lactamase (ESBL) phenotype or resistance to fourth-generation cephalosporins, carbapenems, or aminoglycosides. Similar to the SOT population, bacteremia related to MDR pathogens was associated with an independently increased risk of mortality in this HSCT cohort [44]. PsA represents a major contributor to the overall incidence of MDR Gram-negative infections in HSCT patients as evidenced by a recent European study that identified PsA as the most common (61%) MDR Gram-negative species isolated from any site in HSCT patients [45]. As antibiotic options have become more limited in recent years, carbapenemase production has emerged as another significant concern in HSCT patients. In a 2017 Brazilian study of patients in a bone marrow transplantation unit who experienced BSI related to PsA isolates that produced the SPM-1 carbapenemase, mortality attributed to this infection was extremely high at 79% [46]. While carbapenem resistance is on the rise in the United States, carbapenemase-mediated resistance remains relatively rare, occurring in only 2% of all isolates in a recent multistate laboratory-based surveillance study [47]. However, if resistance rates were to accelerate, it is possible that the HSCT population could eventually experience mortality rates attributed to severe PsA infection that have not been witnessed in the medical field since the 1950s [42].

Host Factors in Hematopoietic Stem Cell Transplant Recipients

In HSCT patients, many risk factors for complications related to PsA infection become established well before transplant takes place. Chemotherapy, total body irradiation, cytopenias, immune dysregulation related to underlying hematologic disease, suboptimal nutrition, mucositis, prolonged hospitalization, prior antibiotic exposure, and indwelling catheters all serve to disturb the human microbiome and promote bacterial infection in this population. Complications directly related to the status of the patient's marrow including graft failure and recurrent primary disease can further predispose to infection. The type of HSCT also affects the risk of infection. As one example, a recent study identified haploidentical transplant as being associated with a greater risk of bacteremia related to pathogens including MDR PsA after HSCT [48]. The authors posited that a greater incidence of significant mucositis in haploidentical patients compared to HLA-identical siblings may have contributed to this finding. The use of anti-thymocyte globulin and the duration of neutropenia were also hypothesized to have also contributed to some of the effect observed in this study.

Of special note is antibiotic prophylaxis in this population. Fluoroquinolones have the capability to promote carbapenem resistance in PsA by inducing the expression of efflux pumps such as MexAB-OprM and promoting the loss of porins including OprD (Fig. 2). In a recent cohort of patients with hematologic malignancy and/or a history of HSCT, 84.6% of PsA isolates that contributed to bacteremia despite levofloxacin prophylaxis were resistant to meropenem; in contrast, only 27.6% of PsA isolates cultured from the blood of patients who were not treated

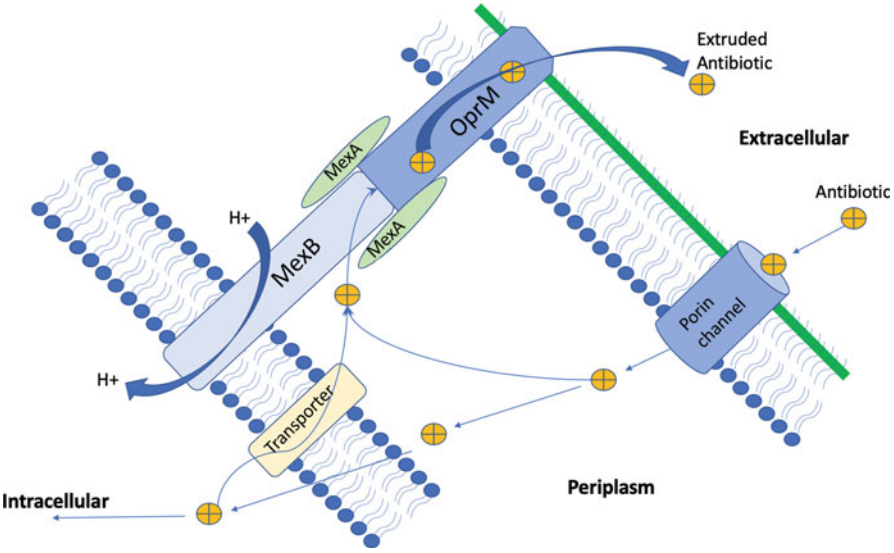


Fig. 2 Pseudomonas wall, with Eflux pump and Porin channel. https://www.semanticscholar.org/paper/Pseudomonas-aeruginosa%3A-arsenal-of-resistance-of-Zowalaty-Thani/cf8665b4be63a2822afed1933d2b77f00ee733f2/figure/0

with quinolone prophylaxis were resistant to meropenem in this work [49]. Changes in both efflux pump and porin expression were noted in isolates that were resistant to meropenem. These data emphasize the importance of prior antibiotic exposure including medications used for prophylaxis when crafting an empiric antibiotic regimen targeting PsA in the HSCT population.

GVHD is another significant risk factor for bacterial infection in the HSCT population. As an obligate aerobe, PsA is typically present at lower titers in stool [50]. As a result, this organism is less commonly associated with invasion of the gastrointestinal tract in normal hosts. In compromised hosts, however, especially those experiencing GVHD of the gut or mucositis and neutropenia related to chemotherapy and/or radiation, PsA has the capability to translocate across the gut. A recent study from the Dana-Farber Cancer Institute identified high grade GVHD of the gut, but not other sites, as a significant risk factor for BSI related to PsA and several common enteric pathogens [51]. Additionally, a recent study that included 3200 adult HSCT patients reported that both acute and chronic GVHD were associated with bacterial infections, including pseudomonal infections [52]. Gut permeability, immune dysregulation, and immunosuppressing therapy may all be involved in increasing the risk of PsA invasion in HSCT patients with GVHD.

Finally, as in the case of SOT patients, CMV infection similarly increases the risk of bacterial infection in HSCT recipients [53]. Immune dysregulation associated with CMV infection may contribute to some of this effect, but antiviral therapy treating CMV may also increase the risk of bacterial infection as it predisposes to leukopenia and renal dysfunction. There exists a clear link between GVHD,

immunosuppressive therapy addressing GVHD, and subsequent CMV reactivation. Corticosteroid dosage treating GVHD directly correlates with the risk of CMV reactivation in HSCT patients [53]. Conversely, in a complex interplay between pathogen and host immunity, primary CMV infection may also predispose to the subsequent development of GVHD. Primary immunologic mechanisms may contribute to this effect, but the reduction of immunosuppression in the setting of CMV infection also has the potential to increase the chance of new onset GVHD. Thus, clinicians can envision CMV and GVHD in HSCT patients as having a cyclical relationship, and PsA infection may become more likely in patients experiencing GVHD, CMV infection, or both of these processes.

Pathogenesis of Infection

The excess morbidity and mortality related to PsA infection observed in the transplant population represents the end result of a complex interaction between a virulent pathogen, host factors, and environmental factors. Recent trends in PsA infection in the immunocompromised population have spurred efforts to gain a deeper understanding of the pathogenesis of this infection.

Microbiology

As an obligate aerobic, flagellated, Gram-negative rod, PsA is most often seen in culture as a single bacterium but can occasionally form short chains (Fig. 3). The

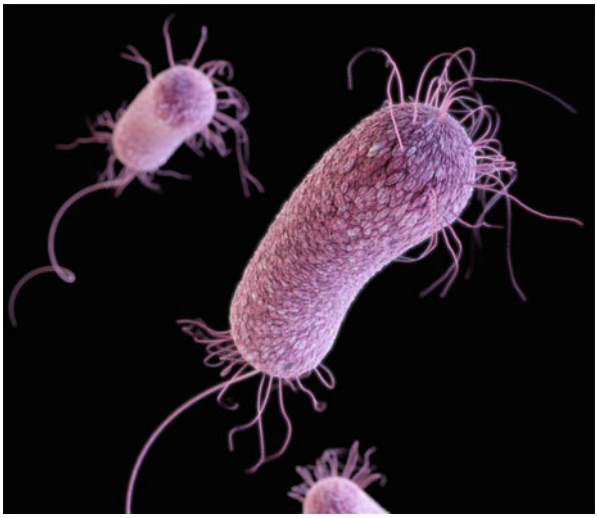


Fig. 3 *Pseudomonas* with flagella and pili. (Picture credit: https://phil.cdc.gov/Details.aspx?pid=16876)

species *aeruginosa*, Latin for verdigris (copper green), refers to the organism's predilection for forming blue/green colonies on solid media [50]. The production of the pigments pyoverdine and pyocyanin confer this characteristic coloration. PsA colonies often have a characteristic odor suggesting corn tortillas or grapes. With a proclivity for growth in aquatic environments, this organism may be identified in nosocomial water sources. Virulence factors in this pathogen are legion. Pili and flagella facilitate mobility, adhesion, and biofilm formation. Biofilm formation becomes particularly important in pulmonary infections and infections related to prosthetic devices. At least one in vitro study assessing biofilm formation along drivelines demonstrated that biofilm formation in PsA was more robust than coagulase-negative staphylococci or *Staphylococcus aureus* [54]. Various exotoxins including phospholipases, adenylate cyclase, elastases, and proteases assist in tissue invasion, induce apoptosis in leukocytes, and promote dysregulation of the host response [34].

Production of these virulence factors is carefully regulated through small, diffusible pheromone-like molecules released in a population-dependent manner. This type of chemical-based cellular communication, known as quorum-sensing, enables bacteria to coordinate expression of behaviors at times of high cell density [50]. While quorum-sensing systems have been shown to be important virulence regulators for a number of both Gram-negative and Gram-positive pathogens, PsA is remarkable for having at least four distinct quorum-sensing systems [55]. It has been suggested that this sophisticated collection of virulence regulators enables PsA to adapt and fine-tune virulence and behavioral traits to a wide range of environmental conditions [55, 56].

Environmental Factors in Transplant Recipients

In addition to virulence factors in the pathogen and risk factors in susceptible hosts, environmental factors can also promote the development of PsA infection in transplant recipients. Given its proclivity for growth in aquatic environments, PsA, including MDR/XDR PsA, may be isolated from water sources and aqueous solutions in the nosocomial setting. Faucets, aerators, showers, baths, therapy pools, drains, and other sources may contribute to colonization or infection [57, 58]. Inhalation of aerosols and ingestion may serve as common modes of transmission. Furthermore, compromised hosts may be at higher risk for the acquisition of PsA from the nosocomial environment [59, 60]. Interventions ranging from patient isolation to enhanced decontamination of patient rooms to expensive changes in facilities construction have attempted to address the broad problem of nosocomial spread. Emphasizing the importance of water sources as reservoirs for drug-resistant PsA and other MDR organisms, recent interventions targeting wastewater management have had some success in limiting transmission [61, 62]. More extensive measures including alterations in the construction of bathroom fixtures have been demonstrated to decrease rates of MDR PsA isolation from the environment, patient colonization, and infection in transplant patients [63]. These recent advances

underscore the importance of robust hospital epidemiology programs in monitoring and limiting the spread of PsA in the nosocomial setting.

Recent Diagnostic Advances

The morbidity and mortality associated with severe PsA infection coupled with rising rates of antimicrobial resistance have fueled recent efforts to advance diagnostic capabilities. Given the significantly increased mortality that can occur with any delay in therapy targeting BSIs, rapid identification of blood isolates remains a priority. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used with some success in compromised hosts to accurately and rapidly identify bacterial species including PsA [64]. MALDI-TOF MS has also been adapted to detect gene products that confer resistance to antimicrobials, including extended-spectrum beta-lactamases [65]. Other rapid molecular diagnostic modalities include the Verigene Gram-negative blood culture (BC-GN) assay (Nanosphere Inc., Northbrook, IL), an FDA-approved multiplex DNA assay that has the capability to identify bacteria isolated from blood culture including PsA and detect molecular markers that predict drug resistance within 2 h of isolation [66]. Several other rapid molecular assays using DNA-based identification or peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) have come to market. Looking to the future, innovative techniques, such as culture-independent whole genome sequencing or breath analysis tools that detect volatile organic compounds associated with PsA pulmonary infection may eventually become available in broader clinical practice [67, 68]. With the development of novel techniques and the optimization of rapid molecular diagnostics, clinicians in the future could enjoy more rapid and accurate diagnostic capabilities. While these technologies should accelerate the time to optimized antibiotics for PsA and other bacterial infections, their impact on patient care outcomes and their financial consequences (both expenses and cost savings) largely remain to be determined.

Resistance to Antimicrobials

Given the strong association between significant antimicrobial resistance and adverse outcomes including excess mortality in the transplant population, research efforts have sought to elucidate the multifarious mechanisms of resistance that PsA employs. Porin loss or modification prevents the transit of antimicrobials to their site of action. Carbapenem resistance, for example, is most often related to porin loss in PsA. Efflux pumps expel antimicrobials from the cell. Enzymes encoded on mobile genetic elements (i.e., transposons, integrons, plasmids) modify or degrade antibiotics (e.g., aminoglycosides and carbapenems, respectively) rendering them ineffective. Mutations in specific antibiotic targets may confer resistance; for example, DNA gyrase and topoisomerase mutations confer resistance to fluoroquinolones [50, 69]. Determining which resistance mechanism or combination of mechanisms is at

play can be a particularly difficult task for clinicians. Regimen selection for treatment of presumed MDR/XDR PsA isolates should be based on prior cultures, recent antibiotic exposure, and response to empiric therapy pending definitive susceptibility data. In such scenarios it is important, if possible, to work closely with clinical microbiology personnel to rapidly identify potential phenotypic and genotypic expressions of resistance.

Beta-lactamases are one of the most important enzyme classes conferring resistance in PsA isolates. One commonly used method of categorizing beta-lactamases, Ambler classification, is based on the structural homology of the enzymes (Table 1). Ambler class A ESBLs are present on mobile genetic elements and hydrolyze penicillins, cephalosporins, and the monobactam aztreonam. Enzymes of this class that have been documented in PsA strains include GES, PER, VEB, SHV, TEM, CTX-M, PME, BEL, and KPC [50]. KPC, though uncommon in PsA, has the additional capability to hydrolyze carbapenem antibiotics, as do certain GES variants [70]. Beta-lactamase inhibitors such as tazobactam and avibactam inhibit Ambler class A enzymes to varying degrees (Table 2). In recent years, plasmid encoded Ambler class B enzymes, also known as metallo-beta-lactamases, have become a greater concern and are the most frequently identified carbapenemases found in PsA (Table 2). These enzymes efficiently hydrolyze all beta-lactams with the exception of aztreonam. IMP, VIM, SIM, FIM, GIM, SPM, and NDM are members of this class that have been described in PsA [50, 71, 72]. AmpC is an important chromosomally encoded class C beta-lactamase that has activity against ticarcillin, piperacillin, and extended-spectrum cephalosporins (Table 2).

Table 1 Ambler classification of beta-lactamases found in *Pseudomonas aeruginosa*

Ambler Class	Activity	Example Enzymes
A	Hydrolyzes penicillins and cephalosporins	CTX-M, SHV, KPC, TEM
B	Hydrolyzes beta-lactam ring using zinc	IMP, VIM, NDM
C	Hydrolyzes broad and extended-spectrum cephalosporins	AmpC
D	Variable hydrolytic activity with ESBL and carbapenemase activity sometimes observed	OXA

Table 2 Beta-lactamase inhibitor activity versus beta-lactamases found in *Pseudomonas aeruginosa*

Ambler Class	Inhibited by avibactam?	Inhibited by tazobactam?	Inhibited by vaborbactam?	Inhibited by relebactam?
A	Yes	Partial	Yes	Yes
B	No	No	No	No
C	Yes	Partial	Yes	Yes
D	Yes	No	No	No

Hyperinduction of AmpC in PsA is caused by mutations in *amp* genes often following exposure to low-level beta-lactam concentrations [73–75]. OXA variants, members of the Ambler class D beta-lactamase family (Table 2), are the final known family of beta-lactamases in PsA isolates. Enzymes in this heterogeneous group can confer resistance patterns ranging from amoxicillin nonsusceptibility to effective late generation cephalosporin and carbapenem resistance [76].

As resistance rates in the transplant population have risen in recent years, it has become increasingly important that clinicians improve their understanding of the mechanisms that confer resistance to antibiotics typically reserved for MDR/XDR isolates. For example, compared to many other beta-lactams, ceftolozane-tazobactam appears to be more resilient to the development of resistance related to new mutations [77, 78]. The development of new resistance in the setting of antibiotic exposure appears to occur primarily via changes that ultimately effect AmpC modification, the upregulation of AmpC production, and porin loss. Paradoxically, while the accumulation of AmpC-related mutations may confer high-level resistance to ceftolozane-tazobactam, certain mutations involved in this process may also increase susceptibility to other beta-lactams including imipenem, cefepime, and piperacillin-tazobactam. The horizontal acquisition of ESBLs, OXA as one example, may also contribute to the development of broad resistance to agents including ceftolozane-tazobactam [78]. Resistance may emerge in patients on therapy, a phenomenon that was reported at rates of 10.6% and 14% in two recent studies [78, 79].

Like ceftolozane-tazobactam, ceftazidime-avibactam is susceptible to cleavage by certain ESBLs. When resistance to ceftazidime-avibactam is related to de novo mutations, AmpC overexpression and efflux pumps appear to contribute. Changes in porin expression seem to be less important factors in this process [80, 81]. Interestingly, ceftazidime-avibactam related selection pressure in vitro was associated with the uniform development of large chromosomal deletions in one study. In some cases, up to 7% of the PsA genome was deleted after prolonged exposure to ceftazidime-avibactam or ceftazidime. Deletion of genetic elements encoding efflux pumps was noted in multiple isolates. As in the case of ceftolozane-tazobactam resistance, the molecular changes that confer ceftazidime-avibactam resistance may be associated with an increased susceptibility to other antimicrobials including aminoglycosides [82]. In patients failing therapy with ceftolozane-tazobactam or ceftazidime-avibactam, including aminoglycoside sensitivities in repeat susceptibility testing may be useful in guiding therapeutic choices.

While ceftolozane-tazobactam and ceftazidime-avibactam are resistant to hydrolysis by most ESBLs, particularly concerning are recent reports describing the new development of resistance related to mobile genetic elements in the Enterobacteriaceae. KPC-3 documented in a *Klebsiella* isolate was recently found to confer resistance to ceftazidime-avibactam, a combination previously thought to be impervious to cleavage by all Ambler class A ESBLs [83]. Reports like this raise concern for the possibility of increasing rates of resistance in common Gram-negative species potentially contributing to worsening trends in PsA resistance related to the interspecies transfer of mobile genetic elements like plasmids.

Upregulation of efflux pumps, located on chromosomes or plasmids, leads to expulsion of antibiotics from inside the cell and is a common method of intrinsic or acquired resistance in PsA. Two major contributors in clinical PsA isolates are MexAB-OprM and MexXY(-OprA). In wild-type strains of PsA, such as PA01, MexAB-OprM is expressed at moderate levels while the MexXY(-OprA) is inducible by ribosome-targeting antibiotics under standard laboratory conditions [84]. The MexAB-OprM is known to expel numerous antibiotics including beta-lactams, carbapenems (except imipenem), fluoroquinolones, macrolides, sulfonamides, and trimethoprim. In addition, the MexXY(-OprA) demonstrates resistance to aminoglycosides, fluoroquinolones, and specific beta-lactams such as cefepime but not ceftazidime. Studies have found that overexpression of such efflux pumps does not necessarily lead to high-level antibiotic resistance but can contribute to the development of MDR PsA. One particular study found a strong association between fluoroquinolone prophylaxis and breakthrough bacteremia with meropenem-nonsusceptible PsA in HSCT recipients [49]. The investigators note the selection of meropenem- nonsusceptible PsA may not only be due to induction of cross-resistance but may be influenced by epidemiology of PsA bloodstream infections at their center.

Resistance to colistin and polymyxin B, antibiotics often reserved for the most resistant MDR/XDR isolates, also occurs in PsA. While resistance to these antimicrobials in wild-type strains is not common, resistance reliably occurs in the setting of gradually escalating antibiotic concentrations. Mutations in *pmrB*, the sensing kinase component of the *pmrCAB* operon, most commonly confer resistance to these antimicrobials [85]. Mutations in *pmrB* ultimately result in a net decrease in the negative charge on lipopolysaccharide in the PsA cell, a process that renders the cationic antibiotics colistin and polymyxin B ineffective [86]. In 2015, researchers in China identified *mcr-1*, a gene that encodes a phosphoethanolamine transferase, which confers resistance to polymyxin B and colistin in an *Escherichia coli* [87, 88]. Although not yet described in clinical PsA isolates, plasmids carrying *mcr-1* are known to be functional in PsA, and mobile genetic elements such as this may facilitate increasing rates of colistin and polymyxin B resistance via horizontal transfer in the future.

Elevated minimum inhibitory concentrations (MICs) to plazomicin, a next-generation aminoglycoside, have been observed in PsA isolates. In the CANWARD study, 64 (10.8%) of 593 PsA isolates were MDR, and the observed plazomicin MICs in these organisms ranging from $\leq$0.12 μg/mL to >64 μg/mL [89]. In Enterobacteriaceae, plazomicin overcomes the effects of multiple antibiotic modifying enzymes, a major source of resistance in these Gram-negative species. However, certain enzymes with ribosome methyltransferase activity do appear to render it ineffective [90]. A similar mechanism may exist in PsA. While the precise mechanisms of plazomicin resistance in PsA have not been completely elucidated, authors have posited that reduced permeability and efflux pumps may be contributing factors [89].

Fosfomycin resistance in PsA is related to the FosA enzyme that confers an antibiotic-modifying effect. Inactivation of the GlpT transport protein may also

contribute to resistance [91]. Even when confronted with pressures exerted by emerging therapeutics, PsA possesses a potent and diverse array of resistance mechanisms that prevent its clearance and promote its pathogenicity.

Emerging Therapeutic Strategies

Delays in appropriate therapy targeting severe PsA infections are clearly associated with adverse clinical outcomes including mortality [92]. Ensuring that initial empiric antibiotic therapy is appropriate becomes especially important in transplant patients, compromised hosts who often have decreased physiologic reserve. Additionally, optimizing the delivery of appropriate therapeutics to the site of infection requires extra care in SOT and HSCT patients. Disseminated infections related to resistant pathogens occasionally require providers to carefully consider the penetration of less commonly employed antibiotics into more challenging sites of infection, such as the central nervous system or the prostate. Complex medication interactions, dysfunction in major organ systems, and malabsorption related to factors including GVHD further complicate therapeutic decisions in this population. In patients with diminished ability to clear bacterial pathogens, source control considerations become extremely important. Retained prosthetic devices, infected fluid collections, and necrotic tissue associated with infection may all predispose to recrudescence if not appropriately managed. With appropriate concern regarding rising rates of resistance in the nosocomial population, multiple recent, high-profile studies have challenged long-held assumptions surrounding the duration of therapy for common clinical syndromes related to infection. Though innovative and insightful, one frequently cited work that addressed the optimal duration of therapy for intraabdominal infections did not incorporate significant numbers of patients treated with immunosuppression or those with substantial cytopenias [93]. Similarly, HSCT providers may find it challenging to extrapolate from studies addressing the duration of therapy for Gram-negative bacteremia that do not include recent recipients of allogeneic stem cell transplants [94]. In general, it is reasonable to consider more conservative courses of therapy in transplant patients with significant infections related to PsA. When devising the duration of therapy for PsA infection in a transplant patient, it is important to contemplate the net state of immunosuppression of the host, the completeness of source control, the effectiveness of the therapy delivered, the risk of incurring resistance, the risk of antibiotic side effects, anatomic factors such as the patency of surgical anastomoses, and the host's physiologic reserve. Robust courses of appropriate therapy become more important in the treatment of MDR and XDR PsA isolates, pathogens that frequently require the use of emerging therapeutics to effect durable clearance.

There are many important patient-, site-, and agent-specific characteristics to consider when treating MDR PsA in immunocompromised patients, as dosing and treatment for this organism is typically more aggressive while concomitant co-morbidities may create difficult-to-manage dosing situations. Knowledge of

specific pharmacokinetic and pharmacodynamic characteristics of antimicrobials and susceptibility data are necessary for therapeutic optimization. Factors affecting efficacy and safety are the site of infection, weight of the patient (e.g., obese, malnourished, edematous), utilization of ECMO, and line access, as extended infusion beta-lactams can complicate therapy. Appropriate evaluation of renal function is paramount. Characteristics that influence renal function to consider include (but are not limited to) serum creatinine in relation to overall muscle mass, patients with a single kidney, falsely appropriate urine output in the setting of diuretics, potential for augmented renal clearance, acute post-operative changes, and in the case of acute or chronic renal failure, the modality of renal replacement therapy. Volume of distribution may also be impacted in critically ill patients due to acute changes in volume status.

Utilization of prolonged- or continuous-infusion dosing of select beta-lactam antibiotics for Gram-negative infections, including pseudomonal infections, is supported by in vitro, in vivo, and human studies demonstrating that increased time of free active drug above an organism's minimum inhibitory concentration (MIC) is associated with improved outcomes [95, 96]. This is reflected in the dosing regimens of newer beta-lactam antibiotics. For example, the duration of infusion for ceftolozane-tazobactam and ceftazidime-avibactam are 1 and 2 h, respectively. For pulmonary infections, some investigators have proposed using inhaled antibiotics as options that reduce risk of adverse effects from agents with important systemic toxicities, such as aminoglycosides and polymyxins. In addition, inhaled antibiotics could increase concentrations at the site of infection (i.e., pulmonary tissues) compared to intravenous administration. However, the role of inhaled agents for the treatment of invasive disease is unclear at best [97, 98].

The twofold crises of rapidly growing antibiotic resistance and a meager antibiotic pipeline have led to renewed interest in combination antibiotic therapy for the treatment of extensively resistant Gram-negative infections, including those due to MDR/XDR PsA. Treatment with more than one anti-pseudomonal agent may be considered for severe PsA infections while awaiting in vitro susceptibility testing. Whether combination therapy (e.g., synergistic) therapy has a role for the treatment of severe, difficult-to-treat PsA infections is unclear and should be considered with careful attention to issues such as severity of infection (rapid progression, relapsed infection, salvage treatment) and the risk of possible adverse effects [99]. Recently, several new anti-pseudomonal drugs have fortunately come to market in the United States and worldwide; however, resistance in PsA to some of these agents has already been reported in case reports and banked collections of isolates. One final important consideration is the limited amount of clinical data with new antibiotics for the treatment of MDR/XDR PsA in immunocompromised populations. For purposes of this chapter, larger studies will be discussed focusing on safety and efficacy of *new agents* targeted against MDR/XDR PsA in general populations. When possible, use of new agents in immunocompromised patients will be highlighted. Some of these agents are already available in North America and/or Europe; they may not all be accessible in all countries.

Ceftolozane-Tazobactam

Ceftolozane-tazobactam is a semisynthetic cephalosporin approved by the FDA in December 2014. Current indications approved for use by the FDA are complicated urinary tract infections (cUTIs), including pyelonephritis, and complicated intraabdominal infections (cIAIs) in combination with metronidazole. Ceftolozane is a novel, broad-spectrum cephalosporin with potent anti-pseudomonal activity including against MDR isolates. Ceftolozane's bactericidal activity results from inhibition of cell wall biosynthesis via binding to penicillin-binding proteins (PBPs), including PBP1b, PBP1c, and PBP3. Tazobactam is an irreversible inhibitor of several beta-lactamases, binding covalently to some chromosomal- and plasmid-mediated bacterial beta-lactamases. Drug adverse events associated with ceftolozane-tazobactam are similar to that of most other cephalosporins (e.g., rash, diarrhea, nausea, headache, pyrexia). Primary monitoring parameters include daily serum creatinine in patients with changing renal function for evaluation of necessary renal dose adjustments, per the FDA-approved package insert.

With the addition of tazobactam, an eightfold increase has been observed in the activity of ceftolozane over ceftazidime against PsA [100]. In previously characterized PsA strains, ceftolozane/tazobactam was superior to piperacillin-tazobactam and imipenem and comparable to doripenem in isolates susceptible to ceftazidime and imipenem-cilastatin. In MDR PsA isolates resistant to ceftazidime and imipenem-cilastatin, ceftolozane-tazobactam demonstrated the most activity followed by doripenem. In fact, ceftolozane-tazobactam retained its overall activity against ceftazidime-resistant isolates [100]. Similarly, a recent study by Farrell et al. tested susceptibilities of 1971 isolates of PsA to ceftazidime, meropenem, piperacillin-tazobactam, levofloxacin, gentamicin, colistin, and ceftazidime-avibactam. In MDR PsA, ceftolozane-tazobactam was found to be second only to colistin in terms of activity [101]. In XDR strains, ceftolozane-tazobactam had maintained "appreciable activity"; however, in metallo-beta-lactamase producing strains no drugs were active [101, 102].

Ceftazidime-Avibactam

Ceftazidime is a third-generation cephalosporin that was approved by the FDA in 1985. In February 2015, ceftazidime combined with the non-beta-lactam beta-lactamase inhibitor, avibactam, received FDA approval for the treatment of cIAI (used in combination with metronidazole) and cUTI, including pyelonephritis. Given ceftazidime's extensive history of clinical use, the approval process for ceftazidime-avibactam relied on ceftazidime historical data plus ceftazidime/avibactam phase I and II data only [103]. The addition of avibactam allows ceftazidime to maintain in vitro activity against class A, some class C, and class D beta-lactamases. A unique feature of avibactam compared to earlier beta-lactamase inhibitors is that avibactam binds *reversibly* to beta-lactamases, creating an

opportunity for the molecule to be recycled with additional beta-lactamases, potentially extending and augmenting its inhibitory effectiveness [103, 104]. Adverse drug reactions associated with ceftazidime-avibactam are similar to that of most other cephalosporins (e.g., rash, diarrhea, nausea, headache, pyrexia). Primary monitoring parameters include at least daily serum creatinine in patients with changing renal function for evaluation of necessary renal dose adjustments, per the FDA-approved package insert. A recent US study evaluating activity of ceftazidime-avibactam against 7868 PsA isolates found that ceftazidime-avibactam inhibited 97.1% of isolates at the susceptible breakpoint of $\leq$8 μg/ml, including 86.5% of MDR, 75.9% of XDR, and 71.8% of isolates resistant to ceftazidime, meropenem, and piperacillin-tazobactam [104]. Among non-beta-lactam compounds tested, only amikacin and colistin showed reasonable in vitro activity against more resistant isolates of PsA.

A multinational, phase III, double-blind, non-inferiority trial demonstrated ceftazidime-avibactam was non-inferior to meropenem in the treatment of nosocomial pneumonia [105]. Thirty percent (107/355) of isolates collected were PsA and 25% of those were nonsusceptible to ceftazidime. Nine PsA isolates were resistant to ceftazidime-avibactam compared to 31 nonsusceptible to meropenem. A recent pooled data analysis of results from phase III trials of ceftazidime-avibactam demonstrated similar clinical and microbiological efficacy versus predominantly carbapenem comparators for management of cIAI and cUTIs caused by PsA, including MDR isolates [106].

Cefiderocol

Cefiderocol is a novel siderophore cephalosporin with affinity mainly for PBP3 and a spectrum of activity that includes MDR organisms such as PsA, *Stenotrophomonas*, and *Acinetobacter baumannii*. A unique characteristic of cefiderocol compared to other cephalosporins is that it does not inhibit cell wall biogenesis but rather chelates iron, essential for bacterial survival, within Gram-negative organisms including PsA [107]. A recent phase II study cefiderocol was found to be non-inferior to imipenem/cilastatin for treatment of cUTI caused by Gram-negative pathogens, including MDR PsA [108]. At test of cure, the proportion of patients who met the composite outcome of clinical and microbiological success for treatment of PsA was similar between the cefiderocol and imipenem-cilastatin groups but lower than the overall response rate (73% for the cefiderocol group and 55% for the imipenem-cilastatin group) [108]. All post-randomization factors that might have been expected to influence outcomes (i.e., proportion of patients with acute uncomplicated pyelonephritis, reduced renal function, concomitant bacteremia, or PsA infection) favored the imipenem/cilastatin group. Cefiderocol was well tolerated with a safety profile similar to that of other cephalosporin antibiotics [108]. Cefiderocol was FDA approved in late 2019 is anticipated to be available to physicians across Europe and other countries soon.

Meropenem-Vaborbactam

In November 2017, the combination of meropenem and a novel non-beta-lactam cyclic boronic acid-based beta-lactamase inhibitor, vaborbactam, received FDA approval for the treatment of adults with cUTI and pyelonephritis due to drug-resistant Enterobacteriaceae expressing class A and class C beta-lactamases [109]. However, it is important to note that the addition of vaborbactam to meropenem does not increase the activity of meropenem against PsA [110, 111]. Therefore, meropenem-vaborbactam is not recommended as therapy against meropenem-resistant PsA and has no additive effects for treatment of meropenem-susceptible isolates.

Imipenem-Relebactam

Imipenem-relebactam was approved by the FDA in July of 2019. Relebactam is a novel non-beta-lactam beta-lactamase inhibitor structurally similar to avibactam with activity against class A and class C beta-lactamases. The addition of relebactam to imipenem has demonstrated significant improvement in activity of imipenem against PsA by reducing the MIC up to ~eightfold [112]. This combination is unique in that both imipenem and relebactam are not subject to removal via efflux pumps in PsA. One of the most significant impacts of the addition of relebactam to imipenem is restoring activity of imipenem to PsA previously identified as imipenem non-susceptible isolates [113]. For PsA, 94% (796/845) and 70.3% (594/845) of isolates were susceptible to imipenem-relebactam and imipenem, respectively; 80.5% (202/251) of imipenem-nonsusceptible isolates were deemed susceptible with addition of relebactam [113]. This combination provides a potential alternative to polymyxins, aminoglycosides, and combination therapy for management of MDR PsA infections.

Plazomicin

Plazomicin, a next-generation aminoglycoside newly FDA approved in 2018, has demonstrated in vitro activity similar to that of amikacin for MDR PsA [89, 114]. Phase III clinical data exists for treatment of cUTIs due to MDR Enterobacteriaceae [115]; however, no clinical data is currently available for treatment of PsA infections. Due to activity similar to amikacin, its utility in MDR *P. aeruginosa* infections is not well established at this time.

Investigational Agents: Aztreonam-Avibactam, Intravenous Fosfomycin

Another agent with broad Gram-negative activity currently in the therapeutic pipeline is aztreonam-avibactam. In addition, intravenous fosfomycin is an agent

available in other countries for MDR Gram-negative bacterial infections but is not yet available in the United States at the time of this chapter writing. Avibactam is a non-beta-lactam beta-lactamase inhibitor structurally similar to relebactam and has been discussed previously as a component of ceftazidime-avibactam [103, 116]. This agent is active in vitro against class A and C beta-lactamases, which is significant because aztreonam can be hydrolyzed by enzymes in both of these classes [116]. A recent study of over 11,842 clinical isolates of PsA collected worldwide found that the in vitro activity of aztreonam-avibactam was less potent in PsA than Enterobacteriaceae [116]. This finding has been replicated in another study [117], suggesting the resistance to aztreonam in PsA may include other mechanisms, such as other beta-lactamases and efflux pumps.

Fosfomycin is a well-known antimicrobial agent historically utilized as an oral sachet for treatment of UTIs; however, intravenous formulations are available in countries outside of the United States for management of non-urinary sources of infection. Fosfomycin acts by inhibiting formation of a peptidoglycan precursor thereby disrupting synthesis of the bacterial cell wall. While intravenous fosfomycin is a potentially promising option for management of MDR PsA infections, it is a bacteriostatic antibiotic and significant resistance has been reported in PsA. Given these issues and the lack of robust clinical data, use of intravenous fosfomycin as monotherapy for MDR PsA in the absence of documented susceptibility is not recommended at this time [118].

Conclusion

P. aeruginosa is one of the most versatile and formidable opportunistic pathogens encountered by solid organ and hematopoietic stem cell transplant patients. Ubiquitous in nature, the organism is a common inhabitant of the hospital-built environment and a frequent nosocomial pathogen. *P. aeruginosa* produces a diverse array of virulence factors and is capable of infecting nearly any tissue in the transplant recipient. In addition to its remarkable versatility and virulence, the bacterium can carry multiple antibiotic drug resistance determinants, which complicates treatment and has led to the emergence of MDR and XDR strains. The combination of virulence, versatility, and increasing drug resistance in *P. aeruginosa* highlight the need for optimized treatment approaches, emerging therapeutics, and infection prevention.

Key Points

- Hematopoietic stem cell and solid organ transplant recipients are at particularly high risk for *P. aeruginosa* infection as a consequence of significant exposure to the healthcare environment and broad spectrum antimicrobial agents.
- Host risk factors for *P. aeruginosa* infection include preexisting comorbidities, anatomic issues related to surgical procedures, the use of indwelling prosthetic

devices, immunosuppression, and post-transplant complications such as rejection, graft-versus-host disease, and opportunistic infection (e.g., CMV infection).
- Despite advances in antimicrobial stewardship, rates of MDR and XDR *P. aeruginosa* continue to rise, particularly in the transplant population. Interdisciplinary collaboration is important when evaluating empirical and directed antimicrobial therapy.
- *P. aeruginosa* has potential to express variety of antibiotic resistance *mechanisms*, including porin loss, beta-lactamases, and efflux pumps. While there is no one-size-fits-all approach for treatment of MDR and XDR *P. aeruginosa*, recently approved antibiotics offer new treatment options for these infections.

References

1. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, Edwards JR, Sievert DM. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol. 2016;37:1288–301.
2. Lodise TP, Wang R, Bhagnani T, Zhao Q, Ye M, Berger A. Clinical and economic burden of multi-drug resistant Pseudomonas sp. (MDRP) among patients with serious infections in United States hospitals. Open Forum Infect Dis. 2017. https://doi.org/10.1093/ofid/ofw172.1593.
3. Hamandi B, Husain S, Grootendorst P, Papadimitropoulos EA. Clinical and microbiological epidemiology of early and late infectious complications among solid-organ transplant recipients requiring hospitalization. Transpl Int. 2016;29:1029–38.
4. Luo A, Zhong Z, Wan Q, Ye Q. The distribution and resistance of pathogens among solid organ transplant recipients with *Pseudomonas aeruginosa* infections. Med Sci Monit. 2016;22:1124.
5. Su H, Ye Q, Wan Q, Zhou J. Predictors of mortality in abdominal organ transplant recipients with *Pseudomonas aeruginosa* infections. Ann Transplant. 2016;21:86–93.
6. Shendi AM, Wallis G, Painter H, Harber M, Collier S. Epidemiology and impact of bloodstream infections among kidney transplant recipients: a retrospective single-center experience. Transpl Infect Dis. 2018;20:e12815.
7. Bodro M, Sabé N, Tubau F, Lladó L, Baliellas C, González-Costello J, Cruzado JM, Carratalà J. Extensively drug-resistant *Pseudomonas aeruginosa* bacteremia in solid organ transplant recipients. Transplantation. 2015;99:616–22.
8. Camargo LFA, Marra AR, Pignatari ACC, et al. Nosocomial bloodstream infections in a nationwide study: comparison between solid organ transplant patients and the general population. Transpl Infect Dis. 2015;17:308–13.
9. Magiorakos A-P, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268–81.
10. Johnson LE, D'Agata EMC, Paterson DL, Clarke L, Qureshi ZA, Potoski BA, Peleg AY. *Pseudomonas aeruginosa* bacteremia over a 10-year period: multidrug resistance and outcomes in transplant recipients. Transpl Infect Dis. 2009. https://doi.org/10.1111/j.1399-3062.2009.00380.x.
11. Tebano G, Geneve C, Tanaka S, Grall N, Atchade E, Augustin P, Thabut G, Castier Y, Montravers P, Desmard M. Epidemiology and risk factors of multidrug-resistant bacteria in respiratory samples after lung transplantation. Transpl Infect Dis. 2016;18:22–30.

12. Liu T, Zhang Y, Wan Q. *Pseudomonas aeruginosa* bacteremia among liver transplant recipients. Infect Drug Resist. 2018;11:2345–56.
13. Vidal E, Torre-Cisneros J, Blanes M, et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. Transpl Infect Dis. 2012;14:595–603.
14. Hashimoto M, Sugawara Y, Tamura S, Kaneko J, Matsui Y, Kokudo N, Makuuchi M. *Pseudomonas aeruginosa* infection after living-donor liver transplantation in adults. Transpl Infect Dis. 2009;11:11–9.
15. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med. 2007;357:2601–14.
16. Moreno A, Cervera C, Gavaldá J, et al. Bloodstream infections among transplant recipients: results of a nationwide surveillance in Spain. Am J Transplant. 2007;7:2579–86.
17. Cervera C, Fernández-Ruiz M, Valledor A, et al. Epidemiology and risk factors for late infection in solid organ transplant recipients. Transpl Infect Dis. 2011;13:598–607.
18. Orlando G, Di Cocco P, Gravante G, D'Angelo M, Famulari A, Pisani F. Fatal hemorrhage in two renal graft recipients with multi-drug resistant *Pseudomonas aeruginosa* infection: case report. Transpl Infect Dis. 2009. https://doi.org/10.1111/j.1399-3062.2009.00412.x.
19. Lewis JD, Sifri CD. Multidrug-resistant bacterial donor-derived infections in solid organ transplantation. Curr Infect Dis Rep. 2016;18:18.
20. Sifri CD, Ison MG. Highly resistant bacteria and donor-derived infections: treading in uncharted territory. Transpl Infect Dis. 2012;14:223–8.
21. Bonvillain RW, Valentine VG, Lombard G, LaPlace S, Dhillon G, Wang G. Post-operative infections in cystic fibrosis and non-cystic fibrosis patients after lung transplantation. J Heart Lung Transplant. 2007;26:890–7.
22. Botha P, Archer L, Anderson RL, Lordan J, Dark JH, Corris PA, Gould K, Fisher AJ. *Pseudomonas aeruginosa* colonization of the allograft after lung transplantation and the risk of bronchiolitis obliterans syndrome. Transplantation. 2008;85:771–4.
23. Gregson AL, Wang X, Weigt SS, et al. Interaction between Pseudomonas and CXC chemokines increases risk of bronchiolitis obliterans syndrome and death in lung transplantation. Am J Respir Crit Care Med. 2013;187:518–26.
24. Dorschner P, McElroy LM, Ison MG. Nosocomial infections within the first month of solid organ transplantation. Transpl Infect Dis. 2014;16:171–87.
25. Yuan X, Liu T, Wu D, Wan Q. Epidemiology, susceptibility, and risk factors for acquisition of MDR/XDR gram-negative bacteria among kidney transplant recipients with urinary tract infections. Infect Drug Resist. 2018;11:707–15.
26. Seehofer D, Eurich D, Veltzke-Schlieker W, Neuhaus P. Biliary complications after liver transplantation: old problems and new challenges. Am J Transplant. 2013;13:253–65.
27. Fejfarová V, Jirkovská A, Petkov V, Bouček P, Skibová J. Comparison of microbial findings and resistance to antibiotics between transplant patients, patients on hemodialysis, and other patients with the diabetic foot. J Diabetes Complicat. 2004. https://doi.org/10.1016/S1056-8727(02)00276-3.
28. Wallen TJ, Habertheuer A, Gottret JP, et al. Sternal wound complications in patients undergoing orthotopic heart transplantation. J Card Surg. 2019;34:186–9.
29. Abad CLR, Lahr BD, Razonable RR. Epidemiology and risk factors for infection after living donor liver transplantation. Liver Transplant. 2017;23:465–77.
30. Alamo JM, Gómez MA, Tamayo MJ, Socas M, Valera Z, Robles JA, Pareja F, García I, Serrano J, Bernardos A. Mycotic pseudoaneurysms after liver transplantation. Transplant Proc. 2005;37:1512–4.
31. Kumar D, Cattral MS, Robicsek A, Gaudreau C, Humar A. Outbreak of *Pseudomonas aeruginosa* by multiple organ transplantation from a common donor. Transplantation. 2003;75:1053–5.
32. Duarte AG, Myers AC. Cough reflex in lung transplant recipients. Lung. 2012;190:23–7.
33. Guzman MB, Vader J, Olsen M, Dubberke ER. Epidemiology and microbiology of first ventricular assist device infection and their effect on outcomes. Open Forum Infect Dis. 2017;4:S652–3.

34. Ramos J-L, Goldberg JB, Filloux A, editors. Pseudomonas; 2015. https://doi.org/10.1007/978-94-017-9555-5.
35. Chang HY, Rodriguez V, Narboni G, Bodey GP, Luna MA, Freireich EJ. Causes of death in adults with acute leukemia. Medicine (Baltimore). 1976;55:259–68.
36. Andrews T, Sullivan KE. Infections in patients with inherited defects in phagocytic function. Clin Microbiol Rev. 2003;16:597–621.
37. Hamilton JR, Overall JC. Synergistic infection with murine cytomegalovirus and *Pseudomonas aeruginosa* in mice. J Infect Dis. 1978;137:775–82.
38. Falagas ME, Snydman DR, Griffith J, Werner BG. Exposure to cytomegalovirus from the donated organ is a risk factor for bacteremia in orthotopic liver transplant recipients. Clin Infect Dis. 1996. https://doi.org/10.1093/clinids/23.3.468.
39. Kalil AC, Levitsky J, Lyden E, Stoner J, Freifeld AG. Meta-analysis: the efficacy of strategies to prevent organ disease by cytomegalovirus in solid organ transplant recipients. Ann Intern Med. 2005;143:870–80.
40. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, Humar A, The Transplantation Society International CMV Consensus Group. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102:900–31.
41. Hakki M, Limaye AP, Kim HW, Kirby KA, Corey L, Boeckh M. Invasive *Pseudomonas aeruginosa* infections: high rate of recurrence and mortality after hematopoietic cell transplantation. Bone Marrow Transplant. 2007;39:687–93.
42. Satlin MJ, Walsh TJ. Multidrug-resistant Enterobacteriaceae, *Pseudomonas aeruginosa*, and vancomycin-resistant *Enterococcus*: three major threats to hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2017;19:e12762.
43. Mikulska M, Del Bono V, Raiola AM, Bruno B, Gualandi F, Occhini D, di Grazia C, Frassoni F, Bacigalupo A, Viscoli C. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of gram-negative rods and increasing antibiotic resistance. Biol Blood Marrow Transplant. 2009;15:47–53.
44. Macesic N, Morrissey CO, Cheng AC, Spencer A, Peleg AY. Changing microbial epidemiology in hematopoietic stem cell transplant recipients: increasing resistance over a 9-year period. Transpl Infect Dis. 2014. https://doi.org/10.1111/tid.12298.
45. Patriarca F, Cigana C, Massimo D, et al. Risk factors and outcomes of infections by multidrug-resistant gram-negative bacteria in patients undergoing hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2017;23:333–9.
46. Chaves L, Tomich LM, Salomão M, et al. High mortality of bloodstream infection outbreak caused by carbapenem-resistant *P. aeruginosa* producing SPM-1 in a bone marrow transplant unit. J Med Microbiol. 2017;66:1722–9.
47. Walters MS, Grass JE, Bulens SN, et al. Carbapenem-resistant *Pseudomonas aeruginosa* at US emerging infections program sites, 2015. Emerg Infect Dis. 2019;25:1281–8.
48. Yan C-H, Wang Y, Mo X-D, et al. Incidence, risk factors, microbiology and outcomes of pre-engraftment bloodstream infection after haploidentical hematopoietic stem cell transplantation and comparison with HLA-identical sibling transplantation. Clin Infect Dis. 2018;67:S162–73.
49. Hakki M, Humphries RM, Hemarajata P, Tallman GB, Shields RK, Mettus RT, Doi Y, Lewis JS. Fluoroquinolone prophylaxis selects for meropenem non-susceptible *Pseudomonas aeruginosa* in patients with hematologic malignancies and hematopoietic-cell transplant recipients. Clin Infect Dis. 2018. https://doi.org/10.1093/cid/ciy825.
50. D'Agata E. *Pseudomonas aeruginosa* and other Pseudomonas species. In: Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 2015. p. 2518–31.e3. Elsevier out of Philadelphia, PA.
51. Cheng MP, Kanjilal S, Paquette K, Issa N, Hammond S, Ho VT, Marty FM. 90-day risk of bloodstream infections with enteric pathogens (BSI-EP) in patients with gastrointestinal graft-versus-host disease (GI-GVHD). Biol Blood Marrow Transplant. 2018;24:S375.

52. Czyzewski K, Styczynski J, Giebel S, et al. Age-dependent determinants of infectious complications profile in children and adults after hematopoietic cell transplantation: lesson from the nationwide study. Ann Hematol. 2019;98:2197–2211.
53. Chan ST, Logan AC. The clinical impact of cytomegalovirus infection following allogeneic hematopoietic cell transplantation: why the quest for meaningful prophylaxis still matters. Blood Rev. 2017;31:173–83.
54. Qu Y, McGiffin DC, Kure CE, Ozcelik B, Thissen H, Fraser JF, Peleg AY. Microbial biofilm formation and migration on ventricular assist device drivelines: implications for infection. J Heart Lung Transplant. 2018;37:S134.
55. Lee J, Zhang L. The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein Cell. 2015;6:26–41.
56. Sifri CD. Healthcare epidemiology: quorum sensing: bacteria talk sense. Clin Infect Dis. 2008;47:1070–6.
57. Decker BK, Palmore TN. The role of water in healthcare-associated infections. Curr Opin Infect Dis. 2013;26:345–51.
58. Kizny Gordon AE, Mathers AJ, Cheong EYL, Gottlieb T, Kotay S, Walker AS, Peto TEA, Crook DW, Stoesser N. The hospital water environment as a reservoir for carbapenem-resistant organisms causing hospital-acquired infections – a systematic review of the literature. Clin Infect Dis. 2017;64:1435–44.
59. Sehulster L, Chinn RYW, CDC, HICPAC. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep. 2003;52:1–42.
60. Suleyman G, Alangaden G, Bardossy AC. The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. Curr Infect Dis Rep. 2018;20:12.
61. Mathers AJ, Vegesana K, German Mesner I, et al. Intensive care unit wastewater interventions to prevent transmission of multispecies *Klebsiella pneumoniae* carbapenemase–producing organisms. Clin Infect Dis. 2018;67:171–8.
62. Fusch C, Pogorzelski D, Main C, Meyer C-L, el Helou S, Mertz D. Self-disinfecting sink drains reduce the *Pseudomonas aeruginosa* bioburden in a neonatal intensive care unit. Acta Paediatr. 2015;104:e344–9.
63. Kossow A, Kampmeier S, Willems S, et al. Control of multidrug-resistant *Pseudomonas aeruginosa* in allogeneic hematopoietic stem cell transplant recipients by a novel bundle including remodeling of sanitary and water supply systems. Clin Infect Dis. 2017;65:935–42.
64. Egli A, Osthoff M, Goldenberger D, Halter J, Schaub S, Steiger J, Weisser M, Frei R. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) directly from positive blood culture flasks allows rapid identification of bloodstream infections in immunosuppressed hosts. Transpl Infect Dis. 2015;17:481–7.
65. Khennouchi NC e H, Loucif L, Boutefnouchet N, Allag H, Rolain J-M. MALDI-TOF MS as a tool to detect a nosocomial outbreak of extended-spectrum-β-lactamase- and armA methyltransferase-producing *Enterobacter cloacae* clinical isolates in Algeria. Antimicrob Agents Chemother. 2015;59:6477.
66. Hill JT, Tran K-DT, Barton KL, Labreche MJ, Sharp SE. Evaluation of the nanosphere verigene BC-GN assay for direct identification of gram-negative bacilli and antibiotic resistance markers from positive blood cultures and potential impact for more-rapid antibiotic interventions. J Clin Microbiol. 2014;52:3805–7.
67. Thalavitiya Acharige MJ, Koshy SS, Koo S. The use of microbial metabolites for the diagnosis of infectious diseases. In: Advanced techniques in diagnostic microbiology. Cham: Springer International Publishing; 2018. p. 261–72.
68. Hauser PM, Bernard T, Greub G, Jaton K, Pagni M, Hafen GM. Microbiota present in cystic fibrosis lungs as revealed by whole genome sequencing. PLoS One. 2014;9:e90934.

69. López-Causapé C, Cabot G, del Barrio-Tofiño E, Oliver A. The versatile mutational resistome of *Pseudomonas aeruginosa*. Front Microbiol. 2018;9:685.
70. Vanegas JM, Cienfuegos AV, Ocampo AM, et al. Similar frequencies of *Pseudomonas aeruginosa* isolates producing KPC and VIM carbapenemases in diverse genetic clones at tertiary-care hospitals in Medellin, Colombia. J Clin Microbiol. 2014;52:3978–86.
71. Gupta V. Metallo beta lactamases in *Pseudomonas aeruginosa* and Acinetobacter species. Expert Opin Investig Drugs. 2008;17:131–43.
72. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. J Clin Microbiol. 2012;50:3773–6.
73. Jacoby GA. AmpC B-lactamases. Clin Microbiol Rev. 2009;22:161–82.
74. Kong K-F, Aguila A, Schneper L, Mathee K. *Pseudomonas aeruginosa* β-lactamase induction requires two permeases, AmpG and AmpP. BMC Microbiol. 2010;10:328.
75. Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. Antimicrob Agents Chemother. 2006;50:1633–41.
76. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. Int J Antimicrob Agents. 2015;45:568–85.
77. Cabot G, Bruchmann S, Mulet X, Zamorano L, Moyà B, Juan C, Haussler S, Oliver A. *Pseudomonas aeruginosa* ceftolozane-tazobactam resistance development requires multiple mutations leading to overexpression and structural modification of AmpC. Antimicrob Agents Chemother. 2014;58:3091–9.
78. Fraile-Ribot PA, Cabot G, Mulet X, Periañez L, Martín-Pena ML, Juan C, Pérez JL, Oliver A. Mechanisms leading to in vivo ceftolozane/tazobactam resistance development during the treatment of infections caused by MDR *Pseudomonas aeruginosa*. J Antimicrob Chemother. 2018;73:658–63.
79. Haidar G, Philips NJ, Shields RK, et al. Ceftolozane-tazobactam for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections: clinical effectiveness and evolution of resistance. Clin Infect Dis. 2017;65:110–20.
80. Winkler ML, Papp-Wallace KM, Hujer AM, Domitrovic TN, Hujer KM, Hurless KN, Tuohy M, Hall G, Bonomo RA. Unexpected challenges in treating multidrug-resistant gram-negative bacteria: resistance to ceftazidime-avibactam in archived isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2015;59:1020–9.
81. Chalhoub H, Sáenz Y, Nichols WW, Tulkens PM, Van Bambeke F. Loss of activity of ceftazidime-avibactam due to MexAB-OprM efflux and overproduction of AmpC cephalosporinase in *Pseudomonas aeruginosa* isolated from patients suffering from cystic fibrosis. Int J Antimicrob Agents. 2018;52:697–701.
82. Sanz-García F, Hernando-Amado S, Martínez JL. Mutation-driven evolution of *Pseudomonas aeruginosa* in the presence of either ceftazidime or ceftazidime-avibactam. Antimicrob Agents Chemother. 2018. https://doi.org/10.1128/AAC.01379-18.
83. Humphries RM, Yang S, Hemarajata P, Ward KW, Hindler JA, Miller SA, Gregson A. First report of ceftazidime-avibactam resistance in a KPC-3-expressing *Klebsiella pneumoniae* isolate. Antimicrob Agents Chemother. 2015;59:6605–7.
84. Goli HR, Nahaei MR, Rezaee MA, Hasani A, Samadi Kafil H, Aghazadeh M, Sheikhalizadeh V. Contribution of mexAB-oprM and mexXY (-oprA) efflux operons in antibiotic resistance of clinical *Pseudomonas aeruginosa* isolates in Tabriz, Iran. Infect Genet Evol. 2016;45:75–82.
85. Dößelmann B, Willmann M, Steglich M, Bunk B, Nübel U, Peter S, Neher RA. Rapid and consistent evolution of colistin resistance in extensively drug-resistant *Pseudomonas aeruginosa* during morbidostat culture. Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.00043-17.
86. Jochumsen N, Marvig RL, Damkiær S, Jensen RL, Paulander W, Molin S, Jelsbak L, Folkesson A. The evolution of antimicrobial peptide resistance in *Pseudomonas aeruginosa* is shaped by strong epistatic interactions. Nat Commun. 2016;7:13002.

87. Phan M-D, Nhu NTK, Achard MES, et al. Modifications in the pmrB gene are the primary mechanism for the development of chromosomally encoded resistance to polymyxins in uropathogenic *Escherichia coli*. J Antimicrob Chemother. 2017;72:2729–36.
88. Liu Y-Y, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16:161–8.
89. Walkty A, Adam H, Baxter M, Denisuik A, Lagace-Wiens P, Karlowsky JA, Hoban DJ, Zhanel GG. In vitro activity of plazomicin against 5,015 gram-negative and gram-positive clinical isolates obtained from patients in Canadian hospitals as part of the CANWARD study, 2011–2012. Antimicrob Agents Chemother. 2014;58:2554–63.
90. Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37:177–92.
91. De Groote VN, Fauvart M, Kint CI, Verstraeten N, Jans A, Cornelis P, Michiels J. *Pseudomonas aeruginosa* fosfomycin resistance mechanisms affect non-inherited fluoroquinolone tolerance. J Med Microbiol. 2011;60:329–36.
92. Lodise TP, Patel N, Kwa A, Graves J, Furuno JP, Graffunder E, Lomaestro B, McGregor JC. Predictors of 30-day mortality among patients with *Pseudomonas aeruginosa* bloodstream infections: impact of delayed appropriate antibiotic selection. Antimicrob Agents Chemother. 2007;51:3510–5.
93. Sawyer RG, Claridge JA, Nathens AB, et al. Trial of short-course antimicrobial therapy for intraabdominal infection. N Engl J Med. 2015;372:1996–2005.
94. Yahav D, Franceschini E, Koppel F, et al. Seven versus 14 days of antibiotic therapy for uncomplicated gram-negative bacteremia: a noninferiority randomized controlled trial. Clin Infect Dis. 2018. https://doi.org/10.1093/cid/ciy1054.
95. Grupper M, Kuti JL, Nicolau DP. Continuous and prolonged intravenous β-lactam dosing: implications for the clinical laboratory. Clin Microbiol Rev. 2016;29:759–72.
96. Lal A, Jaoude P, El-Solh AA. Prolonged versus intermittent infusion of β-lactams for the treatment of nosocomial pneumonia: a meta-analysis. Infect Chemother. 2016;48:81–90.
97. Wenzler E, Fraidenburg DR, Scardina T, Danziger LH. Inhaled antibiotics for gram-negative respiratory infections. Clin Microbiol Rev. 2016;29:581–632.
98. Torres A, Motos A, Battaglini D, Li Bassi G. Inhaled amikacin for severe gram-negative pulmonary infections in the intensive care unit: current status and future prospects. Crit Care. 2018. https://doi.org/10.1186/s13054-018-1958-4.
99. Karaiskos I, Antoniadou A, Giamarellou H. Combination therapy for extensively-drug resistant gram-negative bacteria. Expert Rev Anti-Infect Ther. 2017;15:1123–40.
100. Sorbera M, Chung E, Ho CW, Marzella N. Ceftolozane/tazobactam: a new option in the treatment of complicated gram-negative infections. P T. 2014;39:825–32.
101. Farrell DJ, Flamm RK, Sader HS, Jones RN. Antimicrobial activity of ceftolozane-tazobactam tested against Enterobacteriaceae and *Pseudomonas aeruginosa* with various resistance patterns isolated in U.S. hospitals (2011–2012). Antimicrob Agents Chemother. 2013;57:6305–10.
102. Zhanel GG, Chung P, Adam H, et al. Ceftolozane/tazobactam: a novel cephalosporin/-β-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. Drugs. 2014;74:31–51.
103. Liscio JL, Mahoney MV, Hirsch EB. Ceftolozane/tazobactam and ceftazidime/avibactam: two novel β-lactam/β-lactamase inhibitor combination agents for the treatment of resistant gram-negative bacterial infections. Int J Antimicrob Agents. 2015;46:266–71.
104. Sader HS, Castanheira M, Shortridge D, Mendes RE, Flamm RK. Antimicrobial activity of ceftazidime-avibactam tested against multidrug-resistant Enterobacteriaceae and *Pseudomonas aeruginosa* isolates from U.S. Medical Centers, 2013 to 2016. Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.01045-17.
105. Torres A, Zhong N, Pachl J, et al. Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomised, double-blind, phase 3 non-inferiority trial. Lancet Infect Dis. 2018;18:285–95.

106. Stone GG, Newell P, Gasink LB, Broadhurst H, Wardman A, Yates K, Chen Z, Song J, Chow JW. Clinical activity of ceftazidime/avibactam against MDR Enterobacteriaceae and *Pseudomonas aeruginosa*: pooled data from the ceftazidime/avibactam phase III clinical trial programme. J Antimicrob Chemother. 2018;73:2519–23.
107. Ito A, Sato T, Ota M, et al. In vitro antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against gram-negative bacteria. Antimicrob Agents Chemother. 2018. https://doi.org/10.1128/AAC.01454-17.
108. Portsmouth S, van Veenhuyzen D, Echols R, Machida M, Ferreira JCA, Ariyasu M, Tenke P, Den Nagata T. Cefiderocol versus imipenem-cilastatin for the treatment of complicated urinary tract infections caused by gram-negative uropathogens: a phase 2, randomised, double-blind, non-inferiority trial. Lancet Infect Dis. 2018;18:1319–28.
109. Loutit J, Fusaro K, Zhang S, Morgan E, Alexander E, Griffith D, Lomovskaya O, Dudley MN. Meropenem-vaborbactam (M-V) compared with Piperacillin-tazobactam (P-T) in the treatment of adults with complicated urinary tract infections (cUTI), including acute pyelonephritis (AP) in a phase 3 randomized, double-blind, double-dummy trial (TANGO 1). Open Forum Infect Dis. 2016. https://doi.org/10.1093/ofid/ofw195.07.
110. Lapuebla A, Abdallah M, Olafisoye O, Cortes C, Urban C, Quale J, Landman D. Activity of meropenem combined with RPX7009, a novel β-lactamase inhibitor, against gram-negative clinical isolates in New York city. Antimicrob Agents Chemother. 2015;59:4856–60.
111. Castanheira M, Huband MD, Mendes RE, Flamm RK. Meropenem-vaborbactam tested against contemporary gram-negative isolates collected worldwide during 2014, including carbapenem-resistant, KPC-producing, multidrug-resistant, and extensively drug-resistant Enterobacteriaceae. Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.00567-17.
112. Zhanel GG, Lawrence CK, Adam H, et al. Imipenem–relebactam and meropenem–vaborbactam: two novel carbapenem-β-lactamase inhibitor combinations. Drugs. 2018;78:65–98.
113. Lob SH, Hackel MA, Kazmierczak KM, Young K, Motyl MR, Karlowsky JA, Sahm DF. In vitro activity of imipenem-relebactam against gram-negative ESKAPE pathogens isolated by clinical laboratories in the United States in 2015 (results from the SMART global surveillance program). Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.02209-16.
114. Castanheira M, Deshpande LM, Woosley LN, Serio AW, Krause KM, Flamm RK. Activity of plazomicin compared with other aminoglycosides against isolates from European and adjacent countries, including Enterobacteriaceae molecularly characterized for aminoglycoside-modifying enzymes and other resistance mechanisms. J Antimicrob Chemother. 2018;73:3346–54.
115. Wagenlehner FME, Cloutier DJ, Komirenko AS, et al. Once-daily plazomicin for complicated urinary tract infections. N Engl J Med. 2019;380:729–40.
116. Karlowsky JA, Kazmierczak KM, de Jonge BLM, Hackel MA, Sahm DF, Bradford PA. In vitro activity of aztreonam-avibactam against Enterobacteriaceae and *Pseudomonas aeruginosa* isolated by clinical laboratories in 40 countries from 2012 to 2015. Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.00472-17.
117. Wang X, Zhang F, Zhao C, et al. In vitro activities of ceftazidime-avibactam and aztreonam-avibactam against 372 gram-negative bacilli collected in 2011 and 2012 from 11 teaching hospitals in China. Antimicrob Agents Chemother. 2014;58:1774–8.
118. Walsh CC, McIntosh MP, Peleg AY, Kirkpatrick CM, Bergen PJ. In vitro pharmacodynamics of fosfomycin against clinical isolates of *Pseudomonas aeruginosa*. J Antimicrob Chemother. 2015;70:3042–50.

Management of Acinetobacter Infections in the Immunosuppressed Host

16

Emily Baneman and Meenakshi M. Rana

Contents

Introduction 371
Disease Epidemiology 372
Clinical Impact in Transplant 374
Diagnosis 376
Treatment 377
Carbapenems 377
Polymyxins 379
Sulbactam 380
Tetracyclines and Glycylcyclines 380
Combination Therapy 381
Cefiderocol 382
Infection Prevention 382
Conclusion 383
Key Points 383
References 384

Introduction

Acinetobacter species are an important cause of infection in transplant recipients. *Acinetobacter* infections most often occur in the setting of hospitalization, particularly critical illness and prolonged mechanical ventilatory support. They are notoriously challenging to treat due to intrinsic and acquired resistance to multiple classes of antibiotics. However, with appropriate therapy guided by knowledge of local antimicrobial susceptibilities, successful treatment is possible even in immunocompromised patients.

E. Baneman · M. M. Rana (✉)
Icahn School of Medicine at Mount Sinai, New York, NY, USA
e-mail: emily.baneman@mountsinai.org; meenakshi.rana@mssm.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_21

Disease Epidemiology

Acinetobacter species are aerobic, non-lactose fermenting, catalase-positive, and oxidase-negative gram-negative coccobacilli that have emerged as an important cause of healthcare-associated infections. The genus encompasses more than 50 distinct species that are ubiquitous in the environment, though only a minority of species cause disease in humans [1]. *Acinetobacter baumannii (A. baumannii)* is the major pathogen causing human disease. It is found in soil, vegetables, fish, and meat, but unlike some other species, it is not ubiquitous in the environment, and asymptomatic carriage in the community is uncommon. Rather, it is predominantly associated with infection and epidemic spread in hospitals [2]. *A. pittii*, *A. nosocomialis*, *A. lwoffi*, *A. radioresistans*, *A. ursingii*, and other species can also cause disease in humans, including immunocompromised hosts [3]. *A. baumannii*, *A. calcoaceticus*, *A. pittii*, and *A. nosocomialis* are grouped together into the *A. calcoaceticus-A. baumannii* complex because they are difficult to distinguish by most laboratory methods due to their high degree of phenotypic and genotypic similarity. Unlike the other members of this group, *A. calcoaceticus* is predominantly an environmental organism and rarely causes human disease. *A. seifertii*, the newest addition to this complex, is an emerging pathogen in hospitals [4]. Selected species and their sources are outlined in Table 1.

A. baumannii primarily causes nosocomial infections, particularly hospital-acquired pneumonia and bloodstream infections. It also causes urinary tract infections, skin and soft tissue infections, and neurosurgical infections. Community-acquired infections with *A. baumannii* can occur as well, most commonly in tropical regions with high humidity, and it has caused skin and soft tissue infections in the setting of trauma during warfare and natural disasters. *A. baumannii* accounts for

Table 1 Selected *Acinetobacter* species found in human specimens

Species	Habitat or source
A. baumannii	Humans
A. bereziniae	Human skin, sewage, vegetables, meat, animals
A. calcoaceticus	Soil, water, vegetables, animals, human skin
A. guillouiae	Water, vegetables, human skin
A. haemolyticus	Human skin, water
A. johnsonii	Human skin, water, sewage, animals
A. junii	Human skin, water, sewage, animals, soil
A. lwoffii	Human skin, water, sewage, sea food, animals, vegetables
A. nosocomialis	Human skin, sewage, vegetables
A. pittii	Human skin, sewage, soil, water, vegetables, animals, cheese, meat
A. radioresistens	Human skin, soil, water, animals
A. seifertii	Human skin
A. schindleri	Animals, humans
A. ursingii	Humans

Adapted from [5]

approximately 3–7% of healthcare-associated pneumonia [6, 7], though there is widespread geographic variation and the reported incidence is as low as 2% [8] or as high as 36% of hospital-acquired pneumonia [9]. It is a member of the group of "ESKAPE" pathogens that commonly cause nosocomial and multidrug-resistant infections, along with *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* species [10]. Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been on the rise in recent years. In data from the US National Healthcare Safety Network (NHSN) from 2011 to 2014, 56–64% of *Acinetobacter* isolates from ventilator-associated pneumonias and 47–57% of isolates from central line-associated bloodstream infections were carbapenem-resistant [11]. The prevalence of CRAB is highest in Southern Europe, the Middle East, Asia, and South America [12].

Infection typically occurs in patients with prolonged hospitalization and critical illness, frequently occurring in the intensive care unit. Risk factors for infection with *A. baumannii* include underlying illness severity, mechanical ventilation, prior residence in long-term care facilities, central venous catheterization, recent invasive procedures, and treatment with broad-spectrum antimicrobials [13]. *A. baumannii* is linked to overall high rates of mortality, particularly in patients with bacteremic pneumonia and especially with those isolates found to be multidrug resistant. But given that these infections typically occur in patients with severe underlying illness, the degree of attributable mortality is less clear [2].

The hospital environment is an important reservoir of infection, as *A. baumannii* can survive for several weeks on equipment and surfaces, including tables, sinks, humidifiers, pillows, curtains, bed rails, supply carts, ventilators, and infusion pumps [14]. It can also form biofilm on endotracheal tubing, causing both colonization and infection in the lungs of intubated patients [15]. Patients colonized or infected with MDR *Acinetobacter* can easily contaminate their environment. However, the most common method of transmission is by the hands of healthcare workers [2]. Disinfection of patient care areas and hand hygiene are essential to prevent spread between patients. Due to its ability to persist in the hospital environment, *A. baumannii* frequently causes outbreaks in healthcare facilities [16].

A. baumannii displays intrinsic resistance to penicillins and first- and second-generation cephalosporins and can rapidly acquire resistance to fourth-generation cephalosporins, extended-spectrum penicillins, carbapenems, aminoglycosides, tetracyclines, and fluoroquinolones. Multiple resistance mechanisms can be involved, including β-lactamases, cephalosporinases, carbapenemases, efflux pumps, and porin mutations. Mechanisms of carbapenem resistance are most commonly due to class D oxacilinases (OXA) and class B metallo-β-lactamases, which can spread via plasmid-mediated transfer and have become widely disseminated in many countries [12]. Common mechanisms of resistance are described in Table 2.

The prevalence of multidrug-resistant (MDR) *Acinetobacter* species has been rising since the late 1980s, particularly among critically ill patients. MDR infections with *Acinetobacter* are defined as being resistant to one agent in three different

Table 2 Common resistance mechanisms of *A. baumannii* [17, 18]

Resistance to beta-lactams	• Hydrolysis by beta-lactamases, including class A, class B, class C, and class D • Changes in penicillin-binding proteins • Alterations in the structure and number of porin proteins, resulting in decreased permeability to antibiotics • Activity/overexpression of efflux pumps
Resistance to aminoglycosides	• Aminoglycoside-modifying enzymes
Resistance to quinolones	• Modification in structure of DNA gyrase, resulting in lower affinity of quinolone binding • Activity of efflux pump
Resistance to tetracyclines	• Transposon-mediated efflux pumps (tet A and tet B) • Ribosomal protection protein (shields ribosome from action of tetracycline)
Resistance to polymyxins	• Modification in the lipopolysaccharide that prevents binding of antibiotic to cell membrane

classes of antimicrobials [19]. Accordingly, the CDC has labeled MDR *Acinetobacter* as a serious threat, and the World Health Organization (WHO) has designated *Acinetobacter* as a critical priority organism for research and development of new antibiotics for resistant bacteria [20, 21].

Clinical Impact in Transplant

Acinetobacter infections in solid organ transplant recipients are frequently multidrug- resistant and associated with a high rate of mortality. Infection typically occurs greater than 48 hours after hospitalization, particularly in patients with ICU admission and prolonged mechanical ventilation. Timing of onset posttransplant can vary but is most often early (less than 6 months) after transplantation [22]. Overall 30-day mortality in solid organ transplant recipients with *Acinetobacter* infection ranges from 24% to 65% [22–26], with mortality of up to 91% reported for extremely drug-resistant (XDR) *A. baumannii* infections [27]. XDR *Acinetobacter* is defined as non-susceptibility to at least one agent in all but one or two antimicrobial categories. Delay in appropriate antibiotic therapy is a major contributor to mortality [22, 25, 28], and MDR or XDR infections are associated with higher mortality in some studies [22]. Polymicrobial infections are common, including infection with other multidrug-resistant organisms [24].

Similar to non-transplant recipients, risk factors for *Acinetobacter* infection in transplant recipients include prolonged hospital and ICU admission, mechanical ventilation, central venous catheterization, exposure to broad-spectrum antibiotics, higher illness severity, and invasive procedures [29]. In one single-center study, cardiothoracic transplant recipients were at higher risk than abdominal transplant recipients for *Acinetobacter* infection, particularly for extensively drug-resistant infection [24]. Receiving high-dose corticosteroids may also be a

risk factor [23]. The respiratory tract is the most common site of infection. Other typical sites of infection include bloodstream infection, including central venous catheter-associated infections, urinary tract infection, skin and soft tissue infection, and intra-abdominal infection in abdominal organ recipients [30]. There have also been reports of less common sites of infection including mediastinitis [24, 31], renal abscess [32], endocarditis [33], and necrotizing fasciitis [34]. Risk factors for multi or extensively drug-resistant infection include prolonged use of broad-spectrum antibiotics, particularly carbapenems, and prolonged endotracheal intubation [22, 35]. Higher mortality from *Acinetobacter*-related sepsis has been associated with use of expanded criteria donors and anti-thymocyte globulin [36].

Lung transplant recipients are especially impacted by *Acinetobacter* when it colonizes the respiratory tree. Low rates of microbiologic cure and increased mortality after lung transplant in patients with MDR *A. baumannii* infection have been reported. In one series of six lung transplant recipients with *A. baumannii* pneumonia, four of the six patients failed to clear the infection and ultimately died, despite extended courses of antibiotics and reduction of immunosuppression [37]. In another retrospective study of lung transplant recipients, those infected with *Acinetobacter baumannii* had a lower probability of survival compared to patients with other types of bacterial infection, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *and Stenotrophomonas maltophilia* [38]. Donor-derived *A. baumannii* infections have been associated with prolonged ICU stay and mechanical ventilation [39].

Abdominal organ transplant recipients are also susceptible to *Acinetobacter* and have high rates of MDR infection. One series of 33 abdominal organ transplant recipients with *A. baumannii* infection found that 79% of patients had healthcare-associated infection and 82% had received antibiotics within the preceding 2 weeks. The most common sites of infection were the respiratory tract, blood, urine, and peritoneum. MDR or XDR isolates were predominant, occurring in 85% of cases in a single-center study from North America [22]. In another series of living-related liver transplant recipients, CRAB bacteremia was associated with 50% mortality, which was significantly higher than non-bacteremic CRAB infections and bacteremia due to other organisms [30]. Donor infection with MDR *Acinetobacter* may be considered a relative contraindication to transplant, but this is controversial. Furthermore, donor culture data are frequently not available at the time of transplantation. There have been reports of successful transplantation from organ donors infected with *Acinetobacter* infection, including CRAB infections, when recipients receive appropriate antibiotics within 7 days of transplant [40, 41]. Consequently, we recommend a case-by-case approach, weighing the recognized detrimental impact of *A. baumannii* donor colonization, against the urgency to move to transplant in the intended recipient.

In hematopoietic cell transplant recipients, *A. baumannii* is an uncommon cause of infection. *Acinetobacter* species represent only 1–3% of bloodstream infections in this population [42, 43]; however, once again, multidrug resistance is common, and associated mortality is high. In one South Korean study of 19

hematopoietic stem cell transplant (HSCT) recipients with CRAB bacteremic pneumonia, infection occurred post-engraftment and was associated with longer hospital stay prior to HSCT and ICU level care. Death occurred in 18/19 (95%) of cases [44].

Diagnosis

Ventilator-associated pneumonia and bloodstream infections are the most common clinical presentations of *Acinetobacter* infection. *A. baumannii* can colonize the respiratory tract, gastrointestinal tract, and wound sites, and its presence must be interpreted together with clinical signs to determine whether growth in culture represents colonization or infection. Infection often occurs in patients previously colonized with *A. baumannii*, making it essential to interpret culture results in clinical context. As with other causes of VAP, typical signs include fever, tachypnea, purulent secretions, hypoxia, increased mechanical ventilatory support, and new or worsening infiltrate on chest imaging. Other *Acinetobacter* species are most commonly found in catheter-associated bloodstream infections and have a more benign clinical course compared to *A. baumannii* [13]. For example, in one series of *Acinetobacter* bloodstream infections, *A. baumannii* carried significantly higher mortality than *A. nosocomialis* or *A. pittii* (37% vs 16% and 14%, respectively) [45].

Acinetobacter can be isolated in culture specimens from sputum, blood, cerebrospinal fluid, and other sites and grows readily on standard culture media. It should be suspected when non-fermenting catalase-positive, oxidase-negative gram-negative coccobacilli are seen in the right clinical setting. *Acinetobacter* species are difficult to decolorize and may incorrectly be identified as gram positive. Phenotypic tests can distinguish many species of *Acinetobacter*, but they cannot identify more recently described genomic species and cannot distinguish between closely related members of the *A. calcoaceticus-A. baumannii* complex. Because of their high degree of similarity, *A. pittii* and *A. nosocomialis* are frequently misidentified as *A. baumannii* in clinical laboratories [1]. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) can identify most clinically relevant *Acinetobacter* species accurately since the database was recently expanded [46]. However, MALDI-TOF MS is inadequate to distinguish between different clones of *A. baumannii*, which can be done using pulsed-field gel electrophoresis, amplified fragment length polymorphism analysis, multilocus sequence typing, and other PCR-based methods [47]. In addition, *A. baumannii* has an intrinsic class D OXA-51-like carbapenemase that can be used as a marker for species identification [48]. Real-time PCR of OXA-51 can be used to monitor treatment response, but this requires further study and is likely cost prohibitive for many regions of the world [49, 50]. Antibiotic susceptibility testing should include third- or fourth-generation cephalosporins, beta-lactamase inhibitors, and carbapenems and may also include tetracyclines, glycylcyclines, polymyxins, aminoglycosides, and fluoroquinolones.

Susceptibility testing must be done for each carbapenem individually, as susceptibility to one does not necessarily confer susceptibility to other members of the class. Multiplex PCR for carbapenemase genes has shown promise for rapid detection of carbapenem resistance and tracking spread of carbapenem-resistant strains in an outbreak setting [49]. However, this approach does not encompass all known carbapenem resistance mechanisms and consequently would not capture all CRAB strains.

Treatment

Multiple classes of antimicrobial agents are considered effective against *Acinetobacter* when susceptible. They include beta-lactam/beta-lactamase inhibitor combinations including sulbactam and piperacillin-tazobactam, broad-spectrum cephalosporins, carbapenems, aminoglycosides, quinolones, polymyxins, and tetracyclines and glycylcyclines [18]. However, most *Acinetobacter* strains have the ability to evade treatment with antimicrobials because of intrinsic resistance and/or acquired resistance, resulting in MDR infections and giving *Acinetobacter* its reputation as a difficult-to-treat infection [18].

The most commonly used antimicrobials used to treat multidrug-resistant *Acinetobacter* are discussed below (Table 3). Evidence regarding these agents is limited to mostly retrospective cohort or non-randomized small prospective studies [19]. Additional potential options include fluoroquinolones and aminoglycosides, but neither should be considered as preferred empiric options given high rates of resistance to these classes, in N. America and beyond. In addition, aminoglycosides should not be given as monotherapy and are mostly used as combination therapy due to the rapid acquisition of resistance.

Carbapenems

Carbapenems are generally considered the treatment of choice for infections caused by *Acinetobacter* when susceptible, due to intrinsic and often upregulated expression of chromosomally encoded AmpC cephalosporinases and a favorable safety profile [51, 52]. Discordance between various carbapenems has been reported; however, imipenem, meropenem, and doripenem all have activity. Ertapenem on the other hand is not reliably active, and there is limited data available for oral carbapenems outside the United States. Susceptibility to one carbapenem does not necessarily predict susceptibility to another [53].

Carbapenem resistance in *Acinetobacter* is increasing and is mediated by both plasmid and chromosomally encoded carbapenemases [54]. These include naturally producing oxacillinases (OXA) such as OXA-23, OXA-24 or 40, OXA 51, OXA-58, and OXA 143, which are class D beta-lactamases that are responsible for the majority of carbapenem resistance around the world [1]. Carbapenem-resistance in

Table 3 Currently approved treatment options for MDR *Acinetobacter*

Treatment	Recommended adult dosing (nl CrCl)	Adverse effects	Comments
Carbapenems	Meropenem 1gm IV q8 Imipenem 500 mg IV q6	Rash GI disturbance CNS toxicity/ seizure	• Drug of choice • Can see discordance between various carbapenems
Polymyxins	Colistin 5 mg/kg/day IV or polymyxin B 2.5 mg/kg/day IV	Nephrotoxicity Neurotoxicity	• Requires complex PK/PD dosing with a loading dose • Colistin requires prodrug conversion and is preferred agent for inhalation and for UTI
Sulbactams	6 to 9 grams a day	Rash/ hypersensitivity reaction	Usually given as ampicillin-sulbactam or cefoperazone/ Sulbactam
Tetracyclines	Minocycline 200 mg x 1 followed by 100 mg q12	Neurologic (dizziness) GI disturbance Tooth discoloration	Available in both IV and PO formulations
	Eravacycline 1 mg/kg IV q12	Infusion site reactions Nausea/ vomiting/ diarrhea Tooth discoloration	FDA approved for intra-abdominal infections
Glycylcyclines	Tigecycline 100 mg x 1 followed by 50 mg q12	Nausea/ vomiting	Limited by ability to achieve high serum levels and not recommended for bacteremia

Acinetobacter may also be due to the presence of class B beta-lactamases or metallo-beta-lactamases; these isolates may have had a role in transmission of the *bla* $_{NDM}$ gene to other *Enterobacteriaceae* [1].

Most carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates are usually not resistant to just carbapenems but other commonly used antibiotic classes as well, retaining susceptibility to only tigecycline, polymyxins, and sometimes aminoglycosides. Other mechanisms of resistance that are commonly expressed include efflux pump mechanisms, penicillin-binding protein alterations, and modification of outer membrane proteins or porins [18]. More than one of these resistance mechanisms is typically present, leaving a paucity of active drug options [54]. Infections with CRAB have been increasing, and nearly half of all clinical cultures with Acinetobacter in selected metropolitan regions in the United States were reported as carbapenem non-susceptible, with a crude annual incidence of 1.2 cases/100,000 persons [55]. Worldwide, the proportion of XDR *Acinetobacter* strains has increased from less than 4% in 2000 to greater than 60%. In general these strains are associated with longer hospitalizations,

increased cost, and greater mortality than those caused by carbapenem-susceptible strains [1].

Polymyxins

Polymyxins are cationic polypeptides that interact with the lipid A of the outer membrane of gram-negative bacteria and have been brought back into use because of their activity against multidrug-resistant gram-negative infections, including CRAB [51, 56, 57]. Polymyxins are available in two forms, polymyxin B and colistin (polymyxin E); both are bactericidal and differ only by one amino acid in structure. Colistin, however, is typically administered as its inactive prodrug colistin methanesulfonate (CMS) and undergoes conversion to active drug in plasma, requiring a large loading dose in order to achieve therapeutic concentrations. Polymyxin B is administered as an active drug and is associated with less nephrotoxicity than colistin [58]. Neurotoxicity is also less common with more recent formulations. Both colistin and polymyxin B achieve relatively poor concentrations in the lung, bone, and CSF. Colistin is preferred for urinary tract infections. While resistance against polymyxins remains relatively rare, a surveillance study of US hospitals found that about 5% of all *Acinetobacter* strains were resistant to colistin [59, 60].

A small prospective study evaluated the efficacy and toxicity of intravenous colistin in ventilator-associated pneumonia (VAP) and found no difference in inhospital mortality when comparing patients treated with colistin and patients treated with imipenem [61]. Similar findings were reported in a case-control study comparing patients with VAP secondary to MDR *Acinetobacter* or *Pseudomonas* treated with imipenem versus colistin [95].

Because of concerns over nephrotoxicity and poor penetration into lung tissue, directly administered CMS to the lungs has also been utilized for treatment of pneumonia. Inhaled CMS for the treatment of MDR *Acinetobacter* VAP has been reported in small prospective studies as a safe and effective option [62, 63]. Nebulized CMS can be used either alone or in conjunction with intravenous colistin for the treatment of VAP. In a randomized controlled study of a hundred patients with MDR *Pseudomonas* or *Acinetobacter* VAP, patients who received inhaled CMS in addition to intravenous antibiotics as compared to those who received inhaled normal saline with IV antibiotics had increased rates of microbiological clearance and similar rates of favorable clinical outcome. However, there were more bronchospasm events reported in the group who received inhaled CMS, although not statistically significant [64]. In another case-control study of 80 patients with *Acinetobacter* PNA who received systemic colistin versus 43 patients who received intravenous and inhaled colistin, there were no differences in clinical and microbiological outcomes [65].

Colistin has a low penetration into the CSF, and therefore intravenous colistin is not effective for Acinetobacter meningitis or CNS infections. In limited case series, intrathecal or intraventricular colistin has been used for the management of MDR

Acinetobacter meningitis or ventriculitis, often in conjunction with IV colistin when limited systemic drug therapeutic options exist [66, 67]. Rarely, meningeal irritation has been reported in these cases.

Sulbactam

Of the beta-lactamase inhibitors, sulbactam has the greatest intrinsic bactericidal activity against *Acinetobacter*. Commercially available as ampicillin-sulbactam in the United States, it has been suggested that anywhere from 6 grams to 9 grams of high-dose sulbactam may be effective against *Acinetobacter* infections, although the optimal dose is unknown [56]. In a small prospective study of 28 critically ill patients with MDR *Acinetobacter* VAP, there were no differences in clinical or microbiological outcome in patients treated with sulbactam versus colistin [68]. In a small retrospective cohort study, critically ill trauma patients with *Acinetobacter* VAP were treated with imipenem or ampicillin-sulbactam; there were no differences in regard to associated mortality, duration of mechanical ventilation, or length of stay in the ICU or hospital [69]. In certain locations worldwide, sulbactam is also available as combination cefoperazone/sulbactam; in a recent study in China, patients treated with cefoperazone/sulbactam had lower 28-day mortality rates than those receiving tigecycline for MDR infections [70].

Recent data suggests that higher doses of sulbactam should be considered when treating *Acinetobacter* infections. In an observational study of 58 patients with CRAB VAP, high-dose sulbactam at 8 grams a day was used and shown to be effective with a 30-day mortality of 29% [71]. In a meta-analysis of 12 observational trials involving 1500 patients, those treated with higher doses of sulbactam of greater than 9gm/day had significantly improved clinical response [72]. Sulbactam remains a safe and effective therapeutic option against *Acinetobacter* when susceptible [52].

Tetracyclines and Glycylcyclines

Minocycline and doxycycline have been shown to have activity against *Acinetobacter*. In isolates resistant to tetracycline or doxycycline, minocycline activity may be preserved given that the tet(A) resistance determinant that codes for a tetracycline-specific efflux pump does not confer resistance to minocycline. Minocycline may also retain activity against tigecycline-resistant strains and had activity against 79% and 84% of MDR *Acinetobacter* isolates in two large antimicrobial surveillance studies [73]. Due to its effective penetration into lung tissue, IV minocycline remains an option for treatment of pneumonia with MDR *Acinetobacter* and is FDA approved for this indication [74]. The breakpoint is not well validated based on clinical outcome data, and in the setting of an MIC of 4 μg/mL or greater, caution may be warranted in using minocycline as monotherapy or with bacteremia

[1]. In a small observational study, a total of 53 patients were treated with minocycline, and although the majority received combination therapy, 73% of patients had a successful clinical outcome [75].

Glycylcyclines are related to tetracyclines and include tigecycline, a semisynthetic derivative of minocycline which inhibits protein synthesis. Tigecycline is able to evade common mechanisms of resistance to tetracyclines in *Acinetobacter*, including efflux pumps coded by tet (A-E) and tet (K) resistance determinants and tet (O) and tet (M) determinants that provide ribosomal protection [18, 56]. There is no CLSI breakpoint for *Acinetobacter*, but usually an MIC less than 2 μg/mL is considered susceptible.

However, tigecycline is limited by its ability to achieve high serum levels and therefore should not be routinely used in patients with bacteremia. In a meta-analysis that included 7 controlled and 17 single-arm studies, patients treated with tigecycline had higher inhospital mortality rates, a lower microbial eradication rate, and a trend toward longer hospital stays [76]. Given these finding and limited ability to achieve serum levels, tigecycline should be used with caution, especially in the treatment of bacteremia or VAP [1, 53]. Other pooled analyses indicate that cure rates may be better in intra-abdominal infections and skin and soft-tissue infections [52, 77]. Given this data, a high-dose regimen (200 mg/day) or combination therapy can be considered when using tigecycline to treat MDR *Acinetobacter* infections [78].

Eravacycline, a new antimicrobial in the tetracycline class, was recently approved by the FDA in 2018 for the treatment of intra-abdominal infections and is generally better tolerated than tigecycline, making it an attractive option. Eravacycline has a broad spectrum of activity, including MDROs such as MRSA, VRE, carbapenem-resistant *Enterobacteriaceae*, and *Acinetobacter*. It has demonstrated activity against a large number of *Acinetobacter* isolates, including those with varying patterns of carbapanem resistance, fluoroquinolone, and aminoglycoside resistance and even those with reduced susceptibility to tigecycline [79]. Eravacycline MICs have been found to be two- to eightfold lower than tigecycline MICs against CRAB, but at this time, clinical outcomes are lacking and will likely emerge over the next few years [80].

Combination Therapy

Given *Acinetobacter's* ability to acquire multidrug resistance through various mechanisms, combination therapy is theoretically an attractive treatment option for these infections and is frequently used to treat MDR *Acinetobacter* infections. However, at this time no clear evidence exists to support its approach over monotherapy [81, 78]. Two open-label randomized controlled trials in patients with CRAB infection compared the use of colistin alone to colistin plus rifampin and found no difference in mortality or clinical response, although microbiologic clearance improved with combination therapy [82, 83]. The use of colistin with fosfomycin when compared to

colistin alone for the treatment of patients with CRAB infections had no effect on clinical outcome or mortality [84].

A retrospective single-center study of combination intravenous colistin plus carbapenem treatment of 69 solid organ transplant recipients with XDR *Acinetobacter* infection suggested improved outcomes. In this study, XDR *Acinetobacter* infections were more common among heart and lung transplant recipients with a median time to infection of 172 days after transplant, and the most common site of infection was respiratory. Treatment with a colistin-carbapenem combination was independently associated with survival at 28 days [24].

However, a recent randomized controlled trial in Europe that enrolled 406 patients and compared colistin alone to colistin and meropenem for MDR gram-negative rod infections, including CRAB, showed no difference in clinical outcomes. Immunocompromised patients were included in each arm, including patients on immunosuppression and with malignancy; more than three-fourths of enrolled patients had an infection with *Acinetobacter*. Treatment with colistin monotherapy compared to treatment with colistin and meropenem resulted in no change in clinical outcomes, which was a composite outcome of survival, microbiological cure, hemodynamic stability, improved oxygenation, and severity of illness [85].

Further clinical trials with larger patient populations are needed to evaluate the use of combination therapy in the setting of MDR *Acinetobacter* infections, including close monitoring of polymyxin doses and therapeutic drug monitoring.

Cefiderocol

Cefiderocol is a newly developed cephalosporin, conjugated with a siderophore on its side chain with stability against multiple beta-lactamases providing activity for MDR gram-negative isolates, including CRAB [86]. In vitro studies have demonstrated activity against OXA-23, OXA-40, and OXA-58 isolates as well as NDM- and IMP-producing *Acinetobacter baumannii* isolates. In clinical studies, cefiderocol was found to be superior to imipenem for the treatment of complicated UTIs in a phase III RCT, but no CRAB isolates were included. Ongoing clinical trials are currently recruiting patients for the treatment of other severe infections including bloodstream infections and VAP/HAP and will likely include MDR *Acinetobacter* infections [80]. At least in the United States, FDA approval was obtained in 2019 for the treatment of complicated UTIs.

Infection Prevention

Acinetobacter is a ubiquitous pathogen with an ability to survive for prolonged periods in the environment, contributing to its ability to cause horizontal transmission resulting in infections and outbreaks. Consequently, it is imperative to consider the hospital environment and infection control options. A previous study replicating hospital conditions has shown that *Acinetobacter* could survive for an average of 20 days at a relative humidity of 31% and be isolated from a hospital bed rail 9 days

after discharge of an infected patient [87, 88]. The hospital environment and equipment can therefore serve as a secondary reservoir for *Acinetobacter*, contributing to transmission.

The majority of these outbreaks occur in intensive care units, frequently in patients who require mechanical ventilation. On some occasions, contaminated hospital equipment or shared equipment is identified and may often include a component of the ventilator circuit [87, 89]. Various infection prevention measures, often combined together as a bundle, have been effective in controlling these outbreaks. These include hand hygiene, the implementation of contact precautions, patient cohorting, environmental disinfection, and the use of chlorhexidine bathing [90, 91]. In addition, in some outbreak settings, the use of active surveillance has been utilized to rapidly identify colonized patients; whole genome sequencing can be utilized for strain typing and to assist in outbreak investigations [92]. Lastly, in a Taiwanese cohort, the use of antimicrobial stewardship and judicious antimicrobial use have been associated with decreasing MDR *Acinetobacter* rates [93, 94].

Conclusion

Acinetobacter has emerged as a key transplant pathogen, both in solid organ and bone marrow transplant recipients. *Acinetobacter baumannii* is the major pathogen in humans, concentrates itself in hospital environments, especially intensive care settings, and is prone to epidemic spread within healthcare settings. Because the organism has uniquely significant antimicrobial resistance, with multidrug and extremely drug-resistant isolates being increasingly discovered, treatment is complex, and morbidity and mortality are high. Early identification of infected individuals and instigation of appropriate antibiotics remains the mainstay of therapy.

Key Points

- *Acinetobacter* infection is associated with significant morbidity and mortality, especially *A. baumannii*.
- *A. baumannii* is typically very drug resistant, often exhibiting multi-class resistance or XDR characteristics. Consequently, early identification of the organism and associated sensitivity profile is key to minimize unnecessary antibiotic toxicity and for ultimately successful treatment.
- Acinetobacter infections have a predilection for hospitalized patients, especially bacteremia and hospital-acquired or ventilator-acquired pneumonias.
- Multiple strategies for treatment exist, including antibiotics, perhaps in combination, and strict adherence to infection control protocols to prevent ongoing environmental and nosocomial spread.
- *A. baumannii* remains a significant threat in hospitals and to transplanted patients, even despite the recent development of a number of promising and active antibiotics.

References

1. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges. Clin Microbiol Rev. 2017;30(1):409–47.
2. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol. 2007;5:939–51.
3. Phillips M. Acinetobacter species. In: Mandell, Douglas, and Bennett's principles and practice of infectious diseases, updated edition. Philadelphia: Saunders; 2015. p. 2552–8.
4. Cerezales M, Xanthopoulou K, Ertel J, Nemec A, Bustamante Z, Seifert H, et al. Identification of acinetobacter seifertii isolated from bolivian hospitals. J Med Microbiol. 2018;67(6):834–7.
5. Al Atrouni A, Joly-Guillou ML, Hamze M, Kempf M. Reservoirs of non-baumannii Acinetobacter species. Front Microbiol. 2016;7:49.
6. Weinstein RA, Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005;41(6):848–54. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/432803
7. Kollef MH, Shorr A, Tabak YP et al. Epidemiology and Outcomes of Health-care-Associated Pneumonia*. Chest. 2005;128(6):3854–62.
8. Dananché C, Vanhems P, Machut A, Aupée M, Bervas C, L'Hériteau F, et al. Trends of incidence and risk factors of ventilator-associated pneumonia in elderly patients admitted to French ICUs between 2007 and 2014. Crit Care Med. 2018;46(6):869–77.
9. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, et al. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med. 2011;184(12):1409–17.
10. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016;2016:1–8.
11. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. Infect Control Hosp Epidemiol. 2016;37(11):1288–301.
12. Garnacho-Montero J, Timsit JF. Managing Acinetobacter baumannii infections. Curr Opin Infect Dis. 2019;32(1):69–76.
13. Visca P, Seifert H, Towner KJ. Acinetobacter infection - an emerging threat to human health. IUBMB Life. 2011;63:1048–54.
14. Thom KA, Johnson JK, Lee MS, Harris AD. Environmental contamination because of multidrug-resistant Acinetobacter baumannii surrounding colonized or infected patients. Am J Infect Control. 2011;39(9):711–5.
15. Gil-Perotin S, Ramirez P, Marti V, Sahuquillo JM, Gonzalez E, Calleja I, et al. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. Crit Care. 2012;16(3):R93.
16. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis. 2013;26:338–44.
17. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumanni. Antimicrob Agents Chemother. 2007;51(10):3471–84.
18. Karageorgopoulos DE, Falagas ME. Current control and treatment of multidrug-resistant Acinetobacter baumannii infections. Lancet Infect Dis. 2008;8(12):751–62.
19. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.

20. CDC. Antibiotic resistance threats in the United States, 2013 | antibiotic/antimicrobial resistance | CDC. CDC. 2013.
21. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318–27.. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29276051
22. Kitazono H, Rog D, Grim SA, Clark NM, Reid GE. Acinetobacter baumannii infection in solid organ transplant recipients. Clin Transpl. 2015;29(3):227–32.
23. Serifoglu I, Dedekarginoglu BE, Bozbas SS, Akcay S, Haberal M. Clinical characteristics of Acinetobacter baumannii infection in solid-organ transplant recipients. Exp Clin Transplant. 2018;16:171–5.
24. Shields RK, Clancy CJ, Gillis LM, Kwak EJ, Silveira FP, Massih RCA, et al. Epidemiology, clinical characteristics and outcomes of extensively drug-resistant Acinetobacter baumannii infections among solid organ transplant recipients. PLoS One. 2012;7(12):e52349.
25. Kim YJ, Yoon JH, Kim SI, Hong KW, Kim JI, Choi JY, et al. High mortality associated with acinetobacter species infection in liver transplant patients. Transplant Proc. 2011;43(6):2397–9.
26. Trottier V, Namias N, Pust DG, Nuwayhid Z, Manning R, Marttos AC Jr, et al. Outcomes of Acinetobacter baumannii infection in critically ill surgical patients. Surg Infect. 2007;8(4):437–44.
27. Shields RK, Kwak EJ, Potoski BA, Doi Y, Adams-Haduch JM, Silviera FP, et al. High mortality rates among solid organ transplant recipients infected with extensively drug-resistant Acinetobacter baumannii: using in vitro antibiotic combination testing to identify the combination of a carbapenem and colistin as an effective treatment regimen. Diagn Microbiol Infect Dis. 2011;70(2):246–52.
28. de Gouvêa EF, Martins IS, Halpern M, Ferreira ALP, Basto ST, Gonçalves RT, et al. The influence of carbapenem resistance on mortality in solid organ transplant recipients with Acinetobacter baumannii infection. BMC Infect Dis. 2012;12:351.
29. Ellis D, Cohen B, Liu J, Larson E. Risk factors for hospital-acquired antimicrobial-resistant infection caused by Acinetobacter baumannii. Antimicrob Resist Infect Control. 2015;4(1):40.
30. Kim YJ, Kim SI, Lee YD, Choi HJ, Choi JY, Yoon SK, et al. Carbapenem-resistant Acinetobacter baumannii bacteremia in liver transplant recipients. Transplant Proc. 2018;50(4):1132–5.
31. George RS, Birks EJ, Haj-Yahia S, Bowles CT, Hall A, Khaghani A, et al. Acinetobacter Mediastinitis in a heart transplant patient. Ann Thorac Surg. 2006;82(2):715–6.
32. Choe YM, Park KM, Jeon YS, Cho SG, Hong KC, Shin WY, et al. Abscess of native kidney caused by Carbapenem-resistant Acinetobacter baumannii (CRAB) in renal transplantation: a case report. Transplant Proc. 2016;48(3):949–50.
33. Patel G, Perez F, Hujer AM, Rudin SD, Augustine JJ, Jacobs GH, et al. Fulminant endocarditis and disseminated infection caused by carbapenem-resistant Acinetobacter baumannii in a renal-pancreas transplant recipient. Transpl Infect Dis. 2015;17(2):289–96.
34. Clemente WT, Sanches MD, Coutinho RL, De Oliveira Júnior AR, Lauria MW, Lima CX, et al. Multidrug-resistant acinetobacter baumannii causing necrotizing fasciitis in a pancreas-kidney transplant recipient: a case report. Transplantation. 2012;94(6):e37–8.
35. Zhong L, Men TY, Li H, Peng ZH, Gu Y, Ding X, et al. Multidrug-resistant gram-negative bacterial infections after liver transplantation - Spectrum and risk factors. J Infect. 2012;64(3):299–310.
36. Kroth LV, Barreiro FF, Saitovitch D, Traesel MA, d'Avila DOL, Poli-de-Figueiredo CE. Does thymoglobulin induction increase susceptibility to Carbapenem-resistant Acinetobacter baumannii Sepsis-related death in expanded criteria donors? Transplant Proc. 2016;48(7):2294–7.
37. Nunley DR, Bauldoff GS, Mangino JE, Pope-Harman AL. Mortality associated with Acinetobacter baumannii infections experienced by lung transplant recipients. Lung. 2010;188(5):381–5.

38. Wojarski J, Ochman M, Medrala W, Kulaczkowska Z, Karolak W, Maruszewski M, et al. Bacterial infections during hospital stay and their impact on mortality after lung transplantation: a single-center study. Transplant Proc. 2018;50(7):2064–9.
39. Almutairy, Eid, Alshekaily, Naseer, Khan, Basha, Saleh, Waleed, Nizami I. Prevalence and outcome of donor transmitted Acinetobacter baumannii in lung transplant patients in Saudi Arabia. European Respiratory Journal, Annual Congress 2012; 40:P2448.
40. Cohen J, Michowiz R, Ashkenazi T, Pitlik S, Singer P. Successful organ transplantation from donors with Acinetobacter Baumannii septic shock. Transplantation. 2006;81(6):853–5.
41. Mularoni A, Bertani A, Vizzini G, Gona F, Campanella M, Spada M, et al. Outcome of transplantation using organs from donors infected or colonized with Carbapenem-resistant gram-negative Bacteria. Am J Transplant. 2015;15(10):2674–82.
42. Mikulska M, Viscoli C, Orasch C, Livermore DM, Averbuch D, Cordonnier C, et al. Aetiology and resistance in bacteraemias among adult and paediatric haematology and cancer patients. J Infect. 2014;68(4):321–31.
43. Ortega M, Rovira M, Almela M, Marco F, de la Bellacasa JP, Martínez JA, et al. Bacterial and fungal bloodstream isolates from 796 hematopoietic stem cell transplant recipients between 1991 and 2000. Ann Hematol. 2005;84(1):40–6.
44. Kim SB, Min YH, Cheong JW, Kim JS, Kim SJ, Ku NS, et al. Incidence and risk factors for carbapenem-and multidrug-resistant Acinetobacter baumannii bacteremia in hematopoietic stem cell transplantation recipients. Scand J Infect Dis. 2014;46(2):81–8.
45. Wisplinghoff H, Paulus T, Lugenheim M, Stefanik D, Higgins PG, Edmond MB, et al. Nosocomial bloodstream infections due to Acinetobacter baumannii, Acinetobacter pittii and Acinetobacter nosocomialis in the United States. J Infect. 2012;64(3):282–90.
46. Jeong S, Hong JS, Kim JO, Kim KH, Lee W, Bae IK, et al. Identification of acinetobacter species using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Ann Lab Med. 2016;36(4):325–34.
47. Sousa C, Botelho J, Grosso F, Silva L, Lopes J, Peixe L. Unsuitability of MALDI-TOF MS to discriminate Acinetobacter baumannii clones under routine experimental conditions. Front Microbiol. 2015;6(MAY):481.
48. Howard A, O'Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii. Virulence. 2012;3(3):243–50.
49. Pasanen T, Koskela S, Mero S, Tarkka E, Tissari P, Vaara M, et al. Rapid molecular characterization of Acinetobacter baumannii clones with rep-PCR and evaluation of carbapenemase genes by new multiplex PCR in Hospital District of Helsinki and Uusimaa. PLoS One. 2014; 9(1):e85854.
50. Chuang YC, Chang SC, Wang WK. Using the rate of bacterial clearance determined by real-time polymerase chain reaction as a timely surrogate marker to evaluate the appropriateness of antibiotic usage in critical patients with Acinetobacter baumannii bacteremia. Crit Care Med. 2012;40(8):2273–80.
51. Doi Y, Murray GL, Peleg AY. Acinetobacter baumannii: evolution of antimicrobial resistance — treatment options. Semin Respir Crit Care Med. 2015;36(1):85–98.
52. Pouch SM, Patel G. Multidrug-resistant gram-negative bacterial infections in solid organ transplant recipients – guidelines from the American Society of Transplantation infectious diseases Community of Practice. Clin Transpl. 2019;33(9):e13594.
53. Maragakis LL, Perl TM. Antimicrobial resistance: acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008;46(8):1254–63.
54. Skariyachan S, Taskeen N, Ganta M, Venkata Krishna B. Recent perspectives on the virulent factors and treatment options for multidrug-resistant Acinetobacter baumannii. Crit Rev Microbiol. 2019;45(3):315–333.
55. Bulens SN, Yi SH, Walters MS, Jacob JT, Bower C, Reno J, et al. Carbapenem-nonsusceptible acinetobacter baumannii, 8 US metropolitan areas, 2012-2015. Emerg Infect Dis. 2018; 24(4):727–34.
56. Fishbain J, Peleg AY. Reviews of anti-infective agents: treatment of acinetobacter infections. Clin Infect Dis. 2010;51(1):79–84.

57. Michalopoulos A, Falagas ME. Treatment of *Acinetobacter* infections. Expert Opin Pharmacother. 2010;11(5):779–88.
58. Vardakas KZ, Falagas ME. Colistin versus polymyxin B for the treatment of patients with multidrug-resistant gram-negative infections: a systematic review and meta-analysis. Int J Antimicrob Agents. 2017;49(2):233–8.
59. Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, et al. Colistin-resistant acinetobacter baumannii: beyond carbapenem resistance. Clin Infect Dis. 2015; 60(9):1295–303.
60. Queenan AM, Pillar CM, Deane J, Sahm DF, Lynch AS, Flamm RK, et al. Multidrug resistance among Acinetobacter spp. in the USA and activity profile of key agents: results from CAPITAL surveillance 2010. Diagn Microbiol Infect Dis. 2012;73(3):267–70.
61. Garnacho-Montero J, Ortiz-Leyba C, Jiménez-Jiménez FJ, Barrero-Almodóvar AE, García-Garmendia JL, Bernabeu-WittelI M, et al. Treatment of multidrug-resistant Acinetobacter baumannii ventilator-associated pneumonia (VAP) with intravenous colistin: a comparison with imipenem-susceptible VAP. Clin Infect Dis. 2003;36(9):1111–8.
62. Kwa ALH, Loh C, Low JGH, Kurup A, Tam VH. Nebulized Colistin in the treatment of pneumonia due to multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa. Clin Infect Dis. 2005;41(5):754–7.
63. Lu Q, Luo R, Bodin L, Yang J, Zahr N, Aubry A, et al. Efficacy of high-dose nebulized colistin in ventilator-associated pneumonia caused by multidrug-resistant pseudomonas aeruginosa and acinetobacter baumannii. Anesthesiology. 2012;117(6):1335-47.
64. Rattanaumpawan P, Lorsutthitham J, Ungprasert P, Angkasekwinai N, Thamlikitkul V. Randomized controlled trial of nebulized colistimethate sodium as adjunctive therapy of ventilator-associated pneumonia caused by gram-negative bacteria. J Antimicrob Chemother. 2010; 65(12):2645–9.
65. Demirdal T, Sari US, Nemli SA. Is inhaled colistin beneficial in ventilator associated pneumonia or nosocomial pneumonia caused by Acinetobacter baumannii? Ann Clin Microbiol Antimicrob. 2016;15:11.
66. Karaiskos I, Galani L, Baziaka F, Giamarellou H. Intraventricular and intrathecal colistin as the last therapeutic resort for the treatment of multidrug-resistant and extensively drug-resistant Acinetobacter baumannii ventriculitis and meningitis: a literature review. Int J Antimicrob Agents. 2013;41:499–508.
67. Cascio A, Conti A, Sinardi L, Iaria C, Angileri FF, Stassi G, et al. Post-neurosurgical multidrug-resistant Acinetobacter baumannii meningitis successfully treated with intrathecal colistin. A new case and a systematic review of the literature. Int J Infect Dis. 2010;14(7):e572–9.
68. Betrosian AP, Frantzeskaki F, Xanthaki A, Douzinas EE. Efficacy and safety of high-dose ampicillin/sulbactam vs. colistin as monotherapy for the treatment of multidrug resistant Acinetobacter baumannii ventilator-associated pneumonia. J Infect. 2008;56(6):432–6.
69. Wood GC, Hanes SD, Croce MA, Fabian TC, Boucher BA. Comparison of ampicillin-Sulbactam and imipenem-Cilastatin for the treatment of Acinetobacter ventilator-associated pneumonia. Clin Infect Dis. 2002;34(11):1425–30.
70. Niu T, Luo Q, Li Y, Zhou Y, Yu W, Xiao Y. Comparison of Tigecycline or Cefoperazone/Sulbactam therapy for bloodstream infection due to Carbapenem-resistant Acinetobacter baumannii. Antimicrob Resist Infect Control. 2019;8:52.
71. Jeong IB, Na MJ, Son JW, Jo DY, Kwon SJ. High-dose Sulbactam treatment for ventilator-associated pneumonia caused by Carbapenem-resistant Acinetobacter Baumannii. Korean J Crit Care Med. 2016;31(4):308–16.
72. Chen H, Liu Q, Chen Z, Li C. Efficacy of sulbactam for the treatment of Acinetobacter baumannii complex infection: a systematic review and meta-analysis. J Infect Chemother. 2017;23(5):278–85.
73. Lashinsky JN, Henig O, Pogue JM, Kaye KS. Minocycline for the treatment of multidrug and extensively drug-Resistant A. baumannii: a review. Infect Dis Ther. 2017;6:199–211.
74. Goff DA, Kaye KS. Minocycline: an old drug for a new bug: multidrug-resistant acinetobacter baumannii. Clin Infect Dis. 2014;59(Suppl 6):S365–6.

75. Goff DA, Bauer KA, Mangino JE. Bad bugs need old drugs: a stewardship program's evaluation of minocycline for multidrug-resistant acinetobacter baumannii infections. Clin Infect Dis. 2014;59(Suppl 6):S381–7.
76. Ni W, Han Y, Zhao J, Wei C, Cui J, Wang R, et al. Tigecycline treatment experience against multidrug-resistant Acinetobacter baumannii infections: a systematic review and meta-analysis. Int J Antimicrob Agents. 2016;47(2):107–16.
77. Tucker H, Wible M, Gandhi A, Quintana A. Efficacy of intravenous tigecycline in patients with Acinetobacter complex infections: results from 14 phase III and phase IV clinical trials. Infect Drug Resist. 2017;10:401–417.
78. Garnacho-Montero J, Ortiz-Leyba C, Jiménez-Jiménez FJ, Barrero-Almodóvar AE, García-Garmendia JL, Bernabeu-WittelI M, et al. Treatment of multidrug-resistant Acinetobacter baumannii ventilator-associated pneumonia (VAP) with intravenous colistin: a comparison with imipenem-susceptible VAP. Clin Infect Dis. 2003;36(9):1111–8.
79. Heaney M, Mahoney MV, Gallagher JC. Eravacycline: the tetracyclines strike back. Ann Pharmacother. 2019;53(11):1124–1135.
80. Isler B, Doi Y, Bonomo RA, Paterson DL. New treatment options against carbapenem-resistant acinetobacter baumannii infections. Antimicrob Agents Chemother. 2019;63(1):e01110–8.
81. Perez F, El Chakhtoura NG, Yasmin M, Bonomo RA. Polymyxins: to combine or not to combine? Antibiotics. 2019;8(2):E38.
82. Aydemir H, Akduman D, Piskin N, Comert F, Horuz E, Terzi A, et al. Colistin vs. the combination of colistin and rifampicin for the treatment of carbapenem-resistant Acinetobacter baumannii ventilator-associated pneumonia. Epidemiol Infect. 2013;141(6):1214–22.
83. Durante-Mangoni E, Signoriello G, Andini R, Mattei A, De Cristoforo M, Murino P, et al. Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant Acinetobacter baumannii: a multicenter, randomized clinical trial. Clin Infect Dis. 2013;57(3):349–58.
84. Sirijatuphat R, Thamlikitkul V. Preliminary study of colistin versus colistin plus fosfomycin for treatment of carbapenem-resistant Acinetobacter baumannii infections. Antimicrob Agents Chemother. 2014;58(9):5598–601.
85. Paul M, Daikos GL, Durante-Mangoni E, Yahav D, Carmeli Y, Benattar YD, et al. Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant gram-negative bacteria: an open-label, randomised controlled trial. Lancet Infect Dis. 2018;18(4):391–400.
86. Wright H, Bonomo RA, Paterson DL. New agents for the treatment of infections with gram-negative bacteria: restoring the miracle or false dawn? Clin Microbiol Infect. 2017; 23(10):704–12.
87. Fournier PE, Richet H, Weinstein RA. The epidemiology and control of acinetobacter baumannii in health care facilities. Clin Infect Dis. 2006;42(5):692–9.
88. Jawad A, Snelling AM, Heritage J, Hawkey PM. Exceptional desiccation tolerance of Acinetobacter radioresistens. J Hosp Infect. 1998;39(3):235–40.
89. Villegas MV, Hartstein AI. Acinetobacter outbreaks, 1977–2000. Infect Control Hosp Epidemiol. 2003;24(4):284–95.
90. Rodríguez-Baño J, García L, Ramírez E, Martínez-Martínez L, Muniain MA, Fernández-Cuenca F, et al. Long-term control of hospital-wide, endemic multidrug-resistant Acinetobacter baumannii through a comprehensive "bundle" approach. Am J Infect Control. 2009;37(9):715–22.
91. Gray AP, Allard R, Paré R, Tannenbaum T, Lefebvre B, Lévesque S, et al. Management of a hospital outbreak of extensively drug-resistant Acinetobacter baumannii using a multimodal intervention including daily chlorhexidine baths. J Hosp Infect. 2016;93(1):29–34.
92. Fitzpatrick MA, Ozer EA, Hauser AR. Utility of whole-genome sequencing in characterizing acinetobacter epidemiology and analyzing hospital outbreaks. J Clin Microbiol. 2016; 54(3):593–612.

93. Chen CH, Lin LC, Chang YJ, Liu CE, Soon MS. Long-term effectiveness of infection and antibiotic control programs on the transmission of carbapenem-resistant Acinetobacter calcoaceticus-Acinetobacter baumannii complex in Central Taiwan. Med Mal Infect. 2015; 45(7):264–72.
94. Cheon S, Kim MJ, Yun SJ, Moon JY, Kim YS. Controlling endemic multidrug-resistant acinetobacter baumannii in intensive care units using antimicrobial stewardship and infection control. Korean J Intern Med. 2016;31:367–74.
95. Kallel H, Hergafi L, Bahloul M, Hakim A, Dammak H, Chelly H, et al. Safety and efficacy of colistin compared with imipenem in the treatmentof ventilator-associated pneumonia: a matched case-control study. Intensive Care Med. 2007.

Burkholderia in Transplant: Important to Speciate and Important to Treat

17

Gregory Snell, Olivia Smibert, and Elizabeth Tullis

Contents

Introduction 392
The Importance of *Burkholderia* Speciation 392
B. pseudomallei Complex 392
Transplantation and Bpc 394
Management of Bpc 394
B. gladioli 395
Transplantation and *B. gladioli* 396
Management of *B. gladioli* 396
B. cepacia Complex 396
Bcc Virulence Features 397
Bcc Antibiotic Resistance Mechanisms 399
Bcc Associated with CF 399
Outcomes of Bcc Infection 400
Bcc Associated with Contaminated Medicines, Medical Fluids, and Devices 400
Transplantation and Bcc 400
Current Strategies to Manage Bcc 401
Novel Strategies to Manage Bcc 403
Conclusion 404
Key Points 404
Cross-References 405
References 405

G. Snell (✉)
Lung Transplant Service, Alfred Hospital and Monash University, Melbourne, VIC, Australia
e-mail: g.snell@alfred.org.au

O. Smibert
Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: oliviasmibert@gmail.com

E. Tullis
St Michael's Hospital, Department of Medicine, University of Toronto, Toronto, ON, Canada
e-mail: tullise@smh.ca

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_22

Introduction

The genus of *Burkholderia* continues to attract significant interest from many researchers in a range of fields. *Burkholderia* are found in soil and water and have the ability to cause serious human disease, promote plant growth through fungicidal activity, fix nitrogen, and degrade man-made pollutants [1, 2]. The genus represents some 70 species, including many that are pathogenic to humans. Particular species of interest in the setting of the immunocompromised transplant recipient include the *B. pseudomallei* complex (Bpc) which causes melioidosis, as well as *B. gladioli* and the *B. cepacia* complex (Bcc), both of which can cause pneumonia and septicemia (Table 1). A feature of these organisms is their ability to persist and establish chronic infection within a susceptible host [1, 2]. Infections are difficult to manage as a result of significant resistance to disinfectants and antibiotics. The combination of the intrinsic properties of the bacteria and host factors contributes to significant morbidity and mortality in the transplant setting. This chapter will explore the clinical features of these infections, including consideration of modes and risk factors of acquisition, clinical management strategies, and discussion of emerging novel therapeutic approaches.

The Importance of *Burkholderia* Speciation

Careful characterization and classification of *Burkholderia* species and strains are very important from the point of view of predicting disease extent, prognosis, and the specifics of therapeutic measures that will be required to treat the affected individual. Additionally, understanding the modes of transmission and true pathogenic potential via molecular strain analyses has proven to have relevance to both public health preventative/product safety strategies and the specific transplant risk-benefit for an individual patient [3]. Notwithstanding, there are difficulties identifying these organisms and there are many questions still to be answered.

B. pseudomallei Complex

Bpc are facultative intracellular Gram-negative rods including *B. pseudomallei*, *B. mallei*, and *B. thailandensis* that are saprophytes and that are found in the soil in tropical and subtropical areas, including Thailand, India, and Northern Australia [4] (Fig. 1).

B. pseudomallei causes the most frequent serious infection in humans in the form of the clinical entity called melioidosis. Skin inoculation, inhalation, and ingestion are the most common routes of acquisition, [5] and a variety of disease presentations are possible including acute, subacute, or latent infection. Clinical illness can span a spectrum of presentations including localized or disseminated abscesses, necrotizing pneumonia, osteomyelitis, and overwhelming septicemia, with case fatalities

Table 1 Important *Burkholderia* species [1]

(A) *B. pseudomallei* complex (Bpc)
B. pseudomallei
B. mallei
B. thailandensis
B. humptydoensis
(B) *B. gladioli*
(C) *B. cepacia* complex (Bcc)
B. cenocepacia [genomovar III, includes IIIA (ET12 strain), IIIB (PHDC and Midwest strains)]
B. multivorans (genomovar II)
B. cepacia (genomovar I)
B. vietnamiensis (genomovar V)
B. dolosa (genomovar VI)
B. contaminans
B. ambifaria
B. pyrrocinia

reaching up to 50%. Relapse after apparently treated infection is well described, typically due to reactivation of the original infecting strain [6].

Equines are the only known reservoir of *B. mallei*, and humans can become infected when they have close contact with infected animals via aerosols or ingest contaminated meat or water. This organism is endemic in Africa, Asia, the Middle East, and Central and South America and causes the animal disease entity known as glanders. Similar to melioidosis, glanders can present with a spectrum of illness ranging from localized to disseminated, but is fatal in up to 90% of cases [7]. *B. thailandensis* is generally considered nonpathogenic in humans, but cases of septicemia and pneumonia have been described in the USA from travelers to endemic areas [8].

Clinical risk factors for developing melioidosis include diabetes mellitus, excessive alcohol use, chronic renal or lung disease [including cystic fibrosis (CF) [9]], thalassemia, occupational or environmental exposure, and immunosuppression or immunodeficiency [10]. Animal models suggest survival relates to the host's ability to mount a pro-inflammatory protective interferon-gamma-mediated macrophage response [1, 11].

Although the isolation of *B. pseudomallei* from clinical specimens has been enhanced by the utilization of selective media, even with positive cultures, identification of *B. pseudomallei* can be difficult in clinical microbiology laboratories in non-endemic areas where clinical suspicion is low. Ashdown's medium is commonly used to selective isolate *B. pseudomallei* (Fig. 2). It contains crystal violet and gentamicin and produces characteristic purple, dry, and wrinkled colonies [12]. Commercial identification systems may fail to distinguish between *B. pseudomallei* and closely related species such as *B. thailandensis*. Genotypic identification of suspected isolates by sequencing of gene targets such as *groEL* and *s*pecific PCR-based identification using *B. pseudomallei*-specific gene targets are available [13]. Matrix-assisted laser desorption/ionization time-of-flight

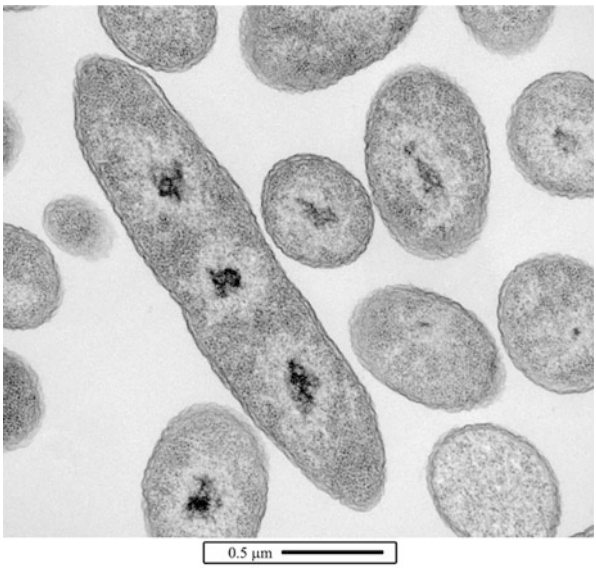


Fig. 1 An example of Bpc – a transmission electron microscope picture of *B. thailandensis* magnified 62,000× [68]. Note the three chromosomes

(MALDI-TOF) mass spectrometry can also be useful for the rapid identification of *Bpc* but has been limited by the availability of a database containing validated reference spectra [3]. Although various serological tests have been developed, they generally have low sensitivities and specificities and are not widely employed [12].

Transplantation and Bpc

From the transplant perspective, Bpc infection is an uncommon pathogen. Donors who have spent time in an endemic area are at risk of transmitting chronic or latently infected Bpc to recipients, so a careful epidemiological history is pertinent, particularly in regard to lung and liver donation. Transplant recipients from an endemic area are at risk of infection due to recrudescence from a latent or chronic focus or primary infection if they visit an endemic area after transplant [1, 10, 14]. While no randomized data exist to support the assertion, it seems probable that in any patient with a latent or chronic nidus, infection will be prevented with routine post-transplant trimethoprim-sulfamethoxazole prophylaxis. Infection due to *B. pseudomallei* needs to be included in the differential diagnosis in transplant recipients presenting with fever of unknown origin who have a history of prior treatment for melioidosis or who travel to endemic regions. Three cases of lung transplantation in the setting of chronic *B. pseudomallei* infection have been reported [15].

Management of Bpc

Bpc is intrinsically resistant to many antibiotics, including penicillin, ampicillin, first- and second-generation cephalosporins, streptomycin, and polymyxin.

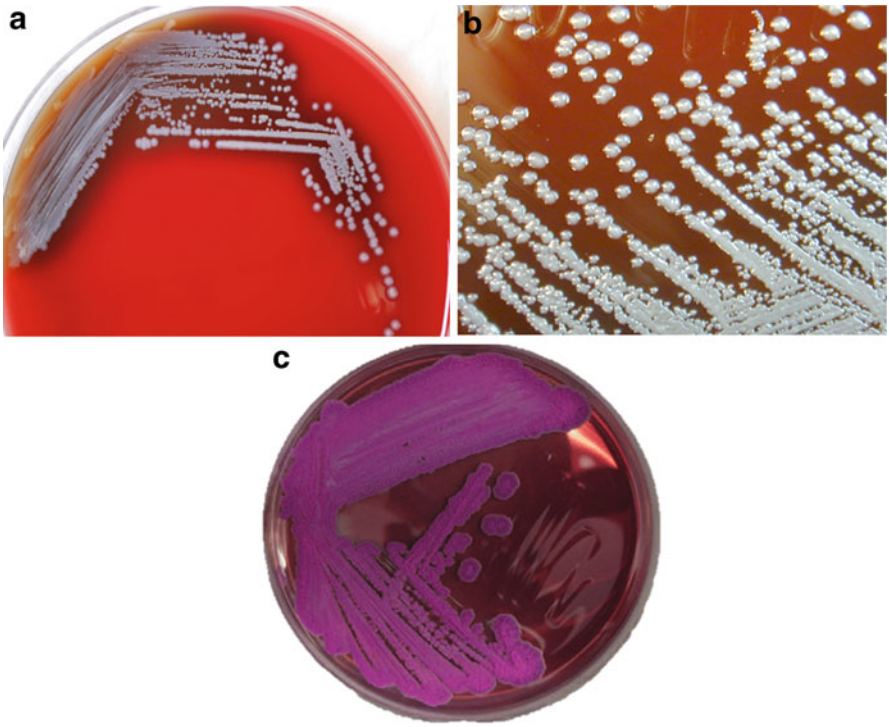


Fig. 2 *B. pseudomallei* (**a**) an enlarged view (**b**) a closeup view on a medium of sheep blood agar, at 48 h, (**c**) on Ashdown's media at 48 h [12]

However, therapy consisting of 10–14 days of an intravenous third-generation cephalosporin or carbapenem as part of an intensive phase, followed by a 3–6-month maintenance phase with oral trimethoprim-sulfamethoxazole (or amoxicillin-clavulanate), is usually effective [1, 2, 10]. Treatment failure occurs in approximately 10–15% of cases and typically represents acquired resistance via a class A β-lactamase in combination with an efflux pump and an outer membrane permeability barrier. In these instances, carbapenems are usually effective [2].

B. gladioli

Although this soil-dwelling organism is an uncommon pathogen, it is an important agent for nosocomially acquired infections in immune-competent and immune-deficient patients [16]. It can infect the respiratory tracts, both transiently and chronically, of patients with CF and chronic granulomatous disease (CGD) pre-transplant. The situation is quite different in the post-lung transplant setting where CF lung transplant recipients in particular have presented with *B. gladioli* abscesses, tracheobronchitis, sinusitis, pneumonia, and bacteremia [16–19]. In a

detailed examination of the effect of the presence of specific *Burkholderia* species on lung transplant outcomes, *B. gladioli* was associated with a statistically significant mortality hazard ratio of 2.2 [18].

It is suggested that oxidation-fermentation-polymyxin B-bacitracin-lactose (OFPBL) medium is the most useful for the recovery of *B. gladioli*, but there is a significant risk of misidentification when phenotypic methods are used alone, with Bcc commonly mistakenly identified as *B. gladioli* [20]. Molecular identification of *B. gladioli* is mainly based on rRNA gene-specific signatures and can be performed by means of species-specific PCR, amplified rRNA gene restriction analysis (ARDRA), or sequencing of the 16S rRNA gene [20].

Transplantation and *B. gladioli*

From the transplant perspective, donors with known B. *gladioli* colonization or infection cultured from any site should be regarded with great caution. Infection control measures, including segregation and avoidance of direct person-to-person contact between *B. gladioli* colonized/infected and pre- or post-transplant patients, are prudent [18]. In any post-transplant recipient, a new *B. gladioli* isolate should be aggressively treated.

Management of *B. gladioli*

There is no direct evidence of patient-to-patient spread, although increased colonization rates have been noted in siblings [18]. The antibiotic susceptibility pattern of *B. gladioli* is quite different from that of both *B. pseudomallei* and *B. cepacia* complex organisms; and the general susceptibility to aminoglycosides, meropenem, and ticarcillin-clavulanic acid is a useful characteristic for use in the differentiation of *B. gladioli* and Bcc in particular. Nevertheless, aminoglycoside-resistant isolates may appear in the course of chronic infections [20], and antibiotic therapy for this species may require consideration of a wider range of agents (see below).

B. cepacia Complex

Bcc organisms are the most infamous *Burkholderia* species and are ubiquitously found in soil and contaminated water (Fig. 3). The group currently consists of approximately 21 species. Notably, species names have replaced the genomovar designations of the early 2000s [1], and that has made comparisons between the literature over this time period confusing. Bcc is a human pathogen almost exclusively in people with CF and CGD where it can cause pneumonitis, bronchitis, septicemia, cutaneous nodules, osteomyelitis, and death. The most common species of Bcc to cause chronic infection in humans are *B. cenocepacia*, *B. multivorans*, and,

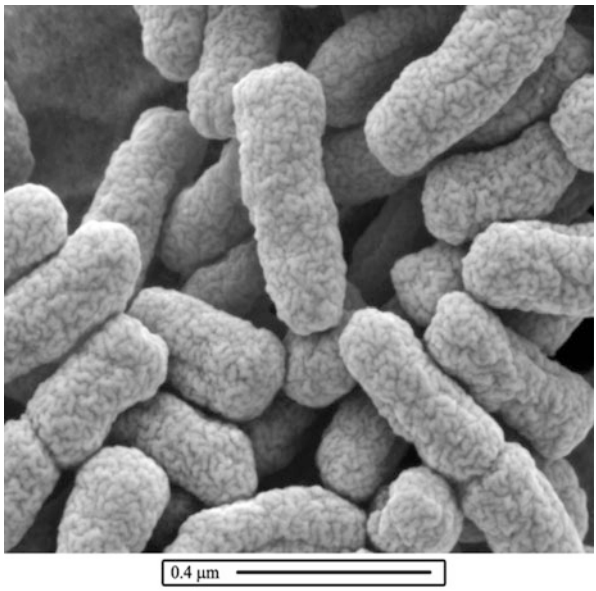


Fig. 3 An example of Bcc – a scanning electron microscope picture of *B. cenocepacia* magnified 137,500× [68]

to a lesser extent, *B. dolosa*, *B. cepacia*, and *B. vietnamensis* although all species of Bcc have been seen in humans.

In routine clinical laboratories, the identification of putative Bcc isolates is generally performed using a combination of selective media, conventional biochemical analysis, and/or commercial systems. Several different media have been developed for the selective isolation of Bcc isolates, in particular *B. cepacia*-selective agar (BCSA) (Fig. 4) or OFPBL agar. Organisms not belonging to the Bcc that are capable of growth on BCSA include *B. gladioli* and *Ralstonia* [21, 22]. Identification of the species requires genotyping with repetitive element PCR (rep-PCR) or multi-locus sequence typing (MLST), and *B. cenocepacia* strain typing requires multi-locus sequence typing [23].

Bcc Virulence Features

While there are some differences in structure and pathogenic potential among the different species of Bcc, there are also common features and mechanisms of virulence (Fig. 5). Bcc demonstrates the ability to invade and can survive within macrophages. Flagella and pili are seen in some species of Bcc and assist in adhesion and motility, the production of biofilms, and the infected host's inflammatory response [24]. Virulence factors that enhance epithelial cell's pathogenicity include metalloproteases, lipases, serine proteases, and lipopolysaccharides. Metalloproteases contribute significantly to Bcc's destruction of lung matrix type IV collagen and fibronectin. Serine protease, just as lipase, has been shown to be a key factor in the intracellular invasion of lung tissue and additionally enhance the ability of Bcc to use

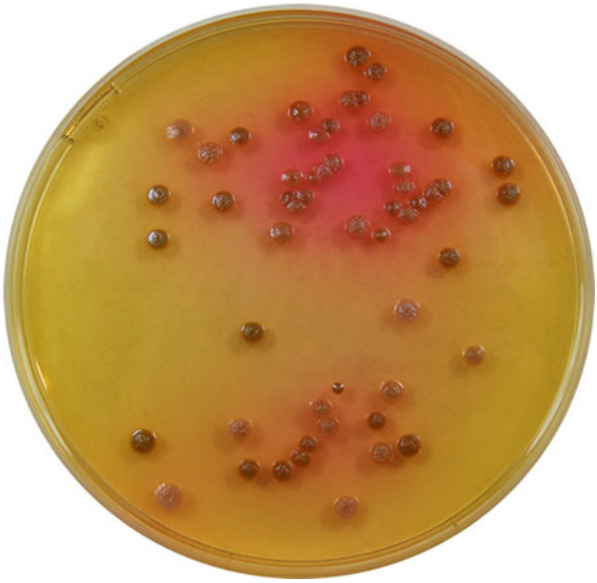


Fig. 4 *B. cepacia* on selective media [13]

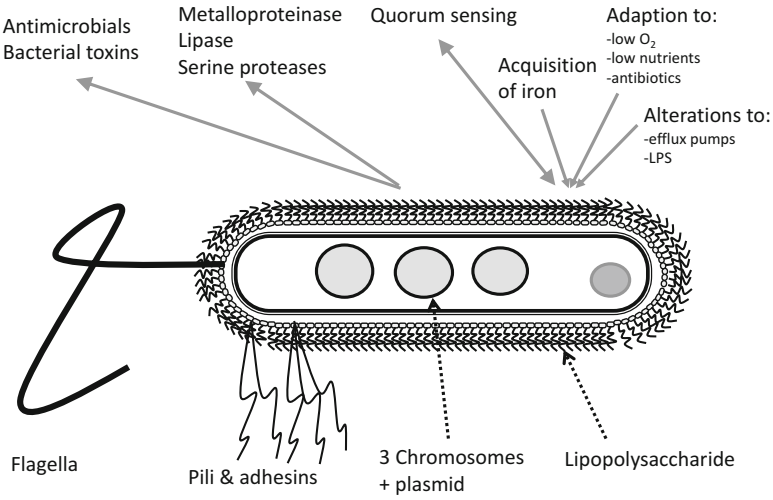


Fig. 5 Virulence features of the Bcc species. Bcc have both physical (lower half of the drawing) and biochemical and pathophysiological tools to survive (upper half of the drawing)

ferritin as an iron source, providing a unique early advantage over other bacteria [25]. Lipopolysaccharides induce an inflammatory response in the lungs of infected patients, allowing the infection to become increasingly necrotizing [25, 26]. The severity of Bcc infection is due in part to its formation of antibiotic-resistant biofilms. The development of the biofilm is orchestrated through the pili and flagella's tissue attachment and lateral movement to enlarge the colony [27]. *B. cepacia* and

B. cenocepacia use quorum sensing to control the expression of their virulence factors such that their pathogenicity is only expressed when a high enough population density exists to take over the host before it can mount a response [1, 25].

Bcc Antibiotic Resistance Mechanisms

Antibiotic resistance in Bcc is mediated via four main mechanisms [2, 28]:

- Antibiotic modification is commonly used by Bcc. The presence of class A β-lactamases provides resistance to β-lactam antibiotics such as penicillins, cephalosporins, clavams, carbapenems, and monobactams.
- Reduced permeability of the cell envelope via a unique lipopolysaccharide structure inhibits the binding of polymyxin to the bacteria's outer membrane.
- Overexpression of efflux pumps due to upregulation of transporters in the presence of antibiotics contributes to resistance to chlorhexidine, ciprofloxacin, tobramycin, minocycline, and chloramphenicol.
- Alterations in antibiotic cellular targets account for a portion of resistance to levofloxacin and trimethoprim.

Bcc Associated with CF

Individuals with CF can become infected with Bcc as a result of transmission from person to person, an infected hospital environment/medical device, or even community environmental contact. The incidence of the Bcc in CF varies from country to country/center to center and is between 3% and 20%. Additionally, the exact proportion of the individual Bcc species varies within that percentage, with *B. multivorans* and *B. cenocepacia* being the commonest species seen in Canada, the USA, and the UK. Following recognition of person-to-person and hospitalization-based transmission, infection control and segregation strategies have been published, and their implementation has been shown to reduce transmissions and alter the epidemiology [29]. Notwithstanding, subsequent outbreaks despite these strategies suggest other routes of transmission might be possible [26]. The prevalence of *B. cenocepacia* (more commonly acquired by person-to-person transmission) has decreased, and *B. multivorans* (more commonly acquired from the environment) has increased [26]. Patients tend to retain the same species and strain over time, but if the strain does change, then *B. cenocepacia* is the most common replacement strain [1]. Using multi-locus sequence typing or whole genome sequencing, it is possible to further define particular B. *cenocepacia* strains including the ET12 strain (ST28) (most commonly seen in Canada and the UK), the PHDC strain (seen in the Mid-Atlantic USA), the Midwest clone (seen in the Western USA), and the Czech strain (ST32) (seen in Europe) [28]. These molecular techniques are particularly useful in investigating Bcc outbreaks [30]. *B. contaminans* is the most frequently seen species in people with CF in Argentina, Portugal, and Spain [1, 31]. The association

of *B. contaminans* with contaminated food sources, medicines, and devices (see below) has raised questions about the source of these organisms [31].

Outcomes of Bcc Infection

In general, all Bcc species are associated with an accelerated chronic loss of lung function and increased mortality when compared to non-Bcc-infected individuals [31, 32]. In addition, there is evidence of lower survival in patients infected with *B. cenocepacia* than in those with *B. multivorans* [33]. Notably the ET12 strain of *B. cenocepacia* is thought to be particularly pathogenic [18, 34]. The notorious "c*epacia* syndrome" of rapidly progressing cavitary pneumonitis, with subsequent septicemia and universal mortality, is linked with *B. cenocepacia*, *B. multivorans*, and *B. dolosa* [1, 18].

Bcc Associated with Contaminated Medicines, Medical Fluids, and Devices

Outbreaks of Bcc have included pneumonia following inoculation of contaminated mouthwash, nasal spray, an intensive care unit sublingual probe, or hospital disinfectants [35–39]. Shampoo, albuterol inhalers, and other products have been found to be contaminated without overt disease transmission [35, 36]. Almost all these contaminations occur in the factory production process. Indeed, Bcc (*B. cepacia* and *B. contaminans*) are among the most frequently isolated microbial species in product recalls as logged by the US Food and Drug Agency; Bcc were present in 22% of non-sterile product recalls and 2.5% of sterile product contaminations [35, 36]. The key features which allow Bcc to survive in such harsh and diverse circumstances include an innate resistance to antimicrobials, efflux-mediated preservative resistance, and an ability to grow in many different substrates over a broad range of temperature.

Infection with Bcc from contaminated medical products may occur in immune-competent and immune-deficient, non-CF individuals with the resultant clinical syndrome being a product of the route of infection (i.e., direct septicemia via infusions or pneumonia following inhalation), the inoculation size, and the immune competency of the host. The use of catheters (hemodialysis, endotracheal tubes, urinary catheters) is a risk factor for acquisition of Bcc which has resulted in reported outbreaks of infection in oncology and dialysis wards and intensive care units.

Transplantation and Bcc

From the general transplantation perspective, donors with known Bcc cultured from any site should be regarded with great caution. From the specific viewpoint of lung transplantation, the isolation of Bcc at any time is a serious "red flag."

Although one US study [18] suggested there was no difference in pre-transplant survival based on the presence of Bcc, a more extensive Canadian review shows an increased waiting list mortality with Bcc [33]. In a recent systematic review and meta-analysis, the pre-transplant presence of Bcc had the most significant association with post-lung transplant mortality, with an adjusted odds ratio of 2.49 [34]. In one large transplant study, outcomes were similar between uninfected recipients and those infected with *B. multivorans*, whereas recipients infected with B. *gladioli* and *B. cenocepacia* had significantly greater post-transplant mortality than uninfected patients [17]. Other studies confirm the importance of *B. cenocepacia* specifically as explaining the excess mortality noted with Bcc [40, 41]. The actual *B. cenocepacia* strain, particularly the presence of the ET12, warrants further study in this regard [18, 34]. A recent small case series places *B. dolosa* in an intermediate position, with an effect between that seen with *B. cenocepacia* and non-Bcc-infected individuals [42]. Further individual case reports describe a fatal outcome in a patient infected with *B. pyrrocinia* [43], a near-fatal but ultimately successful outcome associated with *B. contaminans* [44], and a favorable outcome in a *cepacia* syndrome patient infected with *B. ambifaria* [45].

In all analyses, most of the increased risk of mortality occurs within the first 6 months after transplant, relating to overwhelming chest sepsis in the form of pneumonitis, mediastinitis, empyema, and septicemia. When censored conditionally on 1-year survival, long-term outcomes are similar to patients transplanted without Bcc. It appears that the vast majority of Bcc infectious problems post-lung transplant are rooted in the organisms' present pre-transplant and seeded at the time of surgery, rather than being de novo acquisition at a later stage.

Specific culture for Bcc should be undertaken pre-transplant in all patients and the species present confirmed. In areas of the world with known, highly pathogenic strains (e.g., ET12 in Canada and the UK), strain typing should also be undertaken. The combination of center experience with CF and Bcc, as well as the potential recipient's medical acuity, trajectory, other comorbidities, and specifics of the Bcc species and strain present, must be integrated into the decision regarding whether to transplant or not.

Current Strategies to Manage Bcc

Preventive Strategies

Infection control measures including segregation and avoidance of direct person-to-person or equipment contact between Bcc-infected and non-infected CF patients (pre- or post-transplant) are prudent. Strict staff hand hygiene with gown and glove requirements, as well as patients wearing a mask outside of their consultation or hospital ward room and physically separated from one another, is part of standard preventative practices in North American CF clinics and inpatient areas [46]. Vaccination strategies are under investigation, and although research on a variety of small molecules to modulate host cell-mediated

responses to infection appears promising, no agents are ready yet for clinical practice [1, 28, 47].

Prophylaxis Strategies

There are no proven therapies to reduce CF-associated Bcc exacerbations [48] pre-lung transplant, but a carefully considered antimicrobial regimen of inhaled and intravenous antibiotics (see below) is strongly recommended to reduce the bacterial bio-burden of Bcc peri-transplant [40, 48–50].

Supportive Therapy

Poor glycemic control increases airway bacterial growth and hampers therapy of Bcc infection, so aggressive treatment of CF-related diabetes is recommended [51]. Nutritional support needs to be optimized. Facial sinus drainage procedures should also be considered as the upper airway remains a source of Bcc post-transplant although this strategy has not been demonstrated to reduce postoperative infection.

Interoperative Therapies

Two lung transplant centers suggest taurolidine either as a wash of airways and pleural spaces intraoperatively or as a regular perioperative nebulizer (2 ml 2%) [41, 52], while another suggests a dilute povidone-iodine wash intraoperatively [50]. Careful avoidance of airway spillage intraoperatively and repeated glove changes are highly advisable. Postoperative drainage of any infected/colonized spaces is necessary [50]; however, the potential for inadvertent contamination or seeding via unnecessary tube drainage or bronchoscopic biopsy should also be considered.

Immunosuppressive Therapies Post-Transplant

The transplant team should regard these patients as high risk of sepsis and should strategize the immunosuppression accordingly. This means using the least generally immunosuppressing regimen possible and would typically involve an IL2 blocker (e.g., basiliximab), rather than anti-thymocyte globulin [41], and potentially lower doses of corticosteroids and anti-proliferative agents (e.g., azathioprine or mycophenolate) [53]. Intravenous gamma-globulin or even photopheresis therapy can be used additionally or as alternative safe acute rejection prophylaxis agents [53].

Antibiotic Therapy

Consultation with experts in the management of Bcc infection is recommended with development of a careful plan for antibiotic therapy peri-transplant. There are no evidence-based guidelines for the management of acute or chronic infection with Bcc. Unfortunately, the clinical role of multiple combination bactericidal testing remains unclear, so there is no single best approach or clinical trials to guide therapy [54–57]. General principles for antibiotic therapy of Bcc include using susceptibility testing to guide drug selection, a combination of different classes of antibiotics, and observation of inflammatory markers (e.g., CRP)

to guide therapy duration. Due to the increased drug metabolism seen in people with CF, high doses of antibiotics and extended or continuous infusions of beta-lactams may maximize the antimicrobial action. Antibiotic options for Bcc currently include trimethoprim-sulfamethoxazole, beta-lactams (with ceftazidime and meropenem being the preferred choices), and the bacteriostatic drugs minocycline and chloramphenicol [1, 28, 54, 55]. In vitro susceptibility of Bcc to the combination of a cephalosporin and inhibitor of beta-lactamases, ceftazidime-avibactam, and ceftolozane-tazobactam has been demonstrated [58], and this treatment may restore susceptibility to cetazidime [59].

High doses of aminoglycosides are needed to overcome efflux pump resistance, so inhaled formulations of tobramycin are preferable as higher sputum concentrations are achieved without systemic toxicity [60]. Levofloxacin is often a better choice than ciprofloxacin. A reasonable starting point would be a combination of inhaled tobramycin, trimethoprim-sulfamethoxazole, or minocycline with meropenem or ceftazidime, but up to five agents may be necessary.

Novel Strategies to Manage Bcc

Novel Antibiotics

Cefiderocol is a siderophore cephalosporin with activity against carbapenem-resistant and multidrug-resistant Gram-negative bacilli [61–63], as is meropenem-vaborbactam [64, 65]. While both show promise as potential therapies for resistant organisms, specific efficacy data is yet to be confirmed with regard to the *Burkholderia* species.

Cysteamine

This molecule has been investigated as a potential enhancer of antimicrobial therapy against Bcc due to its ability to disrupt disulfide bonds, thus achieving mucolytic activity and improving biofilm penetration. This, in turn, enhances the activity of tobramycin, ciprofloxacin, and trimethoprim-sulfamethoxazole [66]. This molecule has also been shown to restore cystic fibrosis transmembrane conductance regulator (CFTR) protein function [67].

OligoG

OligoG is a low-molecular-weight oligosaccharide demonstrated in vitro to improve the efficacy of antibiotics used in combination via the perturbation of bacterial biofilms and improvements in mucus properties via the chelation of calcium. The results of a randomized double-blind, placebo-controlled study (NCT02453789) to assess Bcc sputum load are awaited [28].

Phage Therapy

Bacteriophages are viruses that infect and replicate within bacteria. Aerosolized phages decrease Bcc airway load and have been demonstrated to work synergistically with meropenem and ciprofloxacin in vitro [28, 67].

Other Potential Therapies

Inhaled nitric oxide (NCT02498535), cationic antimicrobial peptides, quorum-sensing inhibitors, fish oils, essential oils, and glycol-polymers are all the subject of ongoing Bcc research [28].

Conclusion

Burkholderia species represent potent human systemic pathogens, particularly concerning in the lung transplant population due to establishment of chronicity in the airways and resistance to antibiotics. Bpc, or meliodosis, is uncommonly reported in the transplantation setting which likely reflects the epidemiology of both meliodosis and access to organ transplant. *B. gladioli* is emerging as a contaminant pathogen in the setting of CF, critical illness, and the immune-compromised host. Bcc can be a contaminant and has a predilection to significant clinical infection in patients with CF and CGD. The virulence of Bcc varies with species and, within *B. cenocepacia*, with strain type, and determination of species and strain requires genotyping. Infection with *B. cenocepacia*, in particular the ET12 strain in the setting of lung transplantation, is associated with high morbidity and mortality.

The ability of *Burkholderia* species to survive in harsh macro- and microenvironments and against our best therapies will likely increasingly challenge microbiologists and clinicians. On the other hand, the very broad research being undertaken as a result of these challenges may also provide collateral insights and therapeutic agents against many other pathogens. In the context of transplantation, the high virulence of these organisms challenges us to continue to evolve novel ways of supporting host immunity and avoid, or at least minimize, systemic immunosuppression.

We have definitely not heard the final word regarding this bacterium.

Key Points

- *Burkholderia* species are associated with significant human disease – particularly in an immunosuppressed transplant patient.
- Speciation, and even identification to strain level, has relevance to virulence, prognosis, and therapeutic strategy.
- Bpc causes melioidosis in endemic areas, but if diagnosed is treatable with relatively standard intravenous antibiotics.
- *B. gladioli* is a soil organism and is emerging as a serious pathogen which is challenging to treat with antibiotics.
- Bcc, and in particular *B. cenocepacia*, are associated with serious infection, particularly in patients with suppurative lung disease, and a considered clinical and antibiotic strategy is required if transplantation is being considered or has already been undertaken.

Cross-References

- ▶ Emerging Transplant Infections: Cystic Fibrosis
- ▶ Hospital Epidemiology and Infection Control in the Transplant Center
- ▶ Pretransplant Screening for Infection Exposure: Approach to the Patients with Epidemiologic and Geographic Risk Factors

References

1. Lewis ER, Torres AG. The art of persistence-the secrets to *Burkholderia* chronic infections. Pathog Dis. 2016;74(6):ftw070.
2. Rhodes KA, Schweizer HP. Antibiotic resistance in *Burkholderia* species. Drug Resist Updat. 2016;28:82–90.
3. Coenye M, editor. *Burkholderias*: From genome to function. Cardiff: Caister Academic Press; 2014.
4. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol. 2016;1(1):15008.
5. St John JA, Ekberg JA, Dando SJ, Meedeniya AC, Horton RE, Batzloff M, et al. *Burkholderia pseudomallei* penetrates the brain via destruction of the olfactory and trigeminal nerves: implications for the pathogenesis of neurological melioidosis. MBio. 2014;5(2):e00025.
6. Cheng AC, Currie BJ. Melioidosis: epidemiology, pathophysiology and management. Clin Microbiol Rev. 2005;18(2):383–416.
7. Smith ME, Gossman WG. Glanders and melioidosis. In: StatPearls (Internet). Treasure Island: StatPearls Publishing; 2018.
8. Glass MB, Gee JE, Steigerwalt AG, Cavuoti D, Barton T, Hardy RD, et al. Pneumonia and septicemia caused by *Burkholderia thailandensis* in the United States. J Clin Microbiol. 2006;44(12):4601–4.
9. O'Carroll MR, Kidd TJ, Coulter C, Smith HV, Rose BR, Harbour C, et al. *Burkholderia pseudomallei*: another emerging pathogen in cystic fibrosis. Thorax. 2003;58(12):1087–91.
10. Pitman MC, Luck T, Marshall CS, Anstey NM, Ward L, Currie BJ. Intravenous therapy duration and outcomes in melioidosis: a new treatment paradigm. PLoS Negl Trop Dis. 2015;9(3):e0003586.
11. Conejero L, Patel N, de Reynal M, Oberdorf S, Prior J, Felgner PL, et al. Low-dose exposure of C57BL/6 mice to *Burkholderia pseudomallei* mimics chronic human melioidosis. Am J Pathol. 2011;179(1):270–80.
12. Lau SK, Sridhar S, Ho CC, Chow WN, Lee KC, Lam CW, et al. Laboratory diagnosis of melioidosis: past, present and future. Exp Biol Med (Maywood). 2015;240(6):742–51.
13. Lowe W, March JK, Bunnell AJ, O'Neill KL, Robison RA. PCR-based methodologies used to detect and differentiate the *Burkholderia pseudomallei* complex: *B. pseudomallei*, *B. mallei*, and *B. thailandensis*. Curr Issues Mol Biol. 2014;16:23–54.
14. Franco-Paredes C, Jacob JT, Hidron A, Rodriguez-Morales AJ, Kuhar D, Caliendo AM. Transplantation and tropical infectious diseases. Int J Infect Dis. 2010;14(3):e189–96.
15. Geake JB, Reid DW, Currie BJ, Bell SC, Melioid CFI, Bright-Thomas R, et al. An international, multicentre evaluation and description of *Burkholderia pseudomallei* infection in cystic fibrosis. BMC Pulm Med. 2015;15:116.
16. Kennedy MP, Coakley RD, Donaldson SH, Aris RM, Hohneker K, Wedd JP, et al. *Burkholderia gladioli*: five year experience in a cystic fibrosis and lung transplantation center. J Cyst Fibros. 2007;6(4):267–73.
17. Brizendine KD, Baddley JW, Pappas PG, Leon KJ, Rodriguez JM. Fatal *Burkholderia gladioli* infection misidentified as Empedobacter brevis in a lung transplant recipient with cystic fibrosis. Transpl Infect Dis. 2012;14(4):E13–8.

18. Murray S, Charbeneau J, Marshall BC, LiPuma JJ. Impact of *Burkholderia* infection on lung transplantation in cystic fibrosis. Am J Respir Crit Care Med. 2008;178(4):363–71.
19. Thompson GR 3rd, Wickes BL, Herrera ML, Haman TC, Lewis JS 2nd, Jorgensen JH. Disseminated *Burkholderia gladioli* infection in a lung transplant recipient with underlying hypocomplementemic urticarial vasculitis. Transpl Infect Dis. 2011;13(6):641–5.
20. Segonds C, Clavel-Batut P, Thouverez M, Grenet D, Le Coustumier A, Plesiat P, et al. Microbiological and epidemiological features of clinical respiratory isolates of *Burkholderia gladioli*. J Clin Microbiol. 2009;47(5):1510–6.
21. LiPuma JJ. *Burkholderia cepacia* complex: a contraindication to lung transplantation in cystic fibrosis? Transpl Infect Dis. 2001;3(3):149–60.
22. Shelly DB, Spilker T, Gracely EJ, Coenye T, Vandamme P, LiPuma JJ. Utility of commercial systems for identification of *Burkholderia cepacia* complex from cystic fibrosis sputum culture. J Clin Microbiol. 2000;38(8):3112–5.
23. Kenna DTD, Lilley D, Coward A, Martin K, Perry C, Pike R, et al. Prevalence of *Burkholderia* species, including members of *Burkholderia cepacia* complex, among UK cystic and non-cystic fibrosis patients. J Med Microbiol. 2017;66(4):490–501.
24. Urban TA, Griffith A, Torok AM, Smolkin ME, Burns JL, Goldberg JB. Contribution of *Burkholderia cenocepacia* flagella to infectivity and inflammation. Infect Immun. 2004;72(9):5126–34.
25. McClean S, Callaghan M. *Burkholderia cepacia* complex: epithelial cell-pathogen confrontations and potential for therapeutic intervention. J Med Microbiol. 2009;58(Pt 1):1–12.
26. Maschmeyer G, Gobel UB. *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. In: Principles and practice of infectious disease. Edinburgh: Churchill Livingstone; 2004. p. 2615–20.
27. Cunha MV, Sousa SA, Leitao JH, Moreira LM, Videira PA, Sa-Correia I. Studies on the involvement of the exopolysaccharide produced by cystic fibrosis-associated isolates of the *Burkholderia cepacia* complex in biofilm formation and in persistence of respiratory infections. J Clin Microbiol. 2004;42(7):3052–8.
28. Scoffone VC, Chiarelli LR, Trespidi G, Mentasti M, Riccardi G, Buroni S. *Burkholderia cenocepacia* infections in cystic fibrosis patients: drug resistance and therapeutic approaches. Front Microbiol. 2017;8:1592–600.
29. Salsgiver EL, Fink AK, Knapp EA, LiPuma JJ, Olivier KN, Marshall BC, et al. Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis. Chest. 2016;149(2):390–400.
30. Blanchard AC, Tadros M, Tang L, Muller M, Spilker T, Waters V, Lipuma JJ, Tullis E. New acquisitions of ET-12 *Burkholderia cenocepacia* in adults with cystic fibrosis: role of whole genome sequencing in outbreak investigation. Open Forum Inf Dis. 2018;5(1):S383.
31. Cipolla L, Rocca F, Martinez C, Aguerre L, Barrios R, Prieto M. Prevalence of *Burkholderia cepacia* complex species in cystic fibrosis patients in Argentina during the period 2011–2015. Enferm Infecc Microbiol Clin. 2018;36(7):431–4.
32. Liou TG, Adler FR, Fitzsimmons SC, Cahill BC, Hibbs JR, Marshall BC. Predictive 5-year survivorship model of cystic fibrosis. Am J Epidemiol. 2001;153(4):345–52.
33. Zlosnik JE, Zhou G, Brant R, Henry DA, Hird TJ, Mahenthiralingam E, et al. *Burkholderia* species infections in patients with cystic fibrosis in British Columbia, Canada. 30 years' experience. Ann Am Thorac Soc. 2015;12(1):70–8.
34. Koutsokera A, Varughese RA, Sykes J, Orchanian-Cheff A, Shah PS, Chaparro C, et al. Pre-transplant factors associated with mortality after lung transplantation in cystic fibrosis: a systematic review and meta-analysis. J Cyst Fibros. 2019;18:407–15.
35. https://www.cdc.gov/hai/organisms/bcepacia.html.
36. https://www.fda.gov/Drugs/DrugSafety/ucm570672.htm.
37. LiPuma JJ. *Burkholderia cepacia* epidemiology and pathogenesis: implications for infection control. Curr Opin Pulm Med. 1998;4(6):337–41.
38. Nasser RM, Rahi AC, Haddad MF, Daoud Z, Irani-Hakime N, Almawi WY. Outbreak of *Burkholderia cepacia* bacteremia traced to contaminated hospital water used for dilution of an alcohol skin antiseptic. Infect Control Hosp Epidemiol. 2004;25(3):231–9.

39. Shehabi AA, Abu-Al-Soud W, Mahafzah A, Khuri-Bulos N, Abu Khader I, Ouis IS, et al. Investigation of *Burkholderia cepacia* nosocomial outbreak with high fatality in patients suffering from diseases other than cystic fibrosis. Scand J Infect Dis. 2004;36(3):174–8.
40. Alexander BD, Petzold EW, Reller LB, Palmer SM, Davis RD, Woods CW, et al. Survival after lung transplantation of cystic fibrosis patients infected with *Burkholderia cepacia* complex. Am J Transplant. 2008;8(5):1025–30.
41. De Soyza A, Meachery G, Hester KL, Nicholson A, Parry G, Tocewicz K, et al. Lung transplantation for patients with cystic fibrosis and *Burkholderia cepacia* complex infection: a single-center experience. J Heart Lung Transplant. 2010;29(12):1395–404.
42. Wang R, Welsh SK, Budev M, Goldberg H, Noone PG, Gray A, et al. Survival after lung transplantation of cystic fibrosis patients infected with *Burkholderia dolosa* (genomovar VI). Clin Transpl. 2018;32(5):e13236.
43. Savi D, De Biase RV, Amaddeo A, Anile M, Venuta F, Ruberto F, et al. *Burkholderia pyrrocinia* in cystic fibrosis lung transplantation: a case report. Transplant Proc. 2014;46(1):295–7.
44. Farrell C, Riddell P. Post-lung transplant cepacia syndrome due to *Burkholderia contaminans*. Am J Respir Crit Care Med. 2018;197:A3100.
45. Goodlet KJ, Nailor MD, Omar A, Huang JL, LiPuma JJ, Walia R, et al. Successful lung re-transplant in a patient with Cepacia syndrome due to *Burkholderia ambifaria*. J Cyst Fibros. 2019;18:e1–4.
46. Saiman L, Siegel JD, LiPuma JJ, Brown RF, Bryson EA, Chambers MJ, et al. Infection prevention and control guideline for cystic fibrosis: 2013 update. Infect Control Hosp Epidemiol. 2014;35(Suppl 1):S1–S67.
47. Pradenas GA, Ross BN, Torres AG. *Burkholderia cepacia* complex vaccines: where do we go from here? Vaccines (Basel). 2016;4(2):E10.
48. Tullis DE, Burns JL, Retsch-Bogart GZ, Bresnik M, Henig NR, Lewis SA, et al. Inhaled aztreonam for chronic *Burkholderia* infection in cystic fibrosis: a placebo-controlled trial. J Cyst Fibros. 2014;13(3):296–305.
49. De Soyza A, McDowell A, Archer L, Dark JH, Elborn SJ, Mahenthiralingam E, et al. *Burkholderia cepacia* complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. Lancet. 2001;358(9295):1780–1.
50. Nash EF, Coonar A, Kremer R, Tullis E, Hutcheon M, Singer LG, et al. Survival of *Burkholderia cepacia* sepsis following lung transplantation in recipients with cystic fibrosis. Transpl Infect Dis. 2010;12(6):551–4.
51. Simren K. Increased airway glucose increases airway bacterial load in hyperglycemia. Sci Rep. 2016;6:27636.
52. Zeriouh M, Sabashnikov A, Patil NP, Schmack B, Zych B, Mohite PN, et al. Use of taurolidine in lung transplantation for cystic fibrosis and impact on bacterial colonization. Eur J Cardiothorac Surg. 2018;53(3):603–9.
53. Ivulich S, Westall G, Dooley M, Snell G. The evolution of lung transplant immunosuppression. Drugs. 2018;78(10):965–82.
54. Aaron SD, Ferris W, Henry DA, Speert DP, Macdonald NE. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. Am J Respir Crit Care Med. 2000;161(4 Pt 1):1206–12.
55. El-Halfawy OM, Naguib MM, Valvano MA. Novel antibiotic combinations proposed for treatment of *Burkholderia cepacia* complex infections. Antimicrob Resist Infect Control. 2017;6:120.
56. Regan KH, Bhatt J. Eradication therapy for *Burkholderia cepacia* complex in people with cystic fibrosis. Cochrane Database Syst Rev. 2016;11:CD009876.
57. Horsley A, Jones AM, Lord R. Antibiotic treatment for *Burkholderia cepacia* complex in people with cystic fibrosis experiencing a pulmonary exacerbation. Cochrane Database Syst Rev. 2016;1:CD009529.
58. Van Dalem A, Herpol M, Echahidi F, Peeters C, Wybo I, De Wachter E, et al. In vitro susceptibility of *Burkholderia cepacia* complex isolated from cystic fibrosis patients to ceftazidime-avibactam and Ceftolozane-Tazobactam. Antimicrob Agents Chemother. 2018;62(9):e00590.

59. Papp-Wallace KM, Becka SA, Zeiser ET, Ohuchi N, Mojica MF, Gatta JA, et al. Overcoming an extremely drug resistant (XDR) pathogen: avibactam restores susceptibility to ceftazidime for *Burkholderia cepacia* complex isolates from cystic fibrosis patients. ACS Infect Dis. 2017;3(7):502–11.
60. Ratjen A, Yau Y, Wettlaufer J, Matukas L, Zlosnik JE, Speert DP, et al. In vitro efficacy of high-dose tobramycin against *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients. Antimicrob Agents Chemother. 2015;59(1):711–3.
61. Hackel MA, Lomovskaya O, Dudley MN, Karlowsky JA, Sahm DF. In vitro activity of meropenem-vaborbactam against clinical isolates of KPC-positive Enterobacteriaceae. Antimicrob Agents Chemother. 2018;62(1):e01904.
62. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. In vitro activity of the siderophore cephalosporin, cefiderocol, against carbapenem-nonsusceptible and multidrug-resistant isolates of gram-negative bacilli collected worldwide in 2014 to 2016. Antimicrob Agents Chemother. 2018;62(2):e01968.
63. Karlowsky JA, Hackel MA, Tsuji M, Yamano Y, Echols R, Sahm DF. In vitro activity of cefiderocol, a siderophore cephalosporin, against gram-negative bacilli isolated by clinical laboratories in North America and Europe in 2015–2016: SIDERO-WT-2015. Int J Antimicrob Agents. 2019;53(4):456–66.
64. Athans V, Neuner EA, Hassouna H, Richter SS, Keller G, Castanheira M, et al. Meropenem-vaborbactam as salvage therapy for ceftazidime-avibactam-resistant *Klebsiella pneumoniae* bacteremia and abscess in a liver transplant recipient. Antimicrob Agents Chemother. 2019;63(1):e01551.
65. Petty LA, Henig O, Patel TS, Pogue JM, Kaye KS. Overview of meropenem-vaborbactam and newer antimicrobial agents for the treatment of carbapenem-resistant Enterobacteriaceae. Infect Drug Resist. 2018;11:1461–72.
66. Fraser-Pitt D, Mercer D, Lovie E, Robertson J, O'Neil D. Activity of cysteamine against the cystic fibrosis pathogen *Burkholderia cepacia* complex. Antimicrob Agents Chemother. 2016;60(10):6200–6.
67. Kamal F, Dennis JJ. *Burkholderia cepacia* complex phage-antibiotic synergy (PAS): antibiotics stimulate lytic phage activity. Appl Environ Microbiol. 2015;81(3):1132–8.
68. http://bnbi.org/media-2/image-gallery/.

Mycobacterium tuberculosis in Transplantation

18

Aruna K. Subramanian

Contents

Introduction 409
Disease Epidemiology 410
Clinical Impact in Transplantation 411
Diagnostic Strategies and Screening 412
Treatment of Active TB 414
Prevention/Treatment of Latent TB 416
Conclusion 418
References 418

Introduction

Tuberculosis (TB) in solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients can present a diagnostic challenge due to the immunosuppressed state of the patients, their comorbid conditions, and multiple concomitant medications. Screening and treatment of latent or subclinical TB prior to transplantation is critical in prevention of active TB after transplant, but has diagnostic limitations due to imperfect tests [1–3]. Donor-derived TB is a unique challenge in SOT patients, which requires a high index of suspicion. Treatment of TB after transplantation can be very difficult due to medication interactions, especially with immunosuppressive medications used to prevent rejection in SOT or treat graft-versus-host disease in HSCT [4–7].

Since few studies of latent or active TB treatment have been performed in transplant, clinicians have to rely on consensus guidelines based on expert opinion

A. K. Subramanian (✉)
Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA
e-mail: asubram2@stanford.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_15

[2, 8–16]. New diagnostics and guidance on therapeutic strategies are sorely needed in the area of TB in transplantation.

Disease Epidemiology

TB is caused by members of the *Mycobacterium tuberculosis* complex, most commonly *M. tuberculosis* (MTB), and it is estimated that about 1/3 of the world's population is infected [17]. The pathogen is ubiquitous, often remains latent in the human host, and is spread by airborne exposure [18]. The World Health Organization reported ten million incident cases of TB and 1.57 million TB deaths worldwide in 2017. TB is the leading cause of death from a single infectious agent [19].

In highly endemic areas, the prevalence of TB in transplant recipients is reported as high as 12%, with the prevalence in most developed countries between 1.2 and 6.4% [1, 20]. The highest incidence rates are reported in studies from Asia, with rates of 4 to nearly 30 times that of the general population [21–25]. This increased incidence is noted even when matched to similar non-transplant patients with end-stage organ failure [26]. Among organ transplant recipients, those who receive a lung transplant are at highest risk of TB [24]. While rates of TB in HSCT recipients are not as high as in SOT recipients, HSCT recipients who are from TB-endemic areas are at an increased risk of active disease [27–31]. Overall frequencies of active TB are reported as 2.3%, with the majority of HSCT cases in allogeneic transplant recipients [32].

A transplant recipient can develop active TB through the following mechanisms: [1] reactivation of latent or healed untreated primary TB, [2] undergoing transplant while already having unidentified active TB, [3] transmission of TB through the donated organ, or [4] new exposure to TB leading to primary infection after transplant [10]. Most cases are thought to occur through the first mechanism, endogenous reactivation of latent TB after transplant [25]. Unfortunately the TST reaction or IGRA results prior to transplant may not be a reliable predictor of reactivation post-transplant, likely due to anergy seen in end-stage organ failure [33, 34]. While donor-derived TB is rare (<5% of cases of post-transplant TB), it can cause severe illness or be fatal. TB transmission is reported most frequently through lung transplantation, but has also occurred via kidney, liver, and heart transplant [25, 35–37]. When there is exposure, primary TB infection can be acquired after transplant, and rare nosocomial transmission has been reported on a hospital transplant ward [38].

Risk factors for the acquisition of TB include disease incidence in one's country of birth or prior residence, close contact with persons with TB, medical risks such as diabetes mellitus, malnutrition, chronic kidney disease, and social risk factors such as cigarette smoking, homelessness, incarceration, or institutional residence. Specifically in the transplant setting, risk factors include a history of untreated TB, prior residence in TB-endemic areas, chest imaging with evidence of prior TB, and enhanced immunosuppressive treatment of allograft rejections, especially T-cell-depleting antibodies [10, 24, 39]. Risks specific to kidney transplant include hepatitis

C and duration of hemodialysis pre-transplant [40]. Recipients of lung transplants have the highest risk of active TB post-transplant compared to recipients of other organs. Increased age has been shown to be a risk factor in areas where rates of latent TB are higher in older cohorts [24]. In HSCT, presence of chronic graft-versus-host disease (GVHD) is a risk factor for the development of TB, likely related to the need for more intense immunosuppressive therapy [27, 31, 41].

Clinical Impact in Transplantation

Initial exposure to MTB can lead to primary disease, clearance of the organism, or latent infection with the possibility of reactivation disease many years later. Reactivation occurs in 5–10% of immunocompetent hosts but is much more common in immunocompromised patients [3, 42, 43]. The classic presenting symptoms of TB may be absent in transplant recipients. Fever was seen in 64% of patients with focal disease and 91% of those with disseminated infection in one study of transplant recipients [3]. Imaging findings can be quite varied, and pulmonary cavitary lesions may not be present [44, 45]. While pulmonary disease is still the most common manifestation, SOT patients have rates of extrapulmonary disease and disseminated disease of 30% and 16%, respectively [3, 25]. Similarly, in HSCT patients 34% of TB diagnoses are reported to be extrapulmonary and 23% disseminated [32]. Unusual manifestations of disease can be seen in immunosuppressed patients, including but not limited to cutaneous ulcers, musculoskeletal disease such as pyomyositis or tenosynovitis, and even gastrointestinal tract involvement. Allograft-specific infection can be seen, such as liver abscess in liver transplant or urinary tract involvement in kidney transplant [25].

Lung, heart, and liver transplant recipients have a median time to presentation of 6–11 months, which is shorter than kidney recipients. More than two-thirds of active TB cases in this group occur in the year following transplantation [3, 46]. In kidney transplant recipients, the median time to presentation is greater than 1 year post-transplant, thus requiring an ongoing high index of suspicion in this patient population [25]. As one would expect, donor-derived TB manifests earlier, at a median of less than 3 months [47]. A majority of TB cases seen after HSCT also occur within a year of transplant. In a review of HSCT cases from the last decade, the median time to clinical presentation was 4.6 months (range 3–12.9 months), and one third of cases presented within the first 100 days after HSCT [32].

TB mortality is increased post-transplant, most notably in patients with disseminated disease, prior graft rejection, or receipt of anti-T-cell antibodies [1, 3]. Prior estimates of crude mortality in post-transplant TB have been between 20 and 30% [3, 48], with an attributable mortality of 10% [24]. A recent comprehensive review of reported cases of TB after organ transplant calculated a 19% all-cause mortality and a 15% rate of allograft loss with TB therapy [25]. In HSCT patients, the mortality rate was reported as 28%, with a TB-attributable mortality of 8.5% [32].

Diagnostic Strategies and Screening

Because of the higher frequency of extrapulmonary or disseminated disease and the variability of symptoms and imaging findings, the diagnosis of active TB disease is frequently delayed after transplant unless a high index of suspicion is maintained. Hospitalized patients should be placed in airborne isolation, and three expectorated or induced sputum specimens should be obtained for smear and acid-fast stain, such as Ziehl-Neelsen, and cultured for acid-fast bacilli. If available, sputum should be tested by nucleic acid amplification techniques, such as Xpert MTB/RIF (Cepheid Inc.), an automated molecular test for MTB and resistance to rifampin (RIF). This can increase the sensitivity compared to microscopy alone and decrease the time to diagnosis since MTB can take 4–8 weeks to grow on solid culture media [17, 49]. Of note, a diagnostic invasive procedure is often required to make a diagnosis of TB. With pulmonary TB this may involve bronchoscopy with bronchoalveolar lavage or lung biopsy, or biopsy of skin lesions or lymph nodes, or sampling of abscess fluid in patients with deep collections. In addition to microbiologic exams for smear and culture, specimens should be sent for histopathological evaluation, looking for granulomatous inflammation [17]. Transplant candidates with suggestive symptoms such as fevers, cough, night sweats, and weight loss should be evaluated for active TB. If diagnosed, the candidate should be treated for active TB prior to proceeding with transplantation [11]. Ideally treatment should be completed prior to transplantation; however, this decision needs to be individualized based on the urgency of transplant and response to therapy. There are reports of successful liver transplantation in patients who developed liver failure due to TB medications requiring urgent transplant prior to completion of treatment [50].

Screening for latent TB infection is standard prior to transplant in order to determine the need for LTBI therapy. In patients without signs, symptoms, or chest imaging consistent with active TB, a diagnosis of latent TB is made by documenting a current or prior untreated positive TST or IGRA. IGRAs, including QuantiFERON-TB Gold and Gold Plus (QFT, QFT-Plus, Qiagen) and T-SPOT.TB (Oxford Immunotec Ltd), are currently used as alternatives to the TST in the general population [51]. Diagnostic tests such as the TST and IGRA do not distinguish latent TB infection from active disease, and both can yield falsely negative or indeterminate results in immunosuppressed patients [52]. Therefore, screening for LTBI should be done prior to administration of immunosuppression, and some patients with negative or indeterminate results may be considered for LTBI therapy. In fact, in HSCT patients, screening ideally should be done prior to the receipt of cytotoxic chemotherapy for their underlying malignancy. While TB screening prior to HSCT is not done routinely at most centers, in a review of HSCT patients who developed TB post-transplant, those screened with IGRA or TST pre-transplant had negative test results [32]. It should be noted that QFT and T-SPOT.TB tests are highly specific; therefore, a positive test should be considered evidence of MTB infection.

A thorough history is critical in all transplant candidates, to determine exposure to family members or close contacts with active TB, prior residence or extended travel to areas highly endemic for TB, and a history of prior TST or IGRA results. Any

history of treatment for active or latent TB should be documented, as well as details regarding the length and type of treatment. When a TST is used, it should be considered positive if there is ≥5 mm of induration at 48–72 h [17]. Based on a Spanish study of patients awaiting liver transplant which showed a 4% increase in positive TST when a two-step process is used, a second skin test 2 weeks after may be considered in patients with negative TST who may have had remote exposure to TB if feasible [41]. Many transplant programs prefer IGRA over TST because [1] TST requires 48–72 h follow-up for a read; [2] unlike the TST, results of the IGRA will not be falsely positive due to prior BCG vaccination; (3) IGRA testing appears to be more sensitive in transplant candidates with end-stage renal or liver disease; and [4] IGRA may have a slightly greater predictive value (albeit low) for reactivation of TB post-transplant [34, 53].

Routine screening with chest X-ray (CXR) should be done as part of the pre-transplant evaluation. Based on history, those with epidemiologic risk for latent or asymptomatic active TB should be considered for CT evaluation regardless of TST and IGRA results [39, 54]. A Korean study of patients who received liver transplants showed that CT scans with evidence of prior healed TB were more predictive of post-transplant TB than CXR [55]. Since none of the available screening tests are completely accurate in diagnosing latent or active MTB infection, the decision to treat should be individualized based on the clinical likelihood of infection. Patients with recent conversion of TST or IGRA should be treated for latent TB as promptly as possible. A current negative screening test, especially in patients with organ failure awaiting transplantation or those who have received cytotoxic chemotherapy, does not negate a prior positive test result. Individuals having a documented or reliable prior history of appropriately treated active TB disease or treated LTBI need not undergo further testing with TST or IGRA. However, these individuals should be screened for active TB.

Living organ donors should undergo an evaluation similar to that described for transplant candidates [37]. For living donors, the TST should be interpreted as positive or negative according to CDC guidelines for the general population, or an IGRA should be performed. If either is positive, and symptoms or chest imaging is indicative of TB, active disease should be ruled out. Treatment of LTBI should be considered prior to organ donation if feasible, especially with recent TST or IGRA conversion [10]. With deceased donors, a history of prior TB exposure, active disease, and treatment should be obtained from the family as TST and IGRA are not feasible in deceased donors. Treatment of the recipient for latent TB should be considered, as should recipient monitoring for development of active TB with early diagnosis and treatment as appropriate [37]. If active TB disease is present in either living or deceased donors, organs should not be used due to the high risk of transmission to the recipient. In the rare cases where active TB in the donor is diagnosed post-mortem and transplant has already occurred, treatment of active TB in the recipient should be initiated [37, 56]. Since TB has not been reported to be transmitted by HSCT, guidelines do not recommend routine screening in HSCT donors; however, screening is recommended in recipients who are at high risk of TB exposure [57].

There are many shortcomings of available diagnostics, namely, false-negative or indeterminate TST or IGRA, low predictive value to identify those with latent TB who will develop active TB, inability to distinguish latent from active TB, and lack of quantification of response to therapy. Therefore emerging diagnostics for TB are being developed. Some experts argue that these diagnostics need to be region-specific due to the variability of drug susceptibility patterns of MTB around the world [58]. Urine and serum tests detecting TB biomarkers such as lipoarabinomannan (LAM) and early secretory antigenic target 6 (ESAT6) are being developed for ease of point-of-care testing [59]; cell-free MTB DNA from both urine and blood look promising for diagnosis of active TB [60]. Recently, a novel 3-gene signature of host responses to TB that can be measured in blood has been shown to predict progression of LTBI to active TB up to 6 months prior to sputum conversion. This new assay can detect active TB with high sensitivity and specificity and identify treatment failure [61, 62]. These promising new diagnostics need to be studied in transplant candidates and recipients.

Treatment of Active TB

Standard drug treatment for active TB disease can be found in published guidelines on treatment of TB [8, 11]. While treatment success is achievable, the transplant clinician must plan for drug-drug interactions, additive toxicities to other transplant medications, and the need for dose adjustment with renal dysfunction.

In general, intensive phase treatment with a standard four-drug regimen is recommended for drug-sensitive TB including isoniazid, a rifamycin (rifampin or rifabutin), pyrazinamide, and ethambutol for the first 2 months. Once the susceptibility to isoniazid, rifampin, and pyrazinamide is confirmed, ethambutol can be stopped. This is followed by a continuation phase of isoniazid and the rifamycin for a minimum of 4 additional months [8]. There are some caveats to keep in mind when treating immunosuppressed transplant patients. Due to the risk of progression or dissemination, when the likelihood of TB is high, one may consider starting empiric therapy for active TB while awaiting diagnostic results, given the slow growth of MTB in cultures. Importantly, daily dosing of active TB therapy is recommended since twice- or thrice-weekly administration of TB therapy can increase the risk of relapse [63]. Pyridoxine should be administered daily as comorbid conditions may increase the risk of isoniazid-related neurotoxicity in transplant patients [8]. Transplant patients on treatment should be monitored carefully at least monthly for side effects and laboratory testing. Directly observed therapy (DOT) programs have been shown to improve adherence and outcomes in TB patients and are recommended for transplant recipients. Whenever possible, the involvement of a TB expert is recommended in managing the treatment of active disease, especially in post-transplant patients. Such expertise is essential in caring for patients whose TB is complicated by drug resistance or drug intolerance.

The potential for hepatotoxicity and major drug-drug interactions involving rifampin and immunosuppressive medications are major challenges in treating TB

in transplant recipients. However, rifamycins have potent MTB sterilizing activity, and their use is recommended if possible [17]. Rifampin is a potent inducer of the microsomal enzymes (P450-3A4) that metabolize immunosuppressive agents such as calcineurin inhibitors and mTOR inhibitors, used in both SOT and HSCT. Rifampin may also interfere with corticosteroid metabolism. Allograft rejection occurring in conjunction with rifampin use has been reported due to inadequate levels of immunosuppression resulting from drug-drug interactions with rifampin [25]. Doses of cyclosporine, tacrolimus, and sirolimus need to be increased at least two- to fivefold and therapeutic drug levels followed carefully during concomitant rifampin use in transplant patients [25]. It is possible to replace rifampin with rifabutin given its similar activity against MTB. Since it is a less potent inducer of cytochrome P450-3A4 enzymes, calcineurin inhibitor and mTOR inhibitor levels are somewhat easier to maintain with careful monitoring and dose adjustment [64]. While published clinical reports using rifabutin for TB after transplant are limited [65], clinical experience in patients with HIV infection and TB has shown equal efficacy between regimens containing rifabutin and those contacting rifampin [17, 66, 67]. It should be noted that with the use of either rifamycin, therapeutic drug monitoring (TDM) of immunosuppressants should be used, with appropriate dose adjustment at both the start and end of rifamycin therapy. There are reports of successful use of a non-rifamycin-based regimen for management of post-transplant TB, especially where rifabutin is not available [25, 68, 69].

In many SOT and HSCT recipients, the use of mold-active azoles are quite common, which also poses a challenge due to drug-drug interactions with rifamycins. Rifamycins reduce the serum levels of mold-active azoles; careful TDM is required for achieving therapeutic levels of voriconazole or posaconazole. Isavuconazole may need to be avoided due to lack of widely available TDM. One should note that rifabutin levels increase with the use of mold-active azoles, due to the bidirectional interactions which can pose an additional challenge [70].

Fluoroquinolones including moxifloxacin and levofloxacin have potent activity against MTB in vitro and are currently listed as second-line agents for active TB treatment [8]. These medications may be used in transplant patients with poor liver function and with those who develop hepatotoxicity on standard TB therapy [8]. Although systematic studies of second-line TB therapy in transplant recipients are not available, published case reports indicate that capreomycin, cycloserine, and moxifloxacin have been successfully used [71–73]. Recently, a WHO workshop examined the evidence for dosing of second-line agents for TB. Where there were gaps in knowledge, analyses using pharmacokinetics, pharmacodynamics, and computational and artificial intelligence algorithms were used to generate data to provide guidance on drug combinations and effective dosing [74].

When planning medical therapy for the treatment of active TB, a careful review of all drug-drug interactions is recommended. The hepatotoxicity of isoniazid, rifampin, and pyrazinamide used in combination is greater than isoniazid alone and noted to be particularly severe in liver recipients [75]. A recent review noted overall hepatotoxicity with TB therapy of 20% across all organs and 27.5% in liver transplant recipients [25]. For this reason, liver function tests should be closely

monitored in all transplant recipients during treatment of TB. If pyrazinamide is omitted due to severe baseline liver disease, treatment should be extended to 9 months; if both isoniazid and pyrazinamide are avoided, one could use rifampin, ethambutol, and levofloxacin or moxifloxacin with the regimen extended to 12 months [8]. Of note, among first-line drugs, ethambutol and pyrazinamide require dose adjustment with renal insufficiency, and aminoglycosides used as second-line drugs can cause renal dysfunction.

The recommended duration of treatment for transplant patients is similar to other patients, although longer durations should always be considered if the response to treatment is slow. Extended treatment durations are strongly recommended if second-line drugs are used or if resistance to rifampin and other drugs is documented. Uncomplicated pulmonary TB with sputum clearance before the completion of 2 months of treatment can complete therapy at 6 months; however, bone and joint disease (6–9 months), central nervous system disease (9–12 months), and severe disseminated disease (6–9 months) are treated longer. In cases of cavitary pulmonary TB with persistent TB culture-positive sputum at 2 months, 9 months of treatment is recommended. [17]. Treatment durations of TB after SOT reported in the literature range between 7.3 and 18 months [25]. Fortunately, to date multidrug- and extremely drug-resistant TB remains rare in the transplant population [25]. Recommended durations for treatment of MDR and XDR TB are 20 months long; shorter durations of 10 months have recently been shown to be non-inferior, but have not been studied in transplant recipients [76].

Prevention/Treatment of Latent TB

Identifying and treating latent TB in the setting of transplantation is an important public health measure to reduce the incidence of TB reactivation in transplant recipients. Active TB in the post-transplant setting is difficult to diagnose and treat, can be transmitted to other immunosuppressed contacts, and contributes to morbidity and mortality after transplant [48, 77–79]. In highly endemic areas where TB transmission occurs in the community, universal isoniazid prophylaxis for the first year post-SOT has been studied to prevent TB acquisition during the period of maximum immunosuppression [14]. Please refer to Table 1 for treatment options for latent TB which currently have the greatest amount of data supporting their use;

Table 1 Regimens used for treatment of LTBI [92, 95, 96]

First-line regimens:	**Alternative regimens with disadvantages relative to first-line regimens:**
9H (INH x 9 months) [95]	6H (INH x 6 months) [95]
4R (RIF x 4 months) [95]	RFB x 4 months [95]
3HP (weekly INH/RPT x 12 doses) [95]	3HR (INH/RIF x 3 months) [92, 96]
	4HR (INH/RIF x 4 months) [96]

INH isoniazid, *RIF* rifampin, *RPT* rifapentine, *RFB* rifabutin

however, there is still a paucity of information regarding use in transplant candidates [78, 80–82].

The mainstay of LTBI treatment is isoniazid, with data showing a low risk of isoniazid-related hepatotoxicity in renal transplant recipients without serious underlying liver disease [83] and in patients with compensated liver disease awaiting liver transplantation [84, 85]. Isoniazid preventive treatment for 9 months confers additional benefit over 6 months [78]. Pyridoxine (vitamin B6) 25–50 mg daily should be administered with isoniazid to decrease the risk of neurotoxicity. A 4-month course of rifampin monotherapy can be used for the treatment of latent TB [86, 87] and may be preferred in patients with some underlying liver disease who can complete therapy prior to transplantation. It is limited by drug-drug interactions that preclude continuation of treatment post-transplant for LTBI therapy. A fairly new regimen for treatment of LTBI is a 12-week course of isoniazid and rifapentine [88]. It has been used successfully in renal transplant candidates, with close monitoring of blood pressure due to drug interactions with antihypertensives [89]. Use of this regimen after transplantation is still limited by severe drug interactions between rifamycins and immunosuppressive agents. A clinical trial of levofloxacin for LTBI therapy in transplant candidates was stopped due to development of tenosynovitis; therefore, quinolones are not recommended as first-line therapy [15]. Recently, a 1-month regimen of isoniazid and rifapentine was studied in patients with HIV infection in a TB-endemic region [90]. This has yet to be studied in transplant candidates. Finally, if no treatment is possible, then careful clinical follow-up with prompt diagnostic attention to protracted fever or pulmonary symptoms may be the best course.

The timing of LTBI therapy must be individualized based on the urgency and anticipated timing of transplant and risk of progression to active TB. LTBI treatment should be considered more urgently in those with recent exposure to TB and conversion of the TST or IGRA within the previous year. Since those on hemodialysis are at increased risk for active TB and renal transplant candidates awaiting deceased donor transplants often have long waiting times, they should generally be treated prior to transplant [91]. In patients with chronic lung disease awaiting lung transplantation, it is often preferable to begin treatment prior to transplant once active disease is ruled out [92]. While LTBI treatment is safe in many liver transplant candidates with compensated cirrhosis [84, 85], in those with decompensated liver disease, it may be preferable to delay the administration of isoniazid until after transplantation, when liver function is improved [93]. It should be noted that liver transplant recipients treated with isoniazid who present with elevated serum transaminase levels should be evaluated for a specific diagnosis with liver biopsy, if necessary, without automatically ascribing the effect to isoniazid. HSCT recipients could be started or resumed on LTBI therapy after resolution of mucositis.

Regular clinical and laboratory monitoring of transplant candidates and recipients being treated for LTBI is extremely important. When isoniazid treatment is initiated, it is suggested to obtain at least a blood test for ALT at 2-week intervals for 6 weeks, followed by monthly testing. If transaminases are elevated to 1.5–3 times normal, isoniazid does not require immediate discontinuation, but needs ongoing frequent

laboratory monitoring. If transaminases are increased threefold with signs and symptoms of hepatotoxicity, or there is an asymptomatic fivefold elevation, LTBI treatment should be discontinued [78, 80]. Organ transplantation does not need to be delayed until completion of LTBI therapy. Latent TB treatment with isoniazid can be resumed after transplant once medically possible and the course of therapy can be completed.

Conclusion

Development of diagnostic tests for LTBI that do not rely on an intact T-cell response in the face of organ failure and immunosuppression would greatly improve diagnosis and clinical management, especially in the case of donor-derived infections. Hence, development of new diagnostics based on TB biomarkers, cell-free DNA, and host response gene signatures are very promising. Another important advance would be the development and/or clinical validation of antituberculous drugs that are free of significant organ toxicities and drug-drug interactions. New treatment regimens are on the horizon, including potent drugs that may have the potential to shorten and simplify anti-TB therapy [4]. Evaluation of these in transplant candidates and recipients may provide useful treatment alternatives for this population in the future. Lastly, new TB vaccines are being studied with some promising results, which may eventually lead to more effective prevention strategies [94].

References

1. Munoz P, Rodriguez C, Bouza E. *Mycobacterium tuberculosis* infection in recipients of solid organ transplants. Clin Infect Dis. 2005;40(4):581–7.
2. Subramanian AK, Morris MI, Practice ASTIDCo. *Mycobacterium tuberculosis* infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):68–76.
3. Singh N, Paterson DL. *Mycobacterium tuberculosis* infection in solid-organ transplant recipients: impact and implications for management. Clin Infect Dis. 1998;27(5):1266–77.
4. Ginsberg AM, Spigelman M. Challenges in tuberculosis drug research and development. Nat Med. 2007;13(3):290–4.
5. Horne DJ, Narita M, Spitters CL, Parimi S, Dodson S, Limaye AP. Challenging issues in tuberculosis in solid organ transplantation. Clin Infect Dis. 2013;57(10):1473–82.
6. Dorman SE, Chaisson RE. From magic bullets back to the magic mountain: the rise of extensively drug-resistant tuberculosis. Nat Med. 2007;13(3):295–8.
7. Huaman MA, Brawley R, Ashkin D. Multidrug-resistant tuberculosis in transplant recipients: case report and review of the literature. Transpl Infect Dis. 2017;19(2)
8. Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A, et al. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America clinical practice guidelines: treatment of drug-susceptible tuberculosis. Clin Infect Dis. 2016;63(7):e147–e95.
9. Aguado JM, Torre-Cisneros J, Fortun J, Benito N, Meije Y, Doblas A, et al. Tuberculosis in solid-organ transplant recipients: consensus statement of the group for the study of infection in transplant recipients (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology. Clin Infect Dis. 2009;48(9):1276–84.

10. Bumbacea D, Arend SM, Eyuboglu F, Fishman JA, Goletti D, Ison MG, et al. The risk of tuberculosis in transplant candidates and recipients: a TBNET consensus statement. Eur Respir J. 2012;40(4):990–1013.
11. Subramanian AK, Theodoropoulos NM. *Mycobacterium tuberculosis* infections in solid organ transplantation: guidelines from the infectious diseases community of practice of the American Society of Transplantation. Clin Transpl. 2019:e13513.
12. Currie AC, Knight SR, Morris PJ. Tuberculosis in renal transplant recipients: the evidence for prophylaxis. Transplantation. 2010;90(7):695–704.
13. Vikrant S, Agarwal SK, Gupta S, Bhowmik D, Tiwari SC, Dash SC, et al. Prospective randomized control trial of isoniazid chemoprophylaxis during renal replacement therapy. Transpl Infect Dis. 2005;7(3–4):99–108.
14. Naqvi R, Akhtar S, Noor H, Saeed T, Bhatti S, Sheikh R, et al. Efficacy of isoniazid prophylaxis in renal allograft recipients. Transplant Proc. 2006;38(7):2057–8.
15. Torre-Cisneros J, San-Juan R, Rosso-Fernandez CM, Silva JT, Munoz-Sanz A, Munoz P, et al. Tuberculosis prophylaxis with levofloxacin in liver transplant patients is associated with a high incidence of tenosynovitis: safety analysis of a multicenter randomized trial. Clin Infect Dis. 2015;60(11):1642–9.
16. Kim SH, Lee SO, Park IA, Kim SM, Park SJ, Yun SC, et al. Isoniazid treatment to prevent TB in kidney and pancreas transplant recipients based on an interferon-gamma-releasing assay: an exploratory randomized controlled trial. J Antimicrob Chemother. 2015;70(5):1567–72.
17. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111–5.
18. Comstock GW. Epidemiology of tuberculosis. Am Rev Respir Dis. 1982;125(3 Pt 2):8–15.
19. MacNeil A, Glaziou P, Sismanidis C, Maloney S, Floyd K. Global epidemiology of tuberculosis and Progress toward achieving global targets – 2017. MMWR Morb Mortal Wkly Rep. 2019; 68(11):263–6.
20. Sakhuja V, Jha V, Varma PP, Joshi K, Chugh KS. The high incidence of tuberculosis among renal transplant recipients in India. Transplantation. 1996;61(2):211–5.
21. Jeong JC, Koo TY, Jeon HJ, Park HC, Ryu HJ, Lee JP, et al. Utility of QuantiFERON-TB assay for prediction of tuberculosis development in kidney transplant patients in an intermediate-tuberculosis-burden country: lack of evidence for enhanced prediction for short-term tuberculosis development. Transplant Proc. 2014;46(2):583–7.
22. Jung JY, Joo DJ, Lee CH, Park MS, Kim YS, Kim MS, et al. Pre-transplant risk factors for tuberculosis after kidney transplant in an intermediate burden area. Int J Tuberc Lung Dis. 2012;16(2):248–54.
23. Marques ID, Azevedo LS, Pierrotti LC, Caires RA, Sato VA, Carmo LP, et al. Clinical features and outcomes of tuberculosis in kidney transplant recipients in Brazil: a report of the last decade. Clin Transpl. 2013;27(2):E169–76.
24. Torre-Cisneros J, Doblas A, Aguado JM, San Juan R, Blanes M, Montejo M, et al. Tuberculosis after solid-organ transplant: incidence, risk factors, and clinical characteristics in the RESITRA (Spanish network of infection in transplantation) cohort. Clin Infect Dis. 2009;48 (12):1657–65.
25. Abad CLR, Razonable RR. *Mycobacterium tuberculosis* after solid organ transplantation: a review of more than 2000 cases. Clin Transpl. 2018:e13259.
26. Chen CY, Liu CJ, Feng JY, Loong CC, Liu C, Hsia CY, et al. Incidence and risk factors for tuberculosis after liver transplantation in an endemic area: a Nationwide population-based matched cohort study. Am J Transplant. 2015;15(8):2180–7.
27. Agrawal N, Aggarwal M, Kapoor J, Ahmed R, Shrestha A, Kaushik M, et al. Incidence and clinical profile of tuberculosis after allogeneic stem cell transplantation. Transpl Infect Dis. 2018;20(1)
28. Aki SZ, Sucak GT, Tunccan OG, Kokturk N, Senol E. The incidence of tuberculosis infection in hematopoietic stem cell transplantation recipients: a retrospective cohort study from a center in Turkey. Transpl Infect Dis. 2018;20(4):e12912.

29. Cheng MP, Kusztos AE, Bold TD, Ho VT, Glotzbecker BE, Hsieh C, et al. Risk of Latent Tuberculosis Reactivation After Hematopoietic cell Transplantation. 2019.
30. Liu YC, Wu CJ, Ko PS, Chien SH, Fan NW, Wang HY, et al. Mycobacterial infections in adult recipients of allogeneic hematopoietic stem cell transplantation: a cohort study in a high endemic area. J Microbiol Immunol Infect = Wei mian yu gan ran za zhi. 2018;
31. Fan WC, Liu CJ, Hong YC, Feng JY, Su WJ, Chien SH, et al. Long-term risk of tuberculosis in haematopoietic stem cell transplant recipients: a 10-year nationwide study. Int J Tuberc Lung Dis. 2015;19(1):58–64.
32. Abad CLR, Razonable RR. An update on *Mycobacterium tuberculosis* infection after hematopoietic stem cell transplantation in adults. Clin Transpl. 2018;32(12):e13430.
33. Munoz L, Gomila A, Casas S, Castellote J, Arnan M, Rafecas A, et al. Immunodiagnostic Tests' predictive values for progression to tuberculosis in transplant recipients: a prospective cohort study. Transplant Direct. 2015;1(3):e12.
34. Kim SH, Lee SO, Park JB, Park IA, Park SJ, Yun SC, et al. A prospective longitudinal study evaluating the usefulness of a T-cell-based assay for latent tuberculosis infection in kidney transplant recipients. Am J Transplant. 2011;11(9):1927–35.
35. Kay A, Barry PM, Annambhotla P, Greene C, Cilnis M, Chin-Hong P, et al. Solid organ transplant-transmitted tuberculosis linked to a community outbreak – California, 2015. MMWR Morb Mortal Wkly Rep. 2017;66(30):801–5.
36. Kumar D, Budev M, Koval C, Hellinger WC, Gordon SM, Tomford JW. Donor-derived tuberculosis (TB) infection in lung transplant despite following recommended algorithm. Am J Transplant. 2013;13(8):2225–6.
37. Morris MI, Daly JS, Blumberg E, Kumar D, Sester M, Schluger N, et al. Diagnosis and management of tuberculosis in transplant donors: a donor-derived infections consensus conference report. Am J Transplant. 2012;12(9):2288–300.
38. Jereb JA, Burwen DR, Dooley SW, Haas WH, Crawford JT, Geiter LJ, et al. Nosocomial outbreak of tuberculosis in a renal transplant unit: application of a new technique for restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates. J Infect Dis. 1993;168(5):1219–24.
39. Guirao-Arrabal E, Santos F, Redel-Montero J, Vaquero JM, Cantisan S, Vidal E, et al. Risk of tuberculosis after lung transplantation: the value of pretransplant chest computed tomography and the impact of mTOR inhibitors and azathioprine use. Transpl Infect Dis. 2016;18(4):512–9.
40. Torres J, Aguado JM, San Juan R, Andres A, Sierra P, Lopez-Medrano F, et al. Hepatitis C virus, an important risk factor for tuberculosis in immunocompromised: experience with kidney transplantation. Transpl Int. 2008;21(9):873–8.
41. Lee HJ, Lee DG, Choi SM, Park SH, Cho SY, Choi JK, et al. The demanding attention of tuberculosis in allogeneic hematopoietic stem cell transplantation recipients: high incidence compared with general population. PLoS One. 2017;12(3):e0173250.
42. Sloot R, Schim van der Loeff MF, Kouw PM, Borgdorff MW. Risk of tuberculosis after recent exposure. A 10-year follow-up study of contacts in Amsterdam. Am J Respir Crit Care Med. 2014;190(9):1044–52.
43. Horsburgh CR Jr, Rubin EJ. Clinical practice. Latent tuberculosis infection in the United States. N Engl J Med. 2011;364(15):1441–8.
44. Giacomelli IL, Schuhmacher Neto R, Nin CS, Cassano PS, Pereira M, Moreira JDS, et al. High-resolution computed tomography findings of pulmonary tuberculosis in lung transplant recipients. J Bras Pneumol. 2017;43(4):270–3.
45. Schuhmacher Neto R, Giacomelli IL, Schuller Nin C, da Silva Moreira J, Comaru Pasqualotto A, Marchiori E, et al. High-resolution CT findings of pulmonary tuberculosis in liver transplant patients. Clin Radiol. 2017;72(10):899 e9–e14.
46. Lopez de Castilla D, Schluger NW. Tuberculosis following solid organ transplantation. Transpl Infect Dis. 2010;12(2):106–12.
47. Abad CLR, Razonable RR. Donor derived *Mycobacterium tuberculosis* infection after solid-organ transplantation: a comprehensive review. Transpl Infect Dis. 2018;20(5):e12971.

48. Holty JE, Sista RR. *Mycobacterium tuberculosis* infection in transplant recipients: early diagnosis and treatment of resistant tuberculosis. Curr Opin Organ Transplant. 2009;14(6):613–8.
49. Pfyffer GE, Welscher HM, Kissling P, Cieslak C, Casal MJ, Gutierrez J, et al. Comparison of the mycobacteria growth Indicator tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J Clin Microbiol. 1997;35(2):364–8.
50. Bartoletti M, Martelli G, Tedeschi S, Morelli M, Bertuzzo V, Tadolini M, et al. Liver transplantation is associated with good clinical outcome in patients with active tuberculosis and acute liver failure due to anti-tubercular treatment. Transpl Infect Dis. 2017;19(2)
51. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection – United States, 2010. MMWR Recomm Rep. 2010;59(RR-5):1–25.
52. Sester U, Wilkens H, van Bentum K, Singh M, Sybrecht GW, Schafers HJ, et al. Impaired detection of *Mycobacterium tuberculosis* immunity in patients using high levels of immunosuppressive drugs. Eur Respir J. 2009;34(3):702–10.
53. Casas S, Munoz L, Moure R, Castellote J, Guerra MR, Gonzalez L, et al. Comparison of the 2-step tuberculin skin test and the quantiFERON-TB gold in-tube test for the screening of tuberculosis infection before liver transplantation. Liver Transpl. 2011;17(10):1205–11.
54. Hand J, Sigel K, Huprikar S, Hamula C, Rana M. Tuberculosis after liver transplantation in a large center in New York City: QuantiFERON((R)) -TB Gold-based pre-transplant screening performance and active tuberculosis post-transplant. Transpl Infect Dis. 2018;20(2):e12845.
55. Lyu J, Lee SG, Hwang S, Lee SO, Cho OH, Chae EJ, et al. Chest computed tomography is more likely to show latent tuberculosis foci than simple chest radiography in liver transplant candidates. Liver Transpl. 2011;17(8):963–8.
56. Munoz L, Santin M. Prevention and Management of Tuberculosis in transplant recipients: from guidelines to clinical practice. Transplantation. 2016;100(9):1840–52.
57. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.
58. Manson AL, Abeel T, Galagan JE, Sundaramurthi JC, Salazar A, Gehrmann T, et al. *Mycobacterium tuberculosis* whole genome sequences from southern India suggest novel resistance mechanisms and the need for region-specific diagnostics. Clin Infect Dis. 2017;64(11):1494–501.
59. Broger T, Tsionksy M, Mathew A, Lowary TL, Pinter A, Plisova T, et al. Sensitive electrochemiluminescence (ECL) immunoassays for detecting lipoarabinomannan (LAM) and ESAT-6 in urine and serum from tuberculosis patients. PLoS One. 2019;14(4):e0215443.
60. Fernandez-Carballo BL, Broger T, Wyss R, Banaei N, Denkinger CM. Toward the development of a circulating free DNA-based in vitro diagnostic test for infectious diseases: a review of evidence for tuberculosis. J Clin Microbiol. 2019;57(4)
61. Warsinske H, Vashisht R, Khatri P. Host-response-based gene signatures for tuberculosis diagnosis: a systematic comparison of 16 signatures. PLoS Med. 2019;16(4):e1002786.
62. Warsinske HC, Rao AM, Moreira FMF, Santos PCP, Liu AB, Scott M, et al. Assessment of validity of a blood-based 3-gene signature score for progression and diagnosis of tuberculosis, disease severity, and treatment response. JAMA Netw Open. 2018;1(6):e183779.
63. Chang KC, Leung CC, Yew WW, Chan SL, Tam CM. Dosing schedules of 6-month regimens and relapse for pulmonary tuberculosis. Am J Respir Crit Care Med. 2006;174(10):1153–8.
64. McGregor MM, Olliaro P, Wolmarans L, Mabuza B, Bredell M, Felten MK, et al. Efficacy and safety of rifabutin in the treatment of patients with newly diagnosed pulmonary tuberculosis. Am J Respir Crit Care Med. 1996;154(5):1462–7.
65. Lopez-Montes A, Gallego E, Lopez E, Perez J, Lorenzo I, Llamas F, et al. Treatment of tuberculosis with rifabutin in a renal transplant recipient. Am J Kidney Dis. 2004;44(4):e59–63.
66. Regazzi M, Carvalho AC, Villani P, Matteelli A. Treatment optimization in patients co-infected with HIV and *Mycobacterium tuberculosis* infections: focus on drug-drug interactions with rifamycins. Clin Pharmacokinet. 2014;53(6):489–507.

67. Cohen K, Meintjes G. Management of individuals requiring antiretroviral therapy and TB treatment. Curr Opin HIV AIDS. 2010;5(1):61–9.
68. Vachharajani TJ, Oza UG, Phadke AG, Kirpalani AL. Tuberculosis in renal transplant recipients: rifampicin sparing treatment protocol. Int Urol Nephrol. 2002;34(4):551–3.
69. Jha V, Sakhuja V, Gupta D, Krishna VS, Chakrabarti A, Joshi K, et al. Successful management of pulmonary tuberculosis in renal allograft recipients in a single center. Kidney Int. 1999; 56(5):1944–50.
70. Baciewicz AM, Chrisman CR, Finch CK, Self TH. Update on rifampin, rifabutin, and rifapentine drug interactions. Curr Med Res Opin. 2013;29(1):1–12.
71. Jung BH, Park JI, Lee SG. Urgent living-donor liver transplantation in a patient with concurrent active tuberculosis: a case report. Transplant Proc. 2018;50(3):910–4.
72. Regmi A, Singh P, Harford A. A case of multidrug-resistant monoarticular joint tuberculosis in a renal transplant recipient. Transplant Proc. 2014;46(1):274–7.
73. Garcia-Goez JF, Munera GA, Rojas V, Pacheco R, Cayla JA, Miro JM. Tuberculosis in recipients of solid-organ transplants during 1995–2015 in Cali, Colombia. Int J Tuberc Lung Dis. 2017;21(11):1155–9.
74. Gumbo T, Alffenaar JC. Pharmacokinetic/Pharmacodynamic background and methods and scientific evidence base for dosing of second-line tuberculosis drugs. Clin Infect Dis. 2018; 67(suppl_3):S267–s73.
75. Schluger LK, Sheiner PA, Jonas M, Guarrera JV, Fiel IM, Meyers B, et al. Isoniazid hepatotoxicity after orthotopic liver transplantation. Mt Sinai J Med. 1996;63(5–6):364–9.
76. Nunn AJ, Phillips PPJ, Meredith SK, Chiang CY, Conradie F, Dalai D, et al. A trial of a shorter regimen for rifampin-resistant tuberculosis. N Engl J Med. 2019;380(13):1201–13.
77. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: controlling tuberculosis in the United States. Am J Respir Crit Care Med. 2005;172(9):1169–227.
78. Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, et al. Management of latent *Mycobacterium tuberculosis* infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J. 2015;46(6):1563–76.
79. Holty JE, Gould MK, Meinke L, Keeffe EB, Ruoss SJ. Tuberculosis in liver transplant recipients: a systematic review and meta-analysis of individual patient data. Liver Transpl. 2009;15(8):894–906.
80. Blumberg HM, Leonard MK Jr, Jasmer RM. Update on the treatment of tuberculosis and latent tuberculosis infection. JAMA. 2005;293(22):2776–84.
81. Menzies D, Al Jahdali H, Al Otaibi B. Recent developments in treatment of latent tuberculosis infection. Indian J Med Res. 2011;133:257–66.
82. Jafri SM, Singal AG, Kaul D, Fontana RJ. Detection and management of latent tuberculosis in liver transplant patients. Liver Transpl. 2011;17(3):306–14.
83. Antony SJ, Ynares C, Dummer JS. Isoniazid hepatotoxicity in renal transplant recipients. Clin Transpl. 1997;11(1):34–7.
84. Singh N, Wagener MM, Gayowski T. Safety and efficacy of isoniazid chemoprophylaxis administered during liver transplant candidacy for the prevention of posttransplant tuberculosis. Transplantation. 2002;74(6):892–5.
85. Jahng AW, Tran T, Bui L, Joyner JL. Safety of treatment of latent tuberculosis infection in compensated cirrhotic patients during transplant candidacy period. Transplantation. 2007;83(12):1557–62.
86. Aspler A, Long R, Trajman A, Dion MJ, Khan K, Schwartzman K, et al. Impact of treatment completion, intolerance and adverse events on health system costs in a randomised trial of 4 months rifampin or 9 months isoniazid for latent TB. Thorax. 2010;65(7):582–7.
87. Menzies D, Adjobimey M, Ruslami R, Trajman A, Sow O, Kim H, et al. Four months of rifampin or nine months of isoniazid for latent tuberculosis in adults. N Engl J Med. 2018;379(5):440–53.

88. Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, Bliven-Sizemore E, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med. 2011;365(23):2155–66.
89. Simkins J, Abbo LM, Camargo JF, Rosa R, Morris MI. Twelve-week Rifapentine plus isoniazid versus 9-month isoniazid for the treatment of latent tuberculosis in renal transplant candidates. Transplantation. 2017;101(6):1468–72.
90. Swindells S, Ramchandani R, Gupta A, Benson CA, Leon-Cruz J, Mwelase N, et al. One month of Rifapentine plus isoniazid to prevent HIV-related tuberculosis. N Engl J Med. 2019;380(11): 1001–11.
91. Chitnis AS, Schecter GF, Cilnis M, Robsky K, Flood JM, Barry PM. Epidemiology of tuberculosis cases with end-stage renal disease, California, 2010. Am J Nephrol. 2014;39(4): 314–21.
92. Guirao-Arrabal E, Santos F, Redel J, Vaquero JM, Torre-Cisneros J. Efficacy and safety of short-term treatment with isoniazid and rifampicin for latent tuberculosis infection in lung transplant candidates. Clin Transpl. 2017;31(3)
93. Moon HH, Park SY, Kim JM, Park JB, Kwon CHD, Peck KR, et al. Isoniazid prophylaxis for latent tuberculosis infections in liver transplant recipients in a tuberculosis-endemic area. Ann Transplant. 2017;22:338–45.
94. Van Der Meeren O, Hatherill M, Nduba V, Wilkinson RJ, Muyoyeta M, Van Brakel E, et al. Phase 2b controlled trial of M72/AS01E vaccine to prevent tuberculosis. N Engl J Med. 2018;379(17):1621–34.
95. Centers for Disease Control and Prevention: division of tuberculosis elimination. Latent tuberculosis infection: a guide for primary health care providers, treatment of latent TB infection 2016 updated August 5, 2016. Available from: http://www.cdc.gov/tb/publications/ltbi/treatment.htm
96. Spyridis NP, Spyridis PG, Gelesme A, Sypsa V, Valianatou M, Metsou F, et al. The effectiveness of a 9-month regimen of isoniazid alone versus 3- and 4-month regimens of isoniazid plus rifampin for treatment of latent tuberculosis infection in children: results of an 11-year randomized study. Clin Infect Dis. 2007;45(6):715–22.

Rapidly Growing Mycobacterial Infections in Transplant: Evolving Epidemiology and Treatment Options

19

Nicholas A. Turner and Arthur W. Baker

Contents

Introduction 426
Disease Epidemiology 426
Diagnosis 433
Diagnostic Challenges 433
Species Identification 434
Management 435
The Role for Macrolides 437
Species- and Site-Specific Treatment Considerations 438
Role for Surgery in Extrapulmonary RGM Disease 443
Catheter-Associated Infections 443
Treatment of Less Common RGM Isolates 443
Management Considerations Unique to Transplant Recipients 444
Prevention 447
Clinical Impact in Transplant 448
Impact on Transplant Candidacy 448
Impact on Transplant Recipients and Transplant Centers 449
Conclusion 450
Key Points 450
Cross-References 450
References 451

N. A. Turner · A. W. Baker (✉)
Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, USA

Duke Center for Antimicrobial Stewardship and Infection Prevention, Durham, NC, USA
e-mail: nick.turner@duke.edu; arthur.baker@duke.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_16

Introduction

Nontuberculous mycobacteria (NTM) are widespread in the environment, especially in water-associated biofilms [1]. Rapidly growing mycobacteria (RGM) are a subset of NTM species capable of growing in the laboratory within 7 days. For discussion of other mycobacteria, refer to ▶ Chaps. 18, "*Mycobacterium tuberculosis* in Transplantation," and ▶ 20, "Slow-Growing Nontuberculous Mycobacteria in Transplant." While overall rare, prevalence of RGM infections has increased in recent years both in the United States and throughout the world [2–4]. Immunosuppression and high rates of airway colonization among patients with underlying structural lung disease increase risk of RGM infection among transplant recipients [5, 6]. Transplant patients commonly acquire RGM from the community; however, several recent healthcare facility-associated RGM outbreaks highlight increasing potential for transmission within the modern healthcare system [7–9]. Outbreaks have generated important questions regarding the need for healthcare facility surveillance and infection prevention measures to decrease risk of RGM transmission, particularly for high-risk transplant recipients. Outbreaks have also helped us to better understand diagnosis and management strategies in the immunosuppressed host.

RGM infections classically cause pulmonary disease in patients with abnormal lung architecture, as well as postoperative skin and soft tissue infections [10]. Diagnosis of RGM infection is often challenging, especially in transplant recipients, due to highly variable and indolent clinical presentations. Accurate species identification and susceptibility testing of RGM requires special expertise but provides important data to guide therapy and should be pursued where laboratory capability exists. Treatment is complex, typically requiring months of prolonged combination antimicrobial therapy. Adjunctive surgical management plays an important role in cases of soft tissue disease, osteomyelitis, and involvement of prosthetic material or, at times, for localized pulmonary disease. Clinical outcomes are often poor and can be complicated by antibiotic resistance and toxicities, drug interactions, treatment failure, relapse of infection, and need for long-term step-down or suppressive therapy. A few novel antibiotics with activity against RGM have shown promise *in vitro* or in small clinical studies; however, additional clinical data and treatment options are needed for transplant recipients with RGM infections. We will discuss many of these options in this chapter, highlighting available data and limitations.

Disease Epidemiology

RGM (recognized in some classifications as Runyon group IV) consist of more than 75 described species, classified within 6 major groups: *M. fortuitum, M. chelonae/M. abscessus*, *M. smegmatis, M. mucogenicum*, *M. mageritense/wolinskyi*, and the pigmented species [1, 11]. *M. abscessus* contains three related subspecies: subspecies *abscessus* (formerly *M. abscessus*), *massiliense*, and *bolletii* [1]. RGM are widely distributed in the environment, especially water and soil, are adept at forming

biofilms, and are highly resistant to many common disinfectants and antibiotics [1]. While case reports implicate species from each of the six major RGM groups as potential pathogens, the majority of RGM infections are caused by *M. abscessus*, *M. chelonae*, and *M. fortuitum*. See Table 1 for a detailed listing of recognized clinical syndromes.

RGM cause a wide range of infections, including pulmonary and extrapulmonary infections. Common extrapulmonary infections include skin and soft tissue infection, surgical site infections, osteomyelitis, and disseminated disease, including mycobacteremia. In addition, RGM adhere to foreign material and can cause infections involving prosthetic material, such as central line-associated bloodstream infections (CLABSI) [45], peritoneal dialysis catheter infections [55], and infections of cardiac devices, including ventricular assist devices and other implantable electronic devices [56, 57].

Infections caused by RGM occur worldwide. Variable definitions of incidence or prevalence hinder direct comparisons across regions; however, one of the more widely-available measures is the proportion of RGM among NTM pulmonary isolates, which ranges from 4% to 28% in South America, 6% to 62% in Europe, 9% to 59% in the Middle East, 20% to 60% in Asia, 5% to 10% in Australia, and 5% to 21% in North America [58]. Within the United States, prevalence of *M. abscessus* and *M. chelonae* is highest in the West and South [59]. Several recent studies have suggested that the prevalence of disease caused by RGM is increasing [3, 4]. For example, a US single-site longitudinal survey reported a threefold increase in cutaneous NTM infections from 2000 to 2009, with *M. abscessus* and *M. chelonae* comprising nearly 32% of isolates. Approximately 10% of patients analyzed in this study were solid organ transplant (SOT) recipients.

Defining the true prevalence of RGM infection among transplant patients is challenging, in part because NTM frequently colonize the respiratory tract in the absence of invasive pulmonary infection. However, mycobacteria found in other tissues constitute invasive infection. Epidemiologic studies have been limited by small cohort sizes and inconsistent definitions of disease, stratification of NTM rates by species, and rate calculations. Among transplant recipients, lung and heart transplant patients have the highest rates of NTM infection [60]. Two recent cohort studies of lung transplant recipients reported prevalence rates for RGM pulmonary disease of 1–2%, with a median time to diagnosis between 2 and 3 years post-transplant [5, 12]. Both cohorts found that greater than 80% of NTM pulmonary isolates obtained from lung transplant recipients represented transient colonization and did not require antimicrobial therapy. When invasive infection does occur in lung transplant recipients, pleuropulmonary disease from *M. abscessus* is most common [6, 33, 61]. Prevalence rates of NTM infection reported for heart transplant recipients range from 0.2% to 3%; extrapulmonary infection, including disseminated disease, is more common than isolated pulmonary infection [60, 62].

Compared to lung or heart transplant recipients, the risk of RGM infection is lower for other SOT and hematopoietic cell transplantation (HCT) recipients. Prevalence rates of NTM infection, including both rapidly and slow-growing NTM, have been reported at $<0.1\%$ in liver transplant recipients, $<0.2\%$ in renal transplant

Table 1 Types of rapidly growing mycobacteria and associated clinical syndromes

Group	Species	Pathogenicity	Types of infections
M. chelonae/abscessus	*M. abscessus* subsp. *abscessus*	Commonly reported [7, 12–19]	Pulmonary, lymphadenitis, soft tissue/bone, disseminated
	M. abscessus subsp. *bolletii*	Commonly reported [20]	Pulmonary
	M. abscessus subsp. *massiliense*	Commonly reported [21–26]	Pulmonary, disseminated
	M. chelonae	Commonly reported [12, 27–30]	Pulmonary, lymphadenitis, soft tissue/bone, disseminated
	M. immunogenum	Sporadically reported [31]	Disseminated, pseudo-outbreaks
	M. franklinii	Rarely reported [32]	Sinopulmonary disease
M. fortuitum	*M. fortuitum*	Commonly reported [33–35]	Pulmonary, soft tissue/bone, disseminated, CLABSI
	M. peregrinum	Rarely reported [36]	Pulmonary
	M. senegalense	Rarely reported [37]	CLABSI
	M. porcinum	Rarely reported [38]	Pulmonary
	M. boenickei	Rarely reported [39]	Soft tissue/bone
	M. houstonense	Rarely reported [39]	Soft tissue/bone
	M. brisbanense	Rarely reported [40]	Pulmonary
	M. septicum	Rarely reported [39]	CLABSI
	M. setense	Rarely reported [41]	Soft tissue/bone
M. smegmatis	*M. smegmatis*	Rarely reported [42]	Soft tissue/bone
	M. goodii	Rarely reported [43, 44]	Device infections
M. mucogenicum	*M. mucogenicum*	Sporadically reported [34, 45, 46]	Disseminated, CLABSI
	M. phocaicum	Rarely reported [47]	CLABSI
M. mageritense/wolinskyi	*M. mageritense*	Sporadically reported [48–50]	Skin/soft tissue, CLABSI, prosthetic valve infections
	M. wolinskyi	Rarely reported [51]	Surgical site infections, device infections
Pigmented group	*M. canariasense*	Rarely reported [45]	CLABSI
	M. cosmeticum	Rarely reported [52]	Prosthetic joint infections
	M. monacense	Rarely reported [53]	Soft tissue
	M. bacteremicum	Rarely reported [54]	CLABSI
	M. neoaurum	Rarely reported [34]	CLABSI

CLABSI central line-associated bloodstream infection, *subsp.* subspecies

patients, and <0.5% in HCT patients [5, 60]. Risk of specific clinical syndromes also varies depending on transplant type. For example, RGM infection among HCT recipients commonly manifests as CLABSI (16,18).

Community acquisition of RGM occurs via a variety of mechanisms (Table 2). The municipal water supply is a well-known reservoir of NTM, and molecular epidemiologic studies have isolated the same genotype of RGM from patients with respiratory disease and their residential water outlets [63]. Notably, NTM are relatively chlorine tolerant, and many commonly used water treatment strategies do not prevent NTM colonization of water distribution systems [67]. Cutaneous RGM infections have been linked to a range of other exposures, including acupuncture, pedicures, tattooing, and cosmetic procedures [64, 65, 68, 69].

Hospital acquisition of RGM occurs most commonly when vulnerable patients are exposed to hospital water sources or equipment colonized with NTM (Table 2) [70, 71]. For example, investigators traced one US RGM outbreak occurring among pediatric HCT recipients to colonized ice machines, sinks, and showers [8, 9]. Similarly, a cluster of RGM CLABSIs among adult HCT patients receiving care at a hematology-oncology ward in Japan was linked to a hospital water supply colonized with RGM [45]. Multiple RGM outbreaks of surgical site infections have also been linked to contaminated hospital water sources, but some investigations have been unable to elucidate mechanisms of patient acquisition. A recent cluster of wound infections that occurred in patients who returned to the United States after undergoing cosmetic procedures in the Dominican Republic highlights the risk of postoperative infection and challenges of RGM outbreak investigations [72].

A recent, large, single-hospital outbreak of *M. abscessus* occurred in two distinct phases and affected numerous transplant recipients [7]. The first phase predominantly affected lung transplant patients with respiratory colonization or infection. Molecular fingerprinting confirmed that clinical *M. abscessus* isolates matched environmental isolates taken from biofilms of hospital water sources, including ice machines, water faucets, and showerheads. Use of sterile water, rather than tap water, for patient care tasks potentially associated with aerodigestive water exposure mitigated this phase of the outbreak. The second phase of the outbreak affected cardiac surgery patients, including heart transplant recipients, who developed mediastinal or bloodstream infections from *M. abscessus* after undergoing cardiopulmonary bypass. Infections were suspected to be caused by aerosolization of *M. abscessus* in the operating room from heater-cooler units (HCUs) that were filled with water taken from the colonized plumbing system. This phase of the outbreak resolved after institution of several interventions, including an enhanced disinfection protocol for HCUs and use of sterile water in these devices. Although risk of infection after cardiac surgery related to HCU colonization has been more widely reported for the slow-growing *M. chimaera* [73], *M. abscessus* outbreaks in cardiac surgery patients have demonstrated that RGM can be transmitted by the same mechanism.

Acquisition of RGM has historically been thought to occur via independent transmission events from the environment to patients; however, some recent studies have postulated indirect patient-to-patient transmission of *M. abscessus* [66]. In one large study, global whole genome sequencing (WGS) of *M. abscessus* isolates showed that a

Table 2 Summary of selected community and healthcare facility surveys and outbreak investigations involving rapidly growing mycobacteria

Setting	RGM sources	Study design	Methods/ description	Conclusions
Community	Municipal drinking water	Environmental survey [63]	Survey of *M. abscessus* isolates ($n = 74$) from potable water sources and human clinical isolates; all strains genotyped by rep-PCR	*M. abscessus* drinking water isolates were genetically similar to clinical isolates
	Tattoo ink	Outbreak investigation [64]	Analysis of a cluster of *M. chelonae* infections ($n = 19$) among tattoo recipients; clinical and tattoo ink isolates were compared by PFGE and genomic sequencing	Clinical isolates were identical to *M. chelonae* isolates obtained from premixed tattoo ink, confirming the ink as the outbreak source
	Pedicure baths	Post-outbreak systematic surveillance [65]	Surveillance conducted among pedicure salon clients following a series of cases of pedicure-associated RGM furunculosis ($n = 40$ cases); included comparison of clinical and salon environmental samples	Clinical outbreak consisted of predominantly *M. chelonae/ abscessus*, while multiple different RGM were recovered from salon pedicure baths Presence of biofilms and inadequate cleaning were strongly associated with infection risk
Healthcare-associated	(1) Ice machines (2) Water	Outbreak investigation followed by process improvement intervention [8, 9]	Outbreak investigation of RGM infections among pediatric hematopoietic	RGM isolates were traced to ice and water sources on hospital wards Use of bottled water for consumption and

(continued)

Table 2 (continued)

Setting	RGM sources	Study design	Methods/ description	Conclusions
			cell transplant recipients (n = 15)	installation of distal water outlet filters, copper tubing, and silver-impregnated water components mitigated the outbreak
	(1) Tap water (2) Cardiopulmonary bypass heater-cooler units	Outbreak investigation [7]	Epidemiologic investigation of a two-phase *M. abscessus* outbreak involving cardiothoracic surgery patients (n = 58 lung transplant, n = 8 heart transplant, and n = 4 other solid organ transplant recipients)	Outbreak investigation traced 2 distinct sources: (1) *M. abscessus* among lung transplant recipients: traced to ward tap water exposures; mitigated by elimination of aerodigestive exposure to tap water (2) *M. abscessus* among cardiac surgery patients: traced to heater-cooler units used in cardiopulmonary bypass; mitigated by use of sterile water and improved disinfection process for heater-cooler units
	Ward tap water	Outbreak investigation [45]	Outbreak investigation of RGM central line-associated bloodstream infections among adult hematology-oncology	Bloodstream infections involved uncommon RGM: *M. mucogenicum* (n = 4) and *M. canariasense* (n = 1)

(continued)

Table 2 (continued)

Setting	RGM sources	Study design	Methods/ description	Conclusions
			patients in Japan (n = 5); 16S sequencing used to match strains with environmental isolates	Isolates from tap water sources were matched to clinical isolates by 16S sequencing
	(1) Fomites (2) Aerosols (3) Clinic environment	Multicenter cluster analysis using whole genome sequencing [66]	Multicenter network study of clinical isolates from cystic fibrosis patients coupled with patient-level metadata	Genotyping demonstrated a wide circulation of a limited number of *M. abscessus* clones Embedded proof of concept studies suggested that fomites, aerosols, and clinic environment contamination may have contributed to transmission
	Clinic environment	Outbreak investigation [21]	Molecular epidemiologic investigation of *M. abscessus* subspecies *massiliense* outbreak at a lung transplant/ cystic fibrosis center (n = 5); strains genotyped by rep-PCR and PFGE	All clinical isolates were identical by rep-PCR and PFGE Sole source of shared contact appeared to be an outpatient pulmonary clinic, suggesting indirect transmission via environmental contamination

PFGE pulsed-field gel electrophoresis, *rep-PCR* repetitive element palindromic polymerase chain reaction, *RGM* rapidly growing mycobacteria

large proportion of patients with cystic fibrosis in the United States, Europe, and Australia shared a small number of very closely related *M. abscessus* strains. The authors concluded that widespread transmission of common *M. abscessus* clones may have occurred via fomites, surface contamination of the clinical environments, and generation of aerosols by cystic fibrosis patients. A smaller clonal outbreak of *M. abscessus* subspecies *massiliense* among five cystic fibrosis patients at a single

center similarly implicated contamination of the clinic environment as the most likely mechanism of transmission [21]. Due to concerns over possible healthcare-associated transmission of pulmonary *M. abscessus*, some centers segregate infected patients in single-use outpatient rooms and utilize negative pressure rooms for inpatient care [74]. However, other recent studies analyzing WGS of *M. abscessus* isolates concluded that patient-to-patient transmission of *M. abscessus* is quite uncommon [75, 76]. Hospitals and clinics do not commonly employ specialized infection control practices designed to decrease RGM transmission, and the benefit of such practices has not been proven.

Diagnosis

Diagnostic Challenges

Several factors make diagnosis of RGM infections difficult. First, infections are often indolent, have variable clinical presentations, and can involve a wide range of body sites [1]. Clinical manifestations include pulmonary disease, skin and soft tissue infection, lymphadenitis, osteomyelitis, tenosynovitis, catheter-associated infection, and disseminated disease [77]. Pulmonary disease commonly presents with chronic cough and dyspnea, often accompanied by nodular opacities, bronchiectasis, or cavities on chest imaging. Manifestations of skin and soft tissue RGM infections are particularly diverse and can include cellulitis, delayed surgical site infections, nonhealing ulcers, nodular lesions, lymphadenitis, folliculitis, or furuncles [78]. Particularly for immunosuppressed patients, presenting symptoms and exam findings are often insidious, appearing to represent superficial or limited infection despite the presence of invasive or disseminated disease (Fig. 1).

A second factor complicating RGM diagnosis is the difficulty in differentiating airway colonization from invasive pulmonary infection. The American Thoracic Society (ATS) criteria for NTM lung disease [10] require both clinical and microbiologic evidence of infection. Clinical criteria require that other diagnoses have been appropriately excluded and that patients have pulmonary symptoms, nodular or cavitary opacities on chest radiography, or bronchiectasis and multiple small nodules on high-resolution computed tomography imaging. Microbiologic criteria include either positive sputum cultures from two or more samples, positive culture from at least one bronchoalveolar lavage (BAL), or positive culture from a lung biopsy with consistent histopathologic features (e.g., granulomatous inflammation or acid fast bacilli [AFB] present on tissue stains). These criteria are difficult to apply to transplant recipients, especially lung transplant recipients who have high baseline prevalence of unrelated respiratory symptoms and abnormal lung imaging. Therefore, in addition to considering ATS criteria, we review a number of additional clinical factors to help differentiate NTM colonization from lung disease in transplant recipients, including the number of positive BAL specimens, smear positivity, and degree of immunosuppression.

Third, isolation of RGM in culture typically requires AFB-specific media, designed to inhibit the growth of more common bacteria, as well as prolonged

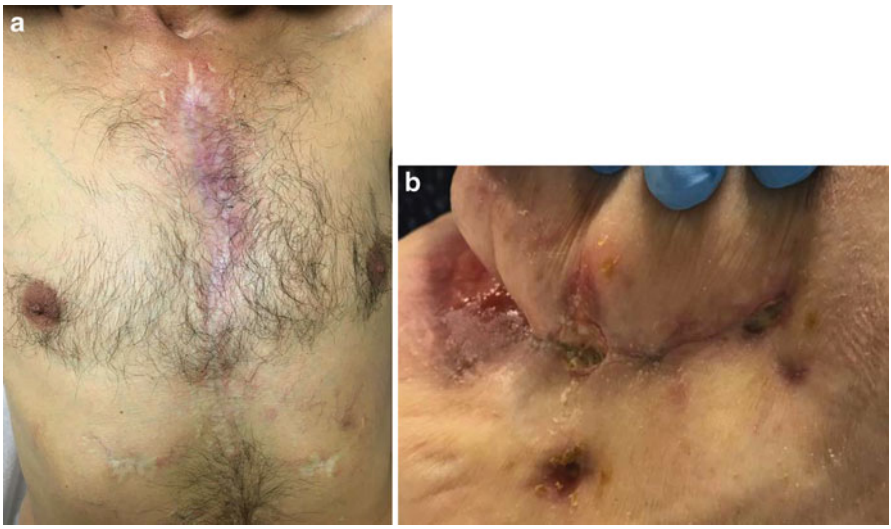


Fig. 1 Appearance of *M. abscessus* surgical wound infections. (**a**) Sternal wound infection following orthotopic heart transplantation. (**b**) Surgical wound infection of clamshell incision following lung transplantation. (Images courtesy of Eileen K. Maziarz, MD, Duke University School of Medicine, Division of Infectious Diseases)

incubation. Even AFB-specific cultures of fluid and tissue samples have limited sensitivity, however, and obtaining samples for histopathology evaluation can be required for diagnosis and should be encouraged [1, 10]. Histopathologic evaluation of NTM infection often reveals granulomatous inflammation. While organism burden may be low, AFB staining can help to confirm the presence of bacilli within tissue samples. Therefore, clinical suspicion of possible NTM infection is needed to ensure appropriate collection and processing of clinical samples for both microbiologic and histopathologic evaluation.

Species Identification

Despite the imperfect sensitivity of culture, isolation of RGM remains critical for species-level identification and subsequent susceptibility testing. In cases of mycobacteremia, RGM tend to grow well with most current blood culture systems; however, the prolonged incubation period used for mycobacterial isolator tubes may still provide increased sensitivity [79]. For specimens that may contain other contaminating organisms (e.g., respiratory samples), processing within one hour is ideal; refrigeration can be used if sample processing is delayed [10]. BAL has higher sensitivity and specificity than sputum samples. For fluid or tissue specimens, either surgical collection or sterile aspiration is preferred over swabs [10]. Culture is often performed on both solid media (either Lowenstein-Jensen or Middlebrook 7H10/11) and broth (usually a modified Middlebrook 7H9 broth) if sufficient specimen is available [10].

RGM species identification traditionally relied on the evaluation of growth rate, colony morphology, pigmentation, and biochemical testing [1]. However, traditional methods fail to separate several RGM beyond the group level (e.g., *M. chelonae/abscessus* group). Due to the difficulty in distinguishing RGM, many clinical laboratories do not identify RGM to the species level. Because antimicrobial susceptibility patterns vary even among closely related species, clinicians or laboratory staff may need to engage reference laboratories to ensure accurate and timely identification. For example, the activity of macrolide antibiotics (azithromycin or clarithromycin) as well as certain aminoglycosides (amikacin and tobramycin) can often be predicted from a more detailed understanding of the species.

Many different biochemical or molecular methods have been developed to promote rapid and accurate species-level identification of RGM. Most methods have had limited clinical utility due to extensive similarities between closely related RGM and lack of widespread availability of specialized testing. High-performance liquid chromatography (HPLC), for example, has been used to analyze mycolic acid signatures but is only capable of accurately identifying a few species [80]. A variety of molecular methods have been developed in reference labs, including restriction fragment length polymorphism (RFLP) analysis of select genes, such as *hsp65* or *rpoB*; however, these tests are not commercially available or approved by the US Food and Drug Administration (FDA). 16S rRNA sequencing has also been used but has limitations. For example, commercial sequencing often analyzes only the first 500 base pairs, which is insufficient to distinguish between closely related RGM, and many commercial databases have limited representation of NTM [1]. Furthermore, the *M. abscessus* subspecies are identical throughout the entire 16S rRNA sequence and cannot be identified to the subspecies level by 16S rRNA sequencing alone.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) may expedite presumptive identification of some RGM. A recent study found that MALDI-TOF could typically identify the *M. fortuitum* group and distinguish between *M. abscessus* and *M. chelonae*; however, in this analysis, MALDI-TOF could not discriminate species within the *M. fortuitum* group, nor could it fully resolve the *M. abscessus* subspecies [81–83]. Whole genome sequencing shows increasing promise for the future, offering maximal discriminatory capacity both for subspecies identification and delineation of outbreaks, but currently remains under development and is limited to reference laboratories.

Management

Treatment of RGM *infections* requires prolonged therapy with multiple antibiotics. Choice of regimen depends on species identification, antimicrobial susceptibilities, site of infection, and patient tolerance of antibiotics associated with multiple potential toxicities.

Table 3 Antimicrobial susceptibilities for commonly isolated rapidly growing mycobacteria[a]

Antimicrobial	*M. abscessus* complex (%)	*M. chelonae* (%)	*M. fortuitum* (%)
Amikacin	90–94	50–80	100
Cefoxitin	70–83	0	50–80
Ciprofloxacin[b]	0–5	20	96–100
Clarithromycin	20–100[c]	100	50–80[c]
Doxycycline	1–5	20–25	40–50
Imipenem	50–60[d]	50–60[d]	100
Linezolid	23–50	54–90	60–90
Trimethoprim-sulfamethoxazole	–	–	100
Tobramycin	–	83–100	–
Tigecycline[e]	99	92–100	100

References: [10, 85–87]

[a]Percentages represent the proportion of isolates with minimum inhibitory concentrations (MICs) in the intermediate to susceptible range

[b]Moxifloxacin and levofloxacin susceptibilities may differ from ciprofloxacin susceptibility for some RGM isolates; susceptibility testing for multiple fluoroquinolones may be considered [88]

[c]*erm*-mediated, inducible macrolide resistance can occur in *M. abscessus* subspecies *abscessus*, *M. abscessus* subspecies *bolletii*, and *M. fortuitum*. Extended incubation or direct assessment for the *erm* gene is needed to ensure accurate results for macrolide susceptibilities. In contrast, *erm*-mediated inducible resistance is not seen with *M. abscessus* subspecies *massiliense*

[d]Imipenem susceptibility testing is problematic in *M. abscessus* and *M. chelonae* due to lack of reproducibility

[e]MIC <1.0 mcg/mL is used as a proxy for tigecycline susceptibility. No widely-accepted interpretable criteria for susceptibility exist

Susceptibility testing often guides therapy, but for most antibiotics with activity against the RGM, there is limited clinical evidence linking *in vitro* susceptibilities to treatment success [84]. Broth micro-dilution remains the gold standard method for antimicrobial susceptibility testing. Presence of multiple organisms on culture, poor isolate growth, strict time windows for accurate minimum inhibitory concentration (MIC) interpretation, and interlaboratory inconsistencies complicate resistance testing [85]. E-tests in particular have shown poor interlaboratory agreement and are discouraged [86]. Due to these complexities, only experienced laboratory personnel should perform RGM antimicrobial susceptibility testing. Susceptibility data are commonly reported for *M. abscessus*, *M. chelonae*, and *M. fortuitum* (Table 3); among less frequently encountered species, data are sparse and less reliable [84].

The following subsections outline the important role of macrolide antibiotics in treating RGM and then address species-specific antibiotic options, including treatment considerations for pulmonary and extrapulmonary disease (Table 4). We also discuss unique challenges encountered in treating RGM infections in transplant recipients, including the often-difficult decision regarding which patients to treat with antibiotic therapy (see section "Management Considerations Unique to Transplant Recipients"). There is a distinct lack of randomized controlled trial data to guide treatment of RGM

Table 4 Antibiotics commonly used to treat rapidly growing mycobacterial infections

Pathogen	Commonly used antibiotics by route
M. abscessus complex	IV: Amikacin Cefoxitin or imipenem Tigecycline PO: Azithromycin/clarithromycin[a] Tedizolid Linezolid Clofazimine Bedaquiline Inhaled: Amikacin
M. chelonae	IV: Tobramycin Imipenem Tigecycline PO: Azithromycin/clarithromycin Fluoroquinolones Tedizolid Linezolid
M. fortuitum	IV: Cefoxitin or imipenem Amikacin PO: Azithromycin/clarithromycin[a] Fluoroquinolones Doxycycline Trimethoprim-sulfamethoxazole
M. mucogenicum	IV: Cefoxitin or imipenem PO: Azithromycin/clarithromycin Doxycycline Fluoroquinolones Trimethoprim-sulfamethoxazole

[a]Inducible resistance to macrolides mediated by the *erm* gene can occur for *M. abscessus* subspecies *abscessus, M. abscessus* subspecies *bolletii*, and *M. fortuitum.* Macrolides are preferred oral agents for these species only if isolates remain susceptible after prolonged incubation

infections, in particular for transplant patients. Guidelines and the following recommendations are therefore largely based on retrospective cohort studies, case series, *in vitro* data, and expert opinion.

The Role for Macrolides

Azithromycin and clarithromycin are often attractive components of multidrug regimens used to treat RGM infections given their high oral bioavailability and

relatively low toxicity. Furthermore, in contrast to other antibiotic options, macrolide susceptibility has been associated with microbiologic response in treatment of NTM disease [22, 89–91]. However, clinicians must be wary of the potential for inducible macrolide resistance: *M. abscessus* subspecies *abscessus, M. abscessus* subspecies *bolletii*, and *M. fortuitum* can express an inducible *erm* gene, which mediates macrolide resistance through ribosomal methylation [92–94]. As a result, MICs may appear low in the first 3–5 days of incubation but rise with extended incubation as *erm* expression increases (i.e., by 14 days). Therefore, accurate macrolide susceptibility testing in these three species requires extended incubation, with or without adjunctive molecular testing for functional *erm* genes.

In contrast, other RGM, such as *M. abscessus* subspecies *massiliense, M. chelonae*, *M. immunogenum*, and *M. mucogenicum*, generally lack a functional *erm* gene and do not exhibit inducible resistance [95]. Nonetheless, isolates without functional *erm* genes can still exhibit macrolide resistance through other mechanisms, such as ribosomal RNA mutations [85]. Detection of non-*erm* gene-mediated macrolide resistance does not require extended incubation.

In vitro and animal model data suggest that compared to clarithromycin, azithromycin is a weaker inducer of the *erm* gene [92]. Furthermore, azithromycin exhibits less inhibition of CYP3A4 compared to clarithromycin and typically is associated with less significant interaction with calcineurin inhibitors [96, 97]. Furthermore, our patients have tolerated a 250 mg daily dose of azithromycin better than clarithromycin or higher doses of azithromycin. Therefore, although clinical studies to definitively inform macrolide preference have not been performed, we favor azithromycin as the macrolide of choice to treat RGM infection in transplant recipients. Importantly, we recommend against monotherapy with a macrolide (or other antibiotic) for induction or maintenance therapy due to high risk of development of resistance and treatment failure [98].

Species- and Site-Specific Treatment Considerations

M. abscessus Complex

Although taxonomically related, the *M. abscessus* complex subspecies have different antimicrobial resistance patterns, particularly for macrolides (Table 3). Macrolides are favored components of regimens used to treat susceptible isolates of *M. abscessus* subspecies *massiliense* and the minority of *M. abscessus* subspecies *abscessus* isolates with a nonfunctional *erm* gene [89]. The utility of macrolides in treating *M. abscessus* complex species possessing an inducible *erm* gene is unclear, but azithromycin may retain more activity than clarithromycin [92]. *M. abscessus* complex species generally have low to intermediate range MICs for cefoxitin. Among the carbapenems, imipenem typically has the best *in vitro* activity, but results of susceptibility testing are inconsistent [86]. Isolates from treatment-naïve patients are usually susceptible to amikacin, but amikacin may not be active against isolates from patients with extensive prior

aminoglycoside exposure (e.g., cystic fibrosis patients) [10]. Widely accepted susceptibility criteria are not available for tigecycline, but MICs typically are low [99].

Several additional antibiotics have been evaluated *in vitro* against *M. abscessus* isolates. Linezolid shows *in vitro* activity for up to 50% of isolates [10], and tedizolid, which shares the same mechanism of action, also has favorable *in vitro* activity [87]. Novel antituberculous agents clofazimine and bedaquiline lack established susceptibility criteria but also demonstrate *in vitro* activity [100, 101]. New semisynthetic tetracyclines with *in vitro* activity against *M. abscessus* have also recently been developed, including omadacycline and eravacycline [102, 103]. *M. abscessus* is almost always resistant to doxycycline and the fluoroquinolones [10].

Many patients fail typical treatment regimens for *M. abscessus* or require a combination of oral antibiotics for long-term suppression. In these scenarios, clinicians are often forced to rely on antibiotics with favorable *in vitro* data but published clinical data that are limited to small cohorts and case reports. For example, although prolonged use of tigecycline is often limited by toxicity, clinical use in treatment of RGM, especially extrapulmonary disease, has become common. An analysis of a small cohort of patients who failed initial therapy for *M. abscessus* or *M. chelonae* infection reported clinical improvement in over 60% of patients who continued tigecycline as part of combination therapy for longer than 1 month [27]. For linezolid and tedizolid, clinical data describing treatment of *M. abscessus* and other RGM are limited to case reports. Compared to linezolid, long-term use of tedizolid may have less toxicity, particularly from myelosuppression and peripheral neuropathy [104].

Limited case series also report use of novel antituberculous agents clofazimine or bedaquiline as salvage therapy for *M. abscessus* [99, 105, 106]. In one observational cohort of patients with NTM infection, inclusion of clofazimine as part of combination therapy resulted in a 50% sputum clearance rate [107]. Notably, patients in this study received aggressive combination therapy with a median of three additional agents for prolonged durations, and the cohort did not include transplant recipients. Another case series reported clofazimine-based combination therapy to be reasonably well-tolerated among five SOT recipients with *Mycobacterium avium* complex (MAC) infection [108]. Bedaquiline was similarly assessed in a small case series of patients with either macrolide-resistant *M. abscessus* or MAC pulmonary disease who had failed prior treatment. Although transplant recipients were excluded, nearly 60% of patients achieved clinical improvement, and 50% converted to negative sputum cultures [100].

Poor outcomes for *M. abscessus* infections, particularly in SOT recipients, highlight the growing need for more effective and better-tolerated therapies [13, 14, 109, 110]. While new antibiotics with activity against *M. abscessus* and other RGM have been developed, clinical effectiveness and impact remain uncertain. Investigators have recently developed and utilized bacteriophage therapy targeting *M. abscessus*, which could represent a treatment option if subsequent clinical data also support use of mycobacteriophage [111].

Pulmonary Disease

M. abscessus is the RGM most frequently isolated from pulmonary specimens [33, 112]. Pulmonary infection from *M. abscessus* is difficult to treat, although likelihood of microbiologic response is at least partly dependent upon the presence of an inducible *erm* gene [89]. For macrolide-susceptible isolates (such as *M. massiliense* and a minority of *M. abscessus* subspecies *abscessus* isolates), optimal therapy includes azithromycin or clarithromycin. We recommend choosing two additional agents from the following: intravenous amikacin (or inhaled amikacin if only intrapulmonary disease is present), cefoxitin (or imipenem), and tedizolid (or linezolid if tedizolid is not available) [10]. Patients with *M. massiliense* or macrolide-susceptible *M. abscessus* have demonstrated sputum conversion rates as high as 80–90%, although larger case series have typically not included transplant recipients [23, 89].

In contrast, *M. abscessus* subspecies *abscessus* isolates with inducible *erm* genes are associated with worse clinical outcomes for pulmonary infections [22, 90, 92]. Optimal combination therapy for transplant patients with macrolide-resistant isolates is not known. Based on our experience, we recommend initial therapy with three-drug regimens, chosen based upon susceptibility results and often including cefoxitin (or imipenem), inhaled or intravenous amikacin, and either azithromycin (despite *in vitro* resistance), tedizolid (or linezolid), or tigecycline.

Use of inhaled amikacin for transplant recipients with *M. abscessus* pulmonary disease may represent a viable alternative to intravenous amikacin with less risk of ototoxicity and nephrotoxicity. However, clinical data are limited to small cohorts, and systemic absorption and associated toxicity can occur with inhalational therapy [13, 113, 114]. Therefore, we recommend surveillance of serum amikacin levels for transplant recipients receiving inhalational therapy who have abnormal renal function or for whom clinical concern of toxicity develops. When random serum amikacin levels are detected, clinicians should decrease the frequency or amount of inhaled amikacin doses.

In the United States, the FDA recently approved a liposomal formulation of inhaled amikacin (amikacin liposome inhalation suspension) for treatment of refractory *M. avium* complex (MAC) based upon improved culture conversion after addition of this agent to standard of care regimens [115, 116]; however, culture conversion was less common for a small subset of patients with *M. abscessus*. Therefore, further study of inhaled amikacin for *M. abscessus* pulmonary disease is needed.

We treat transplant recipients with pulmonary *M. abscessus* infection for a minimum of 6–12 months, but treatment duration depends upon clinical response and tolerance of antibiotics. For patients who exhibit symptomatic and radiographic improvement with good tolerance of antibiotics, we may extend therapy for up to 12 months beyond sputum culture conversion [10]. However, many patients do not achieve culture clearance or are unable to tolerate long durations of therapy, and disease recurrence after cessation of antibiotics is common [117]. In refractory cases, clinicians may revert to periodic courses of therapy lasting approximately 3–6 months with a goal of providing symptomatic management rather than cure.

In other cases, surgery may offer the best opportunity for cure if the infection is localized and resectable [15, 109, 118]. Potential surgical candidates with localized disease must be carefully selected to have sufficient pulmonary reserve to withstand planned resections. However, many transplant recipients present with diffuse, bilateral pulmonary disease and are not candidates for adjunctive surgical therapy.

Extrapulmonary Disease

Skin or soft tissue infection from *M. abscessus* is generally treated with combination antibiotics for 4–6 months. Surgical debridement significantly improves chances of successful therapy, particularly in cases with more extensive disease, necrotic tissue, or abscess formation. We suggest induction therapy with 4–6 weeks of combination therapy, including intravenous therapy for serious disease. Choice of subsequent multidrug maintenance regimens depends upon susceptibilities, extent of infection, surgical source control, patient comorbidities, and clinical response [10].

Bone infections are typically treated for at least 6 months following surgical debridement. Experts recommend induction therapy with three agents (two of which are typically parenteral) for 8–12 weeks before considering oral combination maintenance therapy, based upon susceptibilities [10]. Induction therapy for disseminated *M. abscessus* infection usually consists of 3–4 active agents, including either cefoxitin or imipenem. Induction regimens often include various combinations of the following antibiotics: azithromycin, amikacin, cefoxitin (or imipenem), tigecycline, and tedizolid (or linezolid) [10]. Particular antibiotic combinations depend upon comorbidities, potential for drug interactions, susceptibilities, and patient tolerance. Combination maintenance regimens for severe disease typically include intravenous therapy and are continued for at least 6 months. Many disseminated *M. abscessus* infections are not curable, even with aggressive surgical and medical management. Therefore, maintenance or suppressive regimens are occasionally required, at times including newer oral agents, such as clofazimine or bedaquiline.

M. chelonae

M. chelonae does not express *erm* and is generally susceptible to macrolides (Table 3) [93]. *M. chelonae* is typically resistant to cefoxitin, and, similar to *M. abscessus,* imipenem MIC testing tends to be inconsistent; however, imipenem is often a component of treatment regimens. Tobramycin has excellent *in vitro* activity against most isolates of *M. chelonae* and is the aminoglycoside of choice for treating serious infections from this species. Tigecycline has been used with some success as a component of salvage therapy [27]. Among oral agents, in addition to macrolides, fluoroquinolones and linezolid exhibit good activity for some isolates.

Pulmonary Disease

M. chelonae is a much less frequent cause of pulmonary disease than *M. abscessus*. Treatment duration is similar to other RGM and can extend up to 12 months beyond culture clearance. Combination therapy usually consists of a macrolide plus two additional agents, including parenteral therapy for at least the first 4–6 weeks. Some

providers subsequently transition to maintenance regimens including a macrolide plus two active oral agents, if susceptibilities allow [10].

Extrapulmonary Disease

Similar to *M. abscessus*, skin or soft tissue disease from *M. chelonae* is generally treated for a total of 4–6 months. Surgical debridement is often critical to successful treatment of resectable disease. Patients frequently initiate therapy with 4–6 weeks of combination therapy, including intravenous therapy for serious disease. They may transition to 2–3-drug oral therapy thereafter, based upon susceptibilities, surgical source control, and clinical response [10].

Therapy for bone infection is generally continued for at least 6 months. Similar to treatment of *M. abscessus* osteomyelitis, experts recommend initial therapy with a macrolide plus 2 parenteral agents for 8–12 weeks, followed by conversion to 2–3 active oral agents thereafter [10].

Disseminated infection from *M. chelonae* typically requires at least 6 months of combination therapy. Induction therapy generally includes a macrolide, tobramycin, and an additional active agent for the first 4–6 weeks. Susceptibilities and clinical response help to determine combination maintenance regimens [10].

M. fortuitum

Although variable between isolates, *M. fortuitum* generally exhibits increased susceptibility to oral agents compared to other RGM (Table 3) [10]. Frequently used agents include the fluoroquinolones, trimethoprim-sulfamethoxazole, doxycycline, and, in the absence of *erm* expression, azithromycin. Among parenteral agents, cefoxitin, amikacin, and imipenem are typically active. In contrast to *M. abscessus* and *M. chelonae*, imipenem MICs tend to be reproducible with *M. fortuitum* [10].

Pulmonary Disease

M. fortuitum pulmonary infection is relatively uncommon and can be associated with emesis or aspiration. We recommend initial therapy with three agents, including azithromycin only after excluding *erm*-mediated macrolide resistance. Similar to treatment of pulmonary disease from other RGM, treatment can continue for up to 12 months beyond culture clearance [10].

Extrapulmonary Disease

Treatment duration for skin and soft tissue infection from *M. fortuitum* is typically 4–6 months. Due to favorable susceptibility data for oral agents, it is often possible to treat with combination oral therapy once susceptibility results are available. As with other RGM, surgical debridement of deep infections is key.

For bone infections, we recommend three-drug induction therapy, including at least one parenteral agent, for at least the first 6 weeks. Transitioning to 2–3 active oral drugs to complete a total duration of 6 months thereafter is usually possible [10].

Disseminated disease typically requires at least 6 months of therapy. Induction therapy consists of 4–6 weeks of parenteral therapy, often followed by conversion to oral agents thereafter [10].

Role for Surgery in Extrapulmonary RGM Disease

Due to high rates of drug resistance and a tendency to form biofilms, surgical debridement is recommended for any RGM infection with associated abscess, bony involvement, or extensive tissue involvement [10]. Repeat debridements are often required because the margins of involved tissue can be difficult to ascertain, and obtaining adequate source control from a single debridement may not be possible. One review of cardiac surgical site infections with *M. chelonae*, including deep infections such as mediastinitis, found that nearly all successfully treated patients had undergone surgical debridement [119]. Similarly, any infected prosthetic material should be removed because the retention of foreign material confers a high risk of treatment failure or recurrence. In one small case series of two *M. chelonae* infections involving left ventricular assist device (LVAD) driveline infections, extended courses of combination therapy coupled with repeated debridement were unsuccessful; cure was achieved in both cases only after LVAD removal [56]. A separate analysis of 32 RGM infections of implantable electronic devices reported device removal in all but 3 cases [57].

Catheter-Associated Infections

Catheter-associated RGM infections are common. Regardless of the site or type of infection, catheter removal remains essential to successful treatment given the high risk of biofilm formation. In the setting of isolated CLABSI, catheter removal followed by a short course of therapy may be effective. In one oncology center's experience with 116 RGM CLABSIs, consisting predominantly of *M. mucogenicum* or *M. fortuitum* infections, the majority of patients achieved clinical cures with a median of only 4 weeks of therapy, provided the line was promptly removed [34, 120].

RGM infection of peritoneal dialysis catheters also occurs. These infections can present as culture-negative or treatment-refractory peritonitis. As with CLABSIs, successful treatment requires catheter removal [55]. Duration of therapy for peritoneal dialysis catheter infection with peritonitis is poorly defined. Among published cases, treatment courses after catheter removal ranged from several weeks to 12 months. We suggest at least 3–6 months of therapy for transplant recipients with peritoneal dialysis catheter-related invasive RGM infections.

Treatment of Less Common RGM Isolates

Due to their overall rarity, treatment of less common RGM, such as *M. mucogenicum* or *M. immunogenum*, is often inferred from experience with the more common isolates. One key difference, however, is that susceptibility data are sparse, making the selection of empiric therapy challenging. Initial regimens are generally chosen based upon published susceptibility data, and therapy is individualized according to patient response and susceptibility profile of the specific clinical isolate.

Management Considerations Unique to Transplant Recipients

Treatment of RGM infections in transplant recipients presents several unique challenges. First, the decision to pursue aggressive treatment versus observe off of therapy is often complex. On one hand, some heavily immunosuppressed patients with positive respiratory cultures but a lack of compelling symptoms or radiographic findings indicative of infection may benefit from several months of aggressive combination therapy designed to provide secondary prophylaxis against invasive pulmonary infection. For example, lung transplant recipients with peri-transplant respiratory colonization from RGM may benefit from 3 to 6 months of post-transplant three-drug therapy to decrease risk of chronic RGM infection of the transplanted graft. Ideal duration of therapy in this scenario is not clear and depends upon evaluation of explanted lung histopathology for stigmata of invasive RGM infection, post-transplant surveillance BAL culture results, antibiotic tolerance, and potential for decrease in immunosuppression with time after transplant. On the other hand, some transplant recipients with confirmed invasive or even disseminated infection may benefit from symptom palliation rather than aggressive medical or surgical therapy due to poor overall clinical status, high risk for antibiotic-related toxicities, and lack of potential for cure [121].

Next, transplant recipients who decide to initiate antibiotic therapy for RGM infection are likely to develop clinically significant antibiotic toxicities. A recent study analyzed 55 patients with a variety of underlying diseases who were treated for *M. abscessus* infection [122]. Many patients in this cohort developed clinically significant toxicities related to antibiotics, including 23 (42%) patients with gastrointestinal toxicity. Another recent study of 82 SOT recipients treated for pulmonary ($n = 48$) or extrapulmonary ($n = 34$) *M. abscessus* infection found that 55 (67%) patients developed antibiotic toxicities severe enough to require changes in therapy [13]. Transplant recipients may be at particularly high risk to develop nephrotoxicity from intravenous aminoglycosides, especially given prevalence of pre-existing renal disease and use of concomitant nephrotoxic medications, including calcineurin inhibitors. Potential for drug-drug interactions, such as macrolide inhibition of CYP3A4 and consequent increased calcineurin inhibitor levels, also increase risk of treatment-related adverse events and require close clinical and drug-level monitoring (Table 5).

Duration of therapy decisions are also difficult and must be individualized for transplant recipients with RGM infections. Shorter courses of antibiotics may be appropriate for patients with a removable source of infection, such as a central line, lack of confirmed invasive infection, antibiotic toxicities, or untreatable disease. However, relapsed disease from invasive RGM infection is common, and in some cases, long-term suppressive oral antibiotics can prevent recrudescence of infection and need for re-induction therapy with intravenous antibiotics and surgical debridement.

Several treatment strategies may decrease risk of toxicity from antibiotic regimens commonly used to treat RGM infections (Table 5). For example, patients receiving intravenous aminoglycoside therapy should undergo baseline and

Table 5 Dosing, common adverse effects, and drug interactions for antibiotics commonly used to treat rapidly growing mycobacterial infections

Antimicrobial/dose	Common adverse effects	Transplant-relevant drug interactions
Aminoglycosides		
Amikacin 10–15 mg/kg IV three times per week[a] Adjust dose to maintain trough level <1 μg/mL Amikacin 250–500 mg inhaled daily (free amikacin) Liposomal amikacin 590 mg inhaled daily	Ototoxicity, nephrotoxicity	Potentiates risk of nephrotoxicity with CNI
Tobramycin 7 mg/kg IV three times per week[a] Adjust dose to maintain trough level <1 μg/mL	Ototoxicity, nephrotoxicity	Potentiates risk of nephrotoxicity with CNI
Carbapenems		
Imipenem 500 mg IV Q6h or 1000 mg IV Q12h	GI upset Reduced seizure threshold Hepatitis Hematologic toxicity[b]	May increase CSA levels
Cephalosporins		
Cefoxitin 8–12 g IV daily, in divided doses or via continuous infusion	Artificial increase in creatinine[c] Hematologic toxicity[b]	
Fluoroquinolones	Applicable to all fluoroquinolones:	
Ciprofloxacin 500–750 mg PO twice daily Levofloxacin 500–750 mg PO daily Moxifloxacin 400 mg PO daily	*Clostridioides difficile* infection Tendonitis Aortic aneurysm Prolonged QT	Mildly increases CNI levels Mildly decreases MMF levels
Macrolides	Applicable to all macrolides:	
Azithromycin 250 mg PO daily Clarithromycin 500 mg PO twice daily	GI upset Prolonged QT Ototoxicity	Mildly increases CNI, mTORi levels Increases CNI, mTORi levels

Oxazolidinones		
Linezolid 600 mg PO daily	Peripheral neuropathy Hematologic toxicity[b] Serotonin syndrome	Risk of serotonin syndrome if co-administered with serotonin re-uptake inhibitors or other serotonergic medications
Tedizolid 200 mg PO daily	May have lower risk of the adverse effects associated with linezolid	
Tetracyclines		
Doxycycline 100 mg PO twice daily	Sun sensitivity, esophagitis	
Minocycline 100 mg PO twice daily	GI upset, skin pigmentation	
Tigecycline 50 mg IV daily or Q12h[d]	GI upset	May increase CSA levels
Sulfonamides		
Trimethoprim-sulfamethoxazole 800–1600 mg sulfamethoxazole component PO twice daily	Hyperkalemia Increase in serum creatinine Hematologic toxicity[b]	
Novel agents		
Bedaquiline 400 mg PO daily for 2 weeks and then 200 mg PO three times per week	QT prolongation	
Clofazimine 100 mg PO daily	GI upset Skin pigmentation changes QT prolongation Rare visceral crystallization	

References: [10, 17, 123]

Antibiotic doses provided assume normal renal function and may require adjustment for renal insufficiency

CSA cyclosporine, *CNI* calcineurin inhibitor, *GI* gastrointestinal, *MMF* mycophenolate mofetil, *mTORi* mammalian target of rapamycin inhibitor

[a]Once-daily dosing may be considered, but daily dosing may increase risk of side effects, especially with prolonged use

[b]Hematologic toxicity may include anemia, thrombocytopenia, or leukopenia

[c]Cefoxitin may interfere with the Jaffe method for creatinine measurement; artifactual creatinine elevation can be avoided if blood draws are performed 2–4 h after the last cefoxitin dose, assuming normal renal function

[d]Once-daily tigecycline dose may have less GI toxicity than traditional Q12h dosing

surveillance audiology assessments [124], in addition to close monitoring of renal function and drug levels. We prefer three-times-weekly dosing of amikacin and tobramycin, targeting undetectable trough levels, to decrease risk of toxicity associated with prolonged treatment courses. We also suggest N-acetylcysteine supplements during intravenous aminoglycoside therapy, which may decrease risk of ototoxicity [125]. Reducing dosing frequency for tigecycline to 50 mg once daily [27] and for linezolid to 600 mg once daily [126] may also decrease risk of toxicity. Tedizolid may prove to have similar efficacy as linezolid with improved long-term tolerance [104].

Finally, while immunosuppression is one of the key risk factors for RGM infection, few data are available to guide the management of immunosuppressive medications in the setting of known infection. Reports of immune-reconstitution inflammatory syndrome are exceedingly rare among SOT recipients with NTM infections [127]. Therefore, when feasible, we reduce immunosuppression when treating RGM infections. For SOT recipients, we often decrease the dose or stop the antimetabolite agent (e.g., mycophenolate mofetil or azathioprine).

Prevention

Transplant recipients are at increased risk for community-acquired RGM infection. While RGM are ubiquitous in the environment, municipal water colonization likely represents the most important reservoir for community acquisition [63]. Guidelines recommend that transplant recipients avoid hot tubs due to potential for aerosolization of high concentrations of NTM [128]; however, few data are available to guide outpatient tap water use related to risk of RGM acquisition. Based on our experience, we recommend that recent SOT recipients avoid tap water contact to the surgical wound until it is well-healed and sutures or staples have been removed. For lung transplant recipients living in a geographic area with endemic RGM, we recommend home use of sterile or boiled water for oral care, enteric tube flushes, and consumption, at least until functional status has improved and risk of micro-aspiration of tap water has presumably decreased [7].

Transplant recipients also acquire RGM from healthcare facilities, and recent RGM outbreaks among hospitalized transplant recipients have led to increased scrutiny of hospital water use and equipment that contacts tap water [129]. Tap water should not contact unhealed surgical wounds, central lines, and other catheters or drains that exit the skin [10]. Furthermore, transplant centers should perform clinical surveillance for RGM infections. Suspected healthcare-acquired cases of RGM require investigation of potential mechanisms of transmission, particularly via direct or indirect tap water contact. In the outbreak setting, environmental sampling of biofilms from healthcare facility water sources can help to elucidate mechanisms of transmission; however, given low sensitivity and delays inherent in performing mycobacterial cultures of environmental samples, avoidance of tap water sources or medical equipment potentially responsible for transmission of RGM should occur prior to source confirmation. Tap water avoidance has successfully mitigated RGM

outbreaks at several hospitals [7, 8, 45, 130]. Another potential mitigation measure is use of 0.2 micron filters at distal water outlets. Finally, more proximal interventions on the healthcare facility plumbing infrastructure can improve water flow and disinfectant levels, which may decrease RGM concentration in water [7]. Guidelines for screening healthcare facility water systems for RGM or building new hospital plumbing systems that have intrinsically lower risk for RGM contamination need to be developed, especially for transplant centers.

Clinical Impact in Transplant

RGM infections have unique impact upon transplantation. These infections affect patients undergoing evaluation to assess transplant candidacy, transplant recipients, and transplant centers located in areas where RGM are endemic.

Impact on Transplant Candidacy

The majority of data on pre-transplant RGM infection come from experience with lung transplantation, in particular for patients with cystic fibrosis and *M. abscessus* infection. Pre-transplant RGM infection, most notably from *M. abscessus*, likely increases risk of lung transplantation; therefore, many authorities consider *M. abscessus* infection to be a relative contraindication to lung transplantation [131–134]. Nonetheless, despite risk of recurrent post-transplant RGM infection and related complications, transplantation may offer transplant candidates with complicated RGM infections the best chance for long-term cure. In fact, multiple transplant centers have reported successful transplantation for small cohorts of patients with *M. abscessus* infections [61, 135, 136]. For example, one hospital analyzed clinical courses of 13 patients with cystic fibrosis and positive respiratory cultures for *M. abscessus* prior to transplant and found that 5-year post-transplant survival for this cohort was not different from survival of cystic fibrosis patients without *M. abscessus* who underwent transplantation [135]. In our experience, lung transplant candidates with RGM infection associated with large pulmonary cavities, suspected pleural involvement or risk of soiling during surgery, or poor tolerance of RGM antimicrobial regimens are at highest risk of progressive infection after transplant and may therefore be poor transplant candidates. More data are needed to allow accurate risk stratification of lung transplant candidates based upon clinical characteristics of RGM infection and subspecies identification (e.g., *M. massiliense* vs. *M. abscessus*) [137].

Lung transplant candidates with RGM infection should begin treatment prior to transplant listing with specific therapies chosen based upon species identification, susceptibility patterns, and patient tolerance of particular multidrug therapies [132]. Lung transplant recipients with invasive pulmonary RGM infection at the time of transplant require at least several months of combination therapy after transplant, as well as close clinical and microbiologic surveillance for recurrent or refractory infection.

Pre-transplant evaluation of heart transplant candidates with RGM infections, in particular VAD-associated infections, also presents unique management challenges with minimal data available for guidance. Some centers exclude LVAD recipients with RGM infection from transplantation, but two limited reports describe successful heart transplantation in three patients with chronic, disseminated *M. abscessus* infections involving LVADs [56, 138]. At our center, active intrathoracic RGM infection would likely exclude a patient from transplant due to potential for worsening of infection with immunosuppression. RGM infections limited to LVAD drivelines can be considered for transplantation, however, because LVAD removal would provide surgical source control and opportunity for cure. After LVAD explantation and heart transplantation, we recommend post-transplant antibiotic courses of 6–12 months.

Fewer data are available to help guide management of patients with hematologic malignancy with active RGM infection and potential for HCT. Akin to management prior to SOT, initiation of combination antibiotic therapy to gauge tolerance and clinical response prior to augmentation of immunosuppression is ideal but sometimes not possible due to chemotherapy or transplant timelines. Like SOT candidates, HCT candidates often continue prolonged treatment courses after transplantation.

Impact on Transplant Recipients and Transplant Centers

Substantial morbidity and mortality is associated with RGM infections in SOT or HCT recipients, best studied for *M. abscessus* in the SOT literature [13, 14, 109, 110]. Antibiotic toxicities related to prolonged combination antimicrobial therapy are common, and despite long courses of antibiotics that are difficult to tolerate, failure to achieve cure and relapsed infection occur frequently. Transplant patients are also at increased risk for drug-drug interactions and require close clinical and laboratory surveillance while on therapy. However, despite risks associated with therapy, heavily immunosuppressed patients may be at highest risk for progression from colonization to infection and for development of complications related to infection. Therefore, when level of immunosuppression is highest, such as early after transplant or during treatment for rejection, the relative benefits of aggressive RGM therapy or secondary prophylaxis may be greatest.

Healthcare facility outbreaks of RGM can disproportionately affect transplant recipients due to presence of immunosuppression and frequent healthcare exposures, such as increased number of procedures and need for invasive devices [7]. In addition to causing substantial illness in patients with infection, outbreaks of RGM can profoundly impact SOT and HCT programs, including outcome statistics and other performance metrics [8, 9, 45, 139]. Healthcare facility-associated outbreaks of RGM affecting transplant recipients emphasize the importance of infection prevention, particularly with regard to principles of hospital water safety [70, 129].

Conclusion

RGM are widespread in the environment and well-adapted to form biofilms. While RGM infections are relatively rare, prevalence appears to be increasing, and transplant patients are at elevated risk. RGM infections in transplant recipients are difficult to diagnose due to atypical clinical presentations and limitations of diagnostic testing. Culture using AFB media remains the gold standard for diagnosis and promotes species identification and susceptibility testing, which require specialized laboratory expertise. Treating RGM infections is also challenging because transplant recipients with serious infections require months of combination antibiotic regimens that frequently cause important toxicities. Furthermore, particularly for extrapulmonary infections, patients often require multiple surgical debridements. The high morbidity and mortality associated with RGM infections in transplant patients emphasizes the importance of clinical surveillance and infection prevention strategies. Poor treatment outcomes also underscore the growing need for new treatment agents and approaches.

Key Points

- RGM commonly form biofilms and survive in community and healthcare facility water systems.
- Diagnosis of RGM infection in transplant recipients is difficult because patients often present with atypical symptoms and have indolent clinical courses.
- Culture remains the gold standard for diagnosis, but presence of AFB or granulomatous inflammation on histopathology can provide early evidence of infection.
- Treatment typically requires prolonged courses of combination antibiotic therapy, adjusted based upon susceptibility results, patient tolerance, and clinical response.
- For extrapulmonary infections, removal of infected prosthetic material and debridement of infected tissue is essential for treatment success.
- Transplant can offer cure for some transplant candidates with RGM infections, but active RGM infection also increases risk of complications after transplant.
- Transplant recipients with RGM infections often have poor outcomes and do not achieve cure despite aggressive medical and surgical management.
- To decrease risk of infection, transplant patients living in areas with endemic RGM should avoid high-risk tap water contact in the community and at healthcare facilities.

Cross-References

► *Mycobacterium tuberculosis* in Transplantation
► Slow-Growing Nontuberculous Mycobacteria in Transplant

References

1. Brown-Elliott BA, Philley JV. Rapidly growing mycobacteria. Microbiol Spectr. 2017;5(1):1–19.
2. Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM. Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. Mayo Clin Proc. 2013;88(1):38–45.
3. Alcaide F, Pena MJ, Perez-Risco D, Camprubi D, Gonzalez-Luquero L, Grijota-Camino MD, et al. Increasing isolation of rapidly growing mycobacteria in a low-incidence setting of environmental mycobacteria, 1994–2015. Eur J Clin Microbiol Infect Dis. 2017;36(8):1425–32.
4. Velayati AA, Rahideh S, Nezhad ZD, Farnia P, Mirsaeidi M. Nontuberculous mycobacteria in Middle East: current situation and future challenges. Int J Mycobacteriol. 2015;4(1):7–17.
5. George IA, Santos CA, Olsen MA, Bailey TC. Epidemiology and outcomes of nontuberculous mycobacterial infections in solid organ transplant recipients at a Midwestern center. Transplantation. 2016;100(5):1073–8.
6. Longworth SA, Vinnard C, Lee I, Sims KD, Barton TD, Blumberg EA. Risk factors for nontuberculous mycobacterial infections in solid organ transplant recipients: a case-control study. Transpl Infect Dis. 2014;16(1):76–83.
7. Baker AW, Lewis SS, Alexander BD, Chen LF, Wallace RJ Jr, Brown-Elliott BA, et al. Two-phase hospital-associated outbreak of Mycobacterium abscessus: investigation and mitigation. Clin Infect Dis. 2017;64(7):902–11.
8. Iroh Tam PY, Kline S, Wagner JE, Guspiel A, Streifel A, Ward G, et al. Rapidly growing mycobacteria among pediatric hematopoietic cell transplant patients traced to the hospital water supply. Pediatr Infect Dis J. 2014;33(10):1043–6.
9. Guspiel A, Menk J, Streifel A, Messinger K, Wagner J, Ferrieri P, et al. Management of risks from water and ice from ice machines for the very immunocompromised host: a process improvement project prompted by an outbreak of rapidly growing mycobacteria on a pediatric hematopoietic stem cell transplant (Hsct) unit. Infect Control Hosp Epidemiol. 2017;38(7):792–800.
10. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175(4):367–416.
11. Ward JGMaAC. Bergey's manual of systematics of archaea and bacteria. In: Whitman WB, editor. Mycobacteriaceae. John Wiley and Sons, Hoboken, NJ; 2015.
12. Knoll BM, Kappagoda S, Gill RR, Goldberg HJ, Boyle K, Baden LR, et al. Non-tuberculous mycobacterial infection among lung transplant recipients: a 15-year cohort study. Transpl Infect Dis. 2012;14(5):452–60.
13. Baker AW, Maziarz EK, Saullo JH, Arif S, Anderson DJ, Alexander BD, et al. Mycobacterium abscessus infection in solid organ transplant recipients. Open Forum Infect Dis. 2016;3(suppl_1):2311.
14. Maziarz EK, Baker AW, Patel CB, Patel P, Milano CA, Schroder JN, et al. Invasive Mycobacterium abscessus infection in heart transplant recipients. J Heart Lung Transplant. 2017;36(4, Supplement):S25.
15. Garrison AP, Morris MI, Doblecki Lewis S, Smith L, Cleary TJ, Procop GW, et al. Mycobacterium abscessus infection in solid organ transplant recipients: report of three cases and review of the literature. Transpl Infect Dis. 2009;11(6):541–8.
16. Wallace RJ Jr, Zhang Y, Brown BA, Fraser V, Mazurek GH, Maloney S. DNA large restriction fragment patterns of sporadic and epidemic nosocomial strains of Mycobacterium chelonae and Mycobacterium abscessus. J Clin Microbiol. 1993;31(10):2697–701.
17. Morales P, Gil A, Santos M. Mycobacterium abscessus infection in transplant recipients. Transplant Proc. 2010;42(8):3058–60.
18. Richey LE, Bahadorani J, Mushatt D. Endovascular Mycobacterium abscessus infection in a heart transplant recipient: a case report and review of the literature. Transpl Infect Dis. 2013;15(2):208–13.

19. Uyan ZS, Ersu R, Oktem S, Cakir E, Koksalan OK, Karadag B, et al. Mycobacterium abscessus infection in a cystic fibrosis patient: a difficult to treat infection. Int J Tuberc Lung Dis. 2010;14(2):250–1.
20. Lee MR, Sheng WH, Hung CC, Yu CJ, Lee LN, Hsueh PR. Mycobacterium abscessus complex infections in humans. Emerg Infect Dis. 2015;21(9):1638–46.
21. Aitken ML, Limaye A, Pottinger P, Whimbey E, Goss CH, Tonelli MR, et al. Respiratory outbreak of Mycobacterium abscessus subspecies massiliense in a lung transplant and cystic fibrosis center. Am J Respir Crit Care Med. 2012;185(2):231–2.
22. Choi H, Kim SY, Lee H, Jhun BW, Park HY, Jeon K, et al. Clinical characteristics and treatment outcomes of patients with macrolide-resistant mycobacterium massiliense lung disease. Antimicrob Agents Chemother. 2017;61(2):e02189–16.
23. Park J, Cho J, Lee CH, Han SK, Yim JJ. Progression and treatment outcomes of lung disease caused by Mycobacterium abscessus and Mycobacterium massiliense. Clin Infect Dis. 2017;64(3):301–8.
24. Tettelin H, Davidson RM, Agrawal S, Aitken ML, Shallom S, Hasan NA, et al. High-level relatedness among Mycobacterium abscessus subsp. massiliense strains from widely separated outbreaks. Emerg Infect Dis. 2014;20(3):364–71.
25. Huth RG, Douglass E, Mondy K, Vasireddy S, Wallace RJ Jr. Treatment of Mycobacterium abscessus subsp. massiliense tricuspid valve endocarditis. Emerg Infect Dis. 2015;21(3):535–7.
26. Miyake N, Chong Y, Nishida R, Takenaka K, Kato K, Miyamoto T, et al. Mycobacterium abscessus and massiliense lung infection during macrolide treatment for bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. J Infect Chemother. 2018;24(1):78–81.
27. Wallace RJ Jr, Dukart G, Brown-Elliott BA, Griffith DE, Scerpella EG, Marshall B. Clinical experience in 52 patients with tigecycline-containing regimens for salvage treatment of Mycobacterium abscessus and Mycobacterium chelonae infections. J Antimicrob Chemother. 2014;69(7):1945–53.
28. Somily AM, Al-Anazi AR, Babay HA, Al-Aska AI, Al-Hedaithy MA, Al-Hamoudi WK, et al. Mycobacterium chelonae complex bacteremia from a post-renal transplant patient: case report and literature review. Jpn J Infect Dis. 2010;63(1):61–4.
29. Kim JE, Sung H, Kim MN, Won CH, Chang SE, Lee MW, et al. Synchronous infection with Mycobacterium chelonae and Paecilomyces in a heart transplant patient. Transpl Infect Dis. 2011;13(1):80–3.
30. Chatzikokkinou P, Luzzati R, Sotiropoulos K, Katsambas A, Trevisan G. Disseminated cutaneous infection with Mycobacterium chelonae in a renal transplant recipient. Cutis. 2015;96(5):E6–9.
31. Biggs HM, Chudgar SM, Pfeiffer CD, Rice KR, Zaas AK, Wolfe CR. Disseminated Mycobacterium immunogenum infection presenting with septic shock and skin lesions in a renal transplant recipient. Transpl Infect Dis. 2012;14(4):415–21.
32. Simmon KE, Brown-Elliott BA, Ridge PG, Durtschi JD, Mann LB, Slechta ES, et al. Mycobacterium chelonae-abscessus complex associated with sinopulmonary disease. Northeastern USA Emerg Infect Dis. 2011;17(9):1692–700.
33. Shah SK, McAnally KJ, Seoane L, Lombard GA, LaPlace SG, Lick S, et al. Analysis of pulmonary non-tuberculous mycobacterial infections after lung transplantation. Transpl Infect Dis. 2016;18(4):585–91.
34. El Helou G, Hachem R, Viola GM, El Zakhem A, Chaftari AM, Jiang Y, et al. Management of rapidly growing mycobacterial bacteremia in cancer patients. Clin Infect Dis. 2013;56(6):843–6.
35. Han XY, De I, Jacobson KL. Rapidly growing mycobacteria: clinical and microbiologic studies of 115 cases. Am J Clin Pathol. 2007;128(4):612–21.
36. Adzic-Vukicevic T, Barac A, Blanka-Protic A, Laban-Lazovic M, Lukovic B, Skodric-Trifunovic V, et al. Clinical features of infection caused by non-tuberculous mycobacteria: 7 years' experience. Infection. 2018;46(3):357–63.

37. Oh WS, Ko KS, Song JH, Lee MY, Ryu SY, Taek S, et al. Catheter-associated bacteremia by Mycobacterium senegalense in Korea. BMC Infect Dis. 2005;5:107.
38. Brown-Elliott BA, Wallace RJ Jr, Tichindelean C, Sarria JC, McNulty S, Vasireddy R, et al. Five-year outbreak of community- and hospital-acquired Mycobacterium porcinum infections related to public water supplies. J Clin Microbiol. 2011;49(12):4231–8.
39. Brown-Elliott BA, Wallace RJ Jr. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin Microbiol Rev. 2002;15(4):716–46.
40. Poh ME, Liam CK, Ng KP, Tan R. Mycobacterium brisbanense species nova isolated from a patient with chronic cavitary lung infection. Chest. 2014;145(4):858–60.
41. Toro A, Adekambi T, Cheynet F, Fournier PE, Drancourt M. Mycobacterium setense infection in humans. Emerg Infect Dis. 2008;14(8):1330–2.
42. Saffo Z, Ognjan A. Mycobacterium smegmatis infection of a prosthetic total knee arthroplasty. IDCases. 2016;5:80–2.
43. Ferguson DD, Gershman K, Jensen B, Arduino MJ, Yakrus MA, Cooksey RC, et al. Mycobacterium goodii infections associated with surgical implants at Colorado hospital. Emerg Infect Dis. 2004;10(10):1868–71.
44. Yoo DK, Hosseini-Moghaddam SM. Pacemaker pocket infection due to Mycobacterium goodii, a rapidly growing mycobacteria. BMJ Case Rep. 2017;2017:bcr2016218323.
45. Tagashira Y, Kozai Y, Yamasa H, Sakurada M, Kashiyama T, Honda H. A cluster of central line-associated bloodstream infections due to rapidly growing nontuberculous mycobacteria in patients with hematologic disorders at a Japanese tertiary care center: an outbreak investigation and review of the literature. Infect Control Hosp Epidemiol. 2015;36(1):76–80.
46. Maybrook RJ, Campsen J, Wachs ME, Levi ME. A case of Mycobacterium mucogenicum infection in a liver transplant recipient and a review of the literature. Transpl Infect Dis. 2013;15(6):E260–3.
47. Shachor-Meyouhas Y, Geffen Y, Arad-Cohen N, Zaidman I, Ben-Barak A, Davidson S, et al. Mycobacterium phocaicum bacteremia: an emerging infection in pediatric hematology-oncology patients. Pediatr Infect Dis J. 2014;33(12):1299–301.
48. McMullen AR, Mattar C, Kirmani N, Burnham CA. Brown-pigmented Mycobacterium mageritense as a cause of prosthetic valve endocarditis and bloodstream infection. J Clin Microbiol. 2015;53(8):2777–80.
49. Ali S, Khan FA, Fisher M. Catheter-related bloodstream infection caused by Mycobacterium mageritense. J Clin Microbiol. 2007;45(1):273.
50. Appelgren P, Farnebo F, Dotevall L, Studahl M, Jonsson B, Petrini B. Late-onset posttraumatic skin and soft-tissue infections caused by rapid-growing mycobacteria in tsunami survivors. Clin Infect Dis. 2008;47(2):e11–6.
51. Ariza-Heredia EJ, Dababneh AS, Wilhelm MP, Wengenack NL, Razonable RR, Wilson JW. Mycobacterium wolinskyi: a case series and review of the literature. Diagn Microbiol Infect Dis. 2011;71(4):421–7.
52. Vutescu ES, Koenig KM. Mycobacterium cosmeticum infection after total knee arthroplasty: a case report. JBJS Case Connector. 2017;7(1):e3.
53. Taieb A, Ikeguchi R, Yu VL, Rihs JD, Sharma M, Wolfe J, et al. Mycobacterium monacense: a mycobacterial pathogen that causes infection of the hand. J Hand Surg. 2008;33(1):94–6.
54. Brown-Elliott BA, Wallace RJ Jr, Petti CA, Mann LB, McGlasson M, Chihara S, et al. Mycobacterium neoaurum and Mycobacterium bacteremicum sp. nov. as causes of mycobacteremia. J Clin Microbiol. 2010;48(12):4377–85.
55. Song Y, Wu J, Yan H, Chen J. Peritoneal dialysis-associated nontuberculous mycobacterium peritonitis: a systematic review of reported cases. Nephrol Dial Transplant. 2012;27(4):1639–44.
56. Nunez Breton JD, Hernandez G, Simkins J, Chaparro SV. Mycobacterium abscessus left ventricle assist device driveline infections: an emerging pathogen? Transpl Infect Dis. 2018;20(5):e12957.
57. Phadke VK, Hirsh DS, Goswami ND. Patient report and review of rapidly growing mycobacterial infection after cardiac device implantation. Emerg Infect Dis. 2016;22(3):389–95.

58. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med. 2015;36(1):13–34.
59. Spaulding AB, Lai YL, Zelazny AM, Olivier KN, Kadri SS, Prevots DR, et al. Geographic distribution of nontuberculous mycobacterial species identified among clinical isolates in the United States, 2009–2013. Ann Am Thorac Soc. 2017;14(11):1655–61.
60. Doucette K, Fishman JA. Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. Clin Infect Dis. 2004;38(10):1428–39.
61. Chalermskulrat W, Sood N, Neuringer IP, Hecker TM, Chang L, Rivera MP, et al. Non-tuberculous mycobacteria in end stage cystic fibrosis: implications for lung transplantation. Thorax. 2006;61(6):507–13.
62. Novick RJ, Moreno-Cabral CE, Stinson EB, Oyer PE, Starnes VA, Hunt SA, et al. Nontuberculous mycobacterial infections in heart transplant recipients: a seventeen-year experience. J Heart Transplant. 1990;9(4):357–63.
63. Thomson R, Tolson C, Sidjabat H, Huygens F, Hargreaves M. Mycobacterium abscessus isolated from municipal water – a potential source of human infection. BMC Infect Dis. 2013;13:241.
64. Kennedy BS, Bedard B, Younge M, Tuttle D, Ammerman E, Ricci J, et al. Outbreak of Mycobacterium chelonae infection associated with tattoo ink. N Engl J Med. 2012;367(11):1020–4.
65. Stout JE, Gadkowski LB, Rath S, Alspaugh JA, Miller MB, Cox GM. Pedicure-associated rapidly growing mycobacterial infection: an endemic disease. Clin Infect Dis. 2011;53(8):787–92.
66. Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. Science. 2016;354(6313):751–7.
67. Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V. Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. Appl Environ Microbiol. 2002;68(3):1025–32.
68. Culton DA, Lachiewicz AM, Miller BA, Miller MB, Mackuen C, Groben P, et al. Nontuberculous mycobacterial infection after fractionated CO(2) laser resurfacing. Emerg Infect Dis. 2013;19(3):365–70.
69. Cho SY, Peck KR, Kim J, Ha YE, Kang CI, Chung DR, et al. Mycobacterium chelonae infections associated with bee venom acupuncture. Clin Infect Dis. 2014;58(5):e110–3.
70. Kanamori H, Weber DJ, Rutala WA. Healthcare outbreaks associated with a water reservoir and infection prevention strategies. Clin Infect Dis. 2016;62(11):1423–35.
71. Shin JH, Lee EJ, Lee HR, Ryu SM, Kim HR, Chang CL, et al. Prevalence of non-tuberculous mycobacteria in a hospital environment. J Hosp Infect. 2007;65(2):143–8.
72. Schnabel D, Esposito DH, Gaines J, Ridpath A, Barry MA, Feldman KA, et al. Multistate US outbreak of rapidly growing mycobacterial infections associated with medical tourism to the Dominican Republic, 2013–2014(1). Emerg Infect Dis. 2016;22(8):1340–7.
73. van Ingen J, Kohl TA, Kranzer K, Hasse B, Keller PM, Katarzyna Szafranska A, et al. Global outbreak of severe Mycobacterium chimaera disease after cardiac surgery: a molecular epidemiological study. Lancet Infect Dis. 2017;17(10):1033–41.
74. Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, Inns T, et al. Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study. Lancet. 2013;381(9877):1551–60.
75. Doyle RM, Rubio M, Dixon G, Hartley J, Klein N, Coll P, et al. Cross-transmission is not the source of new Mycobacterium abscessus infections in a multi-centre cohort of cystic fibrosis patients. Clin Infect Dis. 2019. https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciz526/5521616
76. Tortoli E, Kohl TA, Trovato A, Baldan R, Campana S, Cariani L, et al. Mycobacterium abscessus in patients with cystic fibrosis: low impact of inter-human transmission in Italy. Eur Respir J. 2017;50(1):1602525.

77. Chau CL, Griffith JF, Chan PT, Lui TH, Yu KS, Ngai WK. Rice-body formation in atypical mycobacterial tenosynovitis and bursitis: findings on sonography and MR imaging. AJR Am J Roentgenol. 2003;180(5):1455–9.
78. Franco-Paredes C, Marcos LA, Henao-Martinez AF, Rodriguez-Morales AJ, Villamil-Gomez WE, Gotuzzo E, et al. Cutaneous mycobacterial infections. Clin Microbiol Rev. 2018;32(1): e00069–18.
79. Vetter E, Torgerson C, Feuker A, Hughes J, Harmsen S, Schleck C, et al. Comparison of the BACTEC MYCO/F Lytic bottle to the isolator tube, BACTEC Plus Aerobic F/bottle, and BACTEC Anaerobic Lytic/10 bottle and comparison of the BACTEC Plus Aerobic F/bottle to the Isolator tube for recovery of bacteria, mycobacteria, and fungi from blood. J Clin Microbiol. 2001;39(12):4380–6.
80. Toney NC, Toney SR, Butler WR. Utility of high-performance liquid chromatography analysis of mycolic acids and partial 16S rRNA gene sequencing for routine identification of Mycobacterium spp. in a national reference laboratory. Diagn Microbiol Infect Dis. 2010;67(2):143–52.
81. Luo L, Cao W, Chen W, Zhang R, Jing L, Chen H, et al. Evaluation of the VITEK MS knowledge base version 3.0 for the identification of clinically relevant Mycobacterium species. Emerg Microbes Infect. 2018;7(1):114.
82. Body BA, Beard MA, Slechta ES, Hanson KE, Barker AP, Babady NE, et al. Evaluation of the Vitek MS v3.0 matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of Mycobacterium and Nocardia species. J Clin Microbiol. 2018;56(6):e00237–18.
83. Miller E, Cantrell C, Beard M, Derylak A, Babady NE, McMillen T, et al. Performance of Vitek MS v3.0 for identification of mycobacterium species from patient samples by use of automated liquid medium systems. J Clin Microbiol. 2018;56(8):e00219–8.
84. Jeon K, Kwon OJ, Lee NY, Kim BJ, Kook YH, Lee SH, et al. Antibiotic treatment of Mycobacterium abscessus lung disease: a retrospective analysis of 65 patients. Am J Respir Crit Care Med. 2009;180(9):896–902.
85. Brown-Elliott BA, Nash KA, Wallace RJ Jr. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. Clin Microbiol Rev. 2012;25(3):545–82.
86. Brown-Elliott BA, Killingley J, Vasireddy S, Bridge L, Wallace RJ Jr. In vitro comparison of ertapenem, meropenem, and imipenem against isolates of rapidly growing mycobacteria and nocardia by use of broth microdilution and Etest. J Clin Microbiol. 2016;54(6):1586–92.
87. Brown-Elliott BA, Wallace RJ Jr. In vitro susceptibility testing of tedizolid against nontuberculous mycobacteria. J Clin Microbiol. 2017;55(6):1747–54.
88. Heidarieh P, Mirsaeidi M, Hashemzadeh M, Feizabadi MM, Bostanabad SZ, Nobar MG, et al. In vitro antimicrobial susceptibility of nontuberculous mycobacteria in Iran. Microb Drug Resist. 2016;22(2):172–8.
89. Choi H, Jhun BW, Kim SY, Kim DH, Lee H, Jeon K, et al. Treatment outcomes of macrolide-susceptible Mycobacterium abscessus lung disease. Diagn Microbiol Infect Dis. 2018;90(4):293–5.
90. Koh WJ, Jeong BH, Kim SY, Jeon K, Park KU, Jhun BW, et al. Mycobacterial characteristics and treatment outcomes in mycobacterium abscessus lung disease. Clin Infect Dis. 2017;64(3):309–16.
91. Choi H, Kim SY, Kim DH, Huh HJ, Ki CS, Lee NY, et al. Clinical characteristics and treatment outcomes of patients with acquired macrolide-resistant mycobacterium abscessus lung disease. Antimicrob Agents Chemother. 2017;61(10):e01146–17.
92. Choi GE, Shin SJ, Won CJ, Min KN, Oh T, Hahn MY, et al. Macrolide treatment for Mycobacterium abscessus and Mycobacterium massiliense infection and inducible resistance. Am J Respir Crit Care Med. 2012;186(9):917–25.
93. Nash KA, Brown-Elliott BA, Wallace RJ Jr. A novel gene, erm(41), confers inducible macrolide resistance to clinical isolates of Mycobacterium abscessus but is absent from Mycobacterium chelonae. Antimicrob Agents Chemother. 2009;53(4):1367–76.

94. Brown-Elliott BA, Vasireddy S, Vasireddy R, Iakhiaeva E, Howard ST, Nash K, et al. Utility of sequencing the erm(41) gene in isolates of Mycobacterium abscessus subsp. abscessus with low and intermediate clarithromycin MICs. J Clin Microbiol. 2015;53(4):1211–5.
95. Brown-Elliott BA, Hanson K, Vasireddy S, Iakhiaeva E, Nash KA, Vasireddy R, et al. Absence of a functional erm gene in isolates of Mycobacterium immunogenum and the Mycobacterium mucogenicum group, based on in vitro clarithromycin susceptibility. J Clin Microbiol. 2015;53(3):875–8.
96. Rapp RP. Pharmacokinetics and pharmacodynamics of intravenous and oral azithromycin: enhanced tissue activity and minimal drug interactions. Ann Pharmacother. 1998;32(7–8):785–93.
97. Amacher DE, Schomaker SJ, Retsema JA. Comparison of the effects of the new azalide antibiotic, azithromycin, and erythromycin estolate on rat liver cytochrome P-450. Antimicrob Agents Chemother. 1991;35(6):1186–90.
98. Wallace RJ Jr, Meier A, Brown BA, Zhang Y, Sander P, Onyi GO, et al. Genetic basis for clarithromycin resistance among isolates of Mycobacterium chelonae and Mycobacterium abscessus. Antimicrob Agents Chemother. 1996;40(7):1676–81.
99. Ferro BE, Srivastava S, Deshpande D, Pasipanodya JG, van Soolingen D, Mouton JW, et al. Tigecycline is highly efficacious against mycobacterium abscessus pulmonary disease. Antimicrob Agents Chemother. 2016;60(5):2895–900.
100. Philley JV, Wallace RJ Jr, Benwill JL, Taskar V, Brown-Elliott BA, Thakkar F, et al. Preliminary results of bedaquiline as salvage therapy for patients with nontuberculous mycobacterial lung disease. Chest. 2015;148(2):499–506.
101. McGuffin SA, Pottinger PS, Harnisch JP. Clofazimine in nontuberculous mycobacterial infections: a growing niche. Open Forum Infect Dis. 2017;4(3):ofx147.
102. Bax HI, de Vogel CP, Mouton JW, de Steenwinkel JEM. Omadacycline as a promising new agent for the treatment of infections with Mycobacterium abscessus. J Antimicrob Chemother. 2019;74(10):2930–3.
103. Kaushik A, Ammerman NC, Martins O, Parrish NM, Nuermberger EL. In vitro activity of new tetracycline analogs omadacycline and eravacycline against drug-resistant clinical isolates of Mycobacterium abscessus. Antimicrob Agents Chemother. 2019;63(6):e00470–19.
104. Yuste JR, Bertó J, Del Pozo JL, Leiva J. Prolonged use of tedizolid in a pulmonary non-tuberculous mycobacterial infection after linezolid-induced toxicity. J Antimicrob Chemother. 2017;72(2):625–8.
105. Brown-Elliott BA, Wallace RJ Jr. In vitro susceptibility testing of bedaquiline against Mycobacterium abscessus complex. Antimicrob Agents Chemother. 2019;63(2):e01919–8.
106. van Ingen J, Totten SE, Helstrom NK, Heifets LB, Boeree MJ, Daley CL. In vitro synergy between clofazimine and amikacin in treatment of nontuberculous mycobacterial disease. Antimicrob Agents Chemother. 2012;56(12):6324–7.
107. Martiniano SL, Wagner BD, Levin A, Nick JA, Sagel SD, Daley CL. Safety and effectiveness of clofazimine for primary and refractory nontuberculous mycobacterial infection. Chest. 2017;152(4):800–9.
108. Cariello PF, Kwak EJ, Abdel-Massih RC, Silveira FP. Safety and tolerability of clofazimine as salvage therapy for atypical mycobacterial infection in solid organ transplant recipients. Transpl Infect Dis. 2015;17(1):111–8.
109. Osmani M, Sotello D, Alvarez S, Odell JA, Thomas M. Mycobacterium abscessus infections in lung transplant recipients: 15-year experience from a single institution. Transpl Infect Dis. 2018;20(2):e12835.
110. Chandrashekaran S, Escalante P, Kennedy CC. Mycobacterium abscessus disease in lung transplant recipients: diagnosis and management. J Clin Tuberc Other Mycobact Dis. 2017;9:10–8.
111. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat Med. 2019;25(5):730–3.

112. Longworth SA, Blumberg EA, Barton TD, Vinnard C. Non-tuberculous mycobacterial infections after solid organ transplantation: a survival analysis. Clin Microbiol Infect. 2015;21(1):43–7.
113. Olivier KN, Shaw PA, Glaser TS, Bhattacharyya D, Fleshner M, Brewer CC, et al. Inhaled amikacin for treatment of refractory pulmonary nontuberculous mycobacterial disease. Ann Am Thorac Soc. 2014;11(1):30–5.
114. Safdar A. Aerosolized amikacin in patients with difficult-to-treat pulmonary nontuberculous mycobacteriosis. Eur J Clin Microbiol Infect Dis. 2012;31(8):1883–7.
115. Griffith DE, Eagle G, Thomson R, Aksamit TR, Hasegawa N, Morimoto K, et al. Amikacin liposome inhalation suspension for treatment-refractory lung disease caused by Mycobacterium avium complex (CONVERT): a prospective, open-label, randomized study. Am J Respir Crit Care Med. 2018;198(12):1559–69.
116. Olivier KN, Griffith DE, Eagle G, McGinnis JP 2nd, Micioni L, Liu K, et al. Randomized trial of liposomal amikacin for inhalation in nontuberculous mycobacterial lung disease. Am J Respir Crit Care Med. 2017;195(6):814–23.
117. Diel R, Ringshausen F, Richter E, Welker L, Schmitz J, Nienhaus A. Microbiological and clinical outcomes of treating non-Mycobacterium avium complex nontuberculous mycobacterial pulmonary disease: a systematic review and meta-analysis. Chest. 2017;152(1):120–42.
118. Kotecha S, Smibert OC, Doi A, Gooi J, Morrissey O, Paraskeva M, et al. A successful aggressive surgical and medical approach to pulmonary artery obstruction due to Mycobacterium abscessus infection post lung transplantation. Transpl Infect Dis. 2018;20(2):e12838.
119. Unai S, Miessau J, Karbowski P, Bajwa G, Hirose H. Sternal wound infection caused by Mycobacterium chelonae. J Card Surg. 2013;28(6):687–92.
120. El Helou G, Viola GM, Hachem R, Han XY, Raad II. Rapidly growing mycobacterial bloodstream infections. Lancet Infect Dis. 2013;13(2):166–74.
121. Keating MR, Daly JS. Nontuberculous mycobacterial infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):77–82.
122. Novosad SA, Beekmann SE, Polgreen PM, Mackey K, Winthrop KL, Team MaS. Treatment of Mycobacterium abscessus infection. Emerg Infect Dis. 2016;22(3):511–514.
123. De Groote MA, Huitt G. Infections due to rapidly growing mycobacteria. Clin Infect Dis. 2006;42(12):1756–63.
124. Vasconcelos KA, Frota S, Ruffino-Netto A, Kritski AL. The importance of audiometric monitoring in patients with multidrug-resistant tuberculosis. Rev Soc Bras Med Trop. 2017;50(5):646–51.
125. Kranzer K, Elamin WF, Cox H, Seddon JA, Ford N, Drobniewski F. A systematic review and meta-analysis of the efficacy and safety of N-acetylcysteine in preventing aminoglycoside-induced ototoxicity: implications for the treatment of multidrug-resistant TB. Thorax. 2015;70(11):1070–7.
126. Agyeman AA, Ofori-Asenso R. Efficacy and safety profile of linezolid in the treatment of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis: a systematic review and meta-analysis. Ann Clin Microbiol Antimicrob. 2016;15(1):41.
127. Lemoine M, Laurent C, Hanoy M, Leporrier J, Francois A, Guerrot D, et al. Immune reconstitution inflammatory syndrome secondary to Mycobacterium kansasii infection in a kidney transplant recipient. Am J Transplant. 2015;15(12):3255–8.
128. Avery RK, Michaels MG, Practice ASTIDCo. Strategies for safe living following solid organ transplantation. Am J Transplant. 2009;9(Suppl 4):S252–7.
129. Crist MB, Perz JF. Modern healthcare versus nontuberculous mycobacteria: who will have the upper hand? Clin Infect Dis. 2017;64(7):912–3.
130. Baker AW, Smith B, Sexton DJ, Huslage K, Stout JE, Anderson DJ, et al. 927. Tap water avoidance decreases rates of nontuberculous mycobacteria in intensive care units. Open Forum Infect Dis. 2018;5(Suppl 1):S29–30.
131. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the Pulmonary

Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2015;34(1):1–15.
132. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann JL, Nick JA, et al. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. Thorax. 2016;71(Suppl 1):i1–22.
133. Tissot A, Thomas MF, Corris PA, Brodlie M. NonTuberculous Mycobacteria infection and lung transplantation in cystic fibrosis: a worldwide survey of clinical practice. BMC Pulm Med. 2018;18(1):86.
134. Rao M, Silveira FP. Non-tuberculous Mycobacterial infections in thoracic transplant candidates and recipients. Curr Infect Dis Rep. 2018;20(6):14.
135. Lobo LJ, Chang LC, Esther CR Jr, Gilligan PH, Tulu Z, Noone PG. Lung transplant outcomes in cystic fibrosis patients with pre-operative Mycobacterium abscessus respiratory infections. Clin Transpl. 2013;27(4):523–9.
136. Gilljam M, Schersten H, Silverborn M, Jonsson B, Ericsson HA. Lung transplantation in patients with cystic fibrosis and Mycobacterium abscessus infection. J Cyst Fibros. 2010;9(4):272–6.
137. Roux AL, Catherinot E, Soismier N, Heym B, Bellis G, Lemonnier L, et al. Comparing Mycobacterium massiliense and Mycobacterium abscessus lung infections in cystic fibrosis patients. J Cyst Fibros. 2015;14(1):63–9.
138. Wolfe CR, Baker AW, Patel CB, Patel PA, Milano CA, Schroder JN, et al. Mycobacterium abscessus infection in patients with ventricular assist devices. J Heart Lung Transplant. 2017;36(4 Supplement):S257–S8.
139. Shachor-Meyouhas Y, Sprecher H, Eluk O, Ben-Barak A, Kassis I. An outbreak of Mycobacterium mucogenicum bacteremia in pediatric hematology-oncology patients. Pediatr Infect Dis J. 2011;30(1):30–2.

Slow-Growing Nontuberculous Mycobacteria in Transplant

20

Esther Benamu and Joanna Nelson

Contents

Introduction 460
Epidemiology 460
Slow-Growing Mycobacteria: Organisms and Mode of Transmission 460
Incidence of NTM in Transplant Recipients 462
Timing of Infection 463
Risk Factors 463
Clinical Impact 464
Presentation and Diagnosis 466
Clinical Presentation 466
Diagnosis 468
Management 472
General Principles 472
MAC 473
M. chimaera 475
M. kansasii 475
Other Slow-Growing NTM 476
Duration of Therapy 476
Prevention 478
Conclusion 479
Key Points 480
References 480

E. Benamu (✉)
School of Medicine, Division of Infectious Diseases, University of Colorado, Aurora, CO, USA
e-mail: esther.benamu@cuanschutz.edu

J. Nelson
Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
e-mail: joanna2@stanford.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_17

Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms found in soil and water. Although clinical infections with NTM are rare, epidemiologic studies suggest an increase in their incidence in the last four decades [1–3]. Factors such as the continuous expansion of the immunocompromised population, more widespread environmental exposure to these organisms (inhalation of shower-produced aerosols), and improvements in mycobacterial detection and diagnosis have contributed to an increased awareness of NTM as human pathogens and the emergence of these infections in transplant recipients.

Due to impaired cell-mediated immunity, these patients are at increased risk of infection and have higher associated morbidity and mortality. The diagnosis and management of NTM infection in the transplanted host pose a particular challenge to clinicians. Disease manifestations can be protean and nonspecific, invasive infection is often difficult to recognize because of the ubiquitous nature of the organisms, and microbiological diagnosis is frequently delayed. In addition, the necessity of prolonged treatment with multidrug regimens associated with significant drug toxicities and the need to consider drug-drug interactions between antimicrobials and immunosuppressants add to the complexity of managing transplant recipients with NTM.

Our understanding of NTM infections in the immunocompromised host is based on case reports and case series [3–9]. To date, of the over 180 NTM species that have been identified, only 25 are known to have caused disease in transplant recipients [10]. These include rapid and slow-growing NTM, classified as such based on whether the species isolates require less or more than 7 days to form mature colonies on subculture, respectively. This chapter will discuss the epidemiology, clinical presentation, diagnosis, and management of the most common slow-growing NTM described in solid organ transplant (SOT) and hematopoietic stem cell transplant recipients (HSCT) (Table 1).

Epidemiology

Slow-Growing Mycobacteria: Organisms and Mode of Transmission

Approximately 18 species of slow-growing mycobacteria (SGM) are known to cause infection in transplant patients (Table 1). *Mycobacterium avium* and *Mycobacterium intracellulare* (together known as *Mycobacterium avium* complex [MAC]) are the most common NTM species in the United States and are the most common species isolated in SOT recipients [11], followed by *M. kansasii*, *M. haemophilum*, *M. marinum*, *and M. xenopi*. In HSCT recipients, the most frequent species are MAC, *M. haemophilum, M. gordonae*, *and M. xenopi* [3, 7, 12].

NTM are ubiquitous saprophytic organisms widely distributed in the environment, in sources including soil and natural and treated water (e.g., M. *kansasii*,

Table 1 Slow-growing mycobacteria reported in transplant recipients and associated clinical syndromes (SOT, HSCT) [7, 11, 31]

	Pleuropulmonary disease	Skin and soft tissue	Osteoarticular disease	Catheter-related	Lymphadenitis	Disseminated disease, Other
M. avium intracellulare-M. avium	x	x	x		x	x
M. avium intracellulare-M. intracellulare	x	x	x		x	x
M. avium intracellulare-M. chimaera	x					x
M. kansasii	x				x	x
M. haemophilum	x	x	x	x	x	x
M. marinum		X	X			x
M. xenopi	X	x	x			x
M. malmoense	X	x	x		x	X
M. terrae complex [92, 93]	x	x	x			
M. szulgai	X	x	x		x	X
M. asiaticum [94]	x					x
M. genavense		x	x		X	X
M. gordonae	x			x		x
M. scrofulaceum	x				X	x
M. triplex [95]						x
M. gastri [96]			x			
M. thermoresistibile [97]		x				
M. celatum [98]	x					x
M. simiae (lung transplant candidate) [99]	x					

M. ulcerans; M. conspicuum have not been reported in transplant recipients
SOT solid organ transplant, *HSCT* hematopoietic stem cell transplant

M. *xenopi*, and *M. simiae* are recovered almost exclusively from municipal water sources) and also dust, aerosols, plant material, animals, and birds [13, 14]. Most infections arise following environmental exposure. These organisms can be resistant to disinfection and thus can be recovered from drinking water distribution systems. Nosocomial cases due to contaminated surgical equipment, solutions, and cleaning devices and hospital outbreaks from contaminated water systems and hemodialysis units have been reported [15, 16]. In recent years, a global outbreak of disseminated *M. chimaera* (until recently, identified as *M. avium intracellulare* by most laboratories) has been ongoing, associated with contaminated Sorin 3T heater-cooler devices used in open-chest surgery requiring cardiopulmonary bypass. It has affected patients with ventricular-assist devices and heart transplant recipients [17–19]. This outbreak has raised awareness of the emergence of *M. chimaera* as a pathogen, prompting the development of specific guidelines [19] addressing the diagnosis, management, and prevention of infections with this organism. Another pseudo-outbreak of *M. chimaera* originating in aerators and hand-washing machines of a HSCT unit was also recently reported [20]. Recent epidemiologic and genetic evidence of *M. abscessus* spread between individuals with cystic fibrosis (CF) has contested the historical belief that person-to-person transmission of NTM does not occur [21, 22]. The exact mechanism of transmission remains unclear but is thought to be indirect, through contaminated fomites and long-lived infectious aerosols rather than direct human-to-human spread. Moreover, this form of transmission with other NTM species has not been demonstrated. In SOT recipients, transmission from donor to recipient via the allograft can occur but is rare [23].

Incidence of NTM in Transplant Recipients

NTM infection posttransplant is relatively infrequent. However, this disease is on the rise in both the general and transplant population [2, 24–26]. Exact data regarding the incidence of NTM infections in SOT and HSCT recipients is lacking, since reporting of these infections is not mandatory. A large single-center study of SOT recipients of all organ types noted an incidence of NTM infection, defined as any positive culture for NTM, of 1.5%. For slow-growing NTM infection specifically, the incidence was 1% [27]. Among SOT recipients, the incidence of NTM infection varies based on transplanted organ, the highest being reported in lung transplant patients. The only study of NTM infections in liver transplant recipients reported the lowest incidence, of 0.04% [28], which could be reflective of the local epidemiology, underdiagnosis, or true diminished susceptibility to NTM. Among other SOT recipients, studies suggest an incidence of 0.16–0.38% in kidney transplant, 0.24–2.8% in heart transplant, and 0.46–8% in lung transplant recipients. An incidence of 0.4–4.9% has been observed in HSCT patients [3, 7, 26, 29]. Studies have included all NTM species: rapid, intermediate, and slow-growing. Therefore, the incidence of slow-growing NTM is even lower than in these reports.

Timing of Infection

The timing of infection after transplantation varies significantly in the published literature. In general, studies have evaluated infection with all NTM species, and specific data on slow-growing mycobacteria (SGM) is lacking. In a series of 82 SOT recipients with NTM infection, diagnosis was made at a mean of 48 months after transplant (range 10 days to 269 months) [6]. A smaller study of 34 SOT recipients (55% of which had SGM) reported a median time of diagnosis of 8-month post-transplant but in a bimodal distribution, with 18% of infections occurring within 3 months and 44% after 3 years posttransplant [30]. A recent review of NTM infections in SOT recipients reported shorter median times of onset in lung transplant recipients (13 months) than in liver, kidney, and heart recipients (24, 27, and 33 months respectively) [31].

Among 30 allogeneic HSCT recipients with NTM infection, 77% of which were with SGM [MAC ($n = 14$; 46.7%), *M. xenopi* ($n = 8$; 26.7%), *M. gordonae* ($n = 1$; 3.3%)], the median time to diagnosis of NTM infection was 343 days (range 19–1967), and in 83% of patients, the diagnosis was made within 2 years of allogeneic HSCT [26]. In a review of published cases of NTM infection in HSCT recipients, Knoll et al. described a shorter time of onset in this population, with a median onset of 5 months (range 3–11 months) [7].

Risk Factors

Infections with NTM result from the interaction of multiple factors including the type and load of the exposure, the virulence of the pathogen, as well as host-specific factors [32]. As species vary in distribution between regions and across different latitudes and water sources, a determined exposure in a geographic location can predispose to a specific NTM infection. For instance, *M. marinum* infection, commonly known as "fish tank granuloma," is closely related to direct injury from fish or shrimp fins or bites or after cutaneous trauma and subsequent exposure to contaminated saltwater or freshwater from cleaning of fish tanks or swimming pools. *M. kansasii* or *M. avium complex* have been found to be more frequent in states near coastal swamps and estuaries [33]. Certain SGM species such as *M. kansasii* or *M. malmoense* are regarded as being more virulent than others like *M. gordonae* and *M. terrae complex*, usually considered contaminants [11]. However, in the transplanted host, severe infections with species not traditionally considered pathogenic are increasingly reported [23, 34], underscoring the importance of host immune factors.

In the general population, several host-specific predisposing factors for NTM infection have been identified. Structural or preexisting pulmonary diseases such as chronic obstructive pulmonary disease (COPD), bronchiectasis, alpha-1 antitrypsin deficiency, pneumoconiosis, sarcoidosis, pulmonary tuberculosis, and CF have been strongly associated with a risk of pulmonary NTM infection. NTM are recovered in 6.6–20% of CF patients [3], and therefore, these patients are routinely screened for NTM when lung transplantation is considered. Individuals with gastroesophageal

reflux and other esophageal dysmotility syndromes, rheumatoid arthritis, low vitamin D, low body mass index, and malnutrition are also at risk. Nonsmoker elderly females with certain phenotypic features like a slender body type, scoliosis, pectus defects, or mitral valve prolapse (Lady Windermere syndrome) are known to be prone to pulmonary bronchiectatic disease. Immunosuppressed patients are at increased risk of opportunistic infection with NTM. In HIV-infected individuals, a CD4 T-cell count less than 50/μL is associated with an increased risk of disseminated infection [11]. A number of immunodeficiencies have been associated with NTM infection, including inherited disorders and targeted therapies with biologics affecting the IFNγ-IL12 pathway [35], cytokine signaling (e.g., STAT mutations), or the macrophage and dendritic cell function (e.g., GATA2, NRAMP1) [36]. Treatment with corticosteroids and antitumor necrosis factor agents also predisposes to NTM infections [35, 37–40].

In the transplanted host, the disruption of mucosal barriers during surgery or via vascular catheterization can serve as a portal of entry for NTM. Structural abnormalities, notably in lung transplant candidates and recipients, facilitate colonization with NTM. Finally, the net state of immunosuppression in transplant recipients promotes the progression to disease in those who have been environmentally exposed or colonized. Taking this into consideration, it is not surprising that NTM infections are more commonly seen in lung transplant recipients.

Longworth et al. identified lung transplantation and biopsy-proven acute cellular rejection as important risk factors for NTM infection in SOT recipients [30]. Among lung transplant patients, CF, pretransplant NTM infection, and the use of rabbit antithymocyte globulin have been identified as significant risk factors of post-transplant NTM. However, in a different single-center study, African American race and high-risk CMV mismatch status emerged as risk factors, and there was no difference in the incidence of NTM infection among those with and without pre-transplant NTM infection [41].

For HSCT recipients, Beswick et al. identified CMV viremia and chronic graft-versus-host disease as independent risk factors of NTM infection, both surrogate markers of overall immunosuppression [26]. An earlier study suggested total body irradiation (TBI) as a risk factor of cutaneous NTM in this population [12].

Clinical Impact

The clinical impact of SGM depends on the site of culture positivity. A positive culture from a sterile site is considered clinically significant. However, because these organisms are ubiquitous in the environment, it is often a challenge to determine clinical relevance if the organism is isolated from a non-sterile site such as sputum or bronchoalveolar lavage fluid. In this setting, particularly relevant to the lung transplant population, a positive culture is not always indicative of disease and could simply signify transient growth or colonization. Colonization of the airway by NTM is 10 times more common than invasive infection in lung transplant patients, comprising 75–89% of cases with positive culture (the majority of which were

SGM) in two single-center studies [8, 42]. The same percentage of pulmonary colonization was noted among all organ types in the SOT population (75%) [30].

Similar findings have been observed in HSCT patients. A small single-center study found that 20% of patients with NTM isolated in respiratory sample had proven or probable pulmonary infection requiring treatment [34]. Another found that 44% of HSCT patients with NTM isolated on sputum cultures met criteria for NTM pulmonary disease requiring treatment [25]. Based on these studies, positive NTM respiratory culture in HSCT patients represented colonization 56–80% of the time. Underlying GVHD as well as coinfection with additional pathogens such as *Aspergillus* is common in HSCT patients who grow NTM from respiratory cultures, making it difficult to pinpoint the exact cause of symptoms and the effect of NTM specifically [43]. Thus, a positive culture must be interpreted in the context of the patient's overall status to determine the clinical impact of these organisms.

NTM infections are often difficult to treat. Disease cure rates in the literature for all NTM infections range from 32% to 44%, although a greater number of patients do have some clinical improvement without complete resolution of infection on NTM therapy [3]. Outcomes in transplant patients with NTM disease are highly variable and depend on the extent of infection, virulence, and susceptibility of the organism and level of immunosuppression of the host [3, 4, 42]. Studies of the impact of NTM infection on patient outcome show varied results, likely in part due to small numbers of patients examined in single-center studies. Early infection within the first year posttransplant has been associated with decreased survival in SOT patients (half of which were infected with SGM), although NTM infection itself was listed as a cause of death in a minority [44]. The impact of early NTM infection on survival is complex. Multiple factors likely contribute to increase in mortality including indirect immune modulation, transplant rejection, or development of secondary infections [44]. Among lung transplant patients specifically, there are mixed results regarding NTM infection impact on mortality, with one single-center study showing that NTM infection (both colonization and pulmonary disease) at any time point is associated with increased mortality (although in no patients was death a direct result of NTM infection) and another showing no effect of NTM infection on mortality[41, 42]. Both studies showed no significant independent increased risk of bronchiolitis obliterates syndrome (BOS) when controlling for other potential contributing factors.

Interestingly, in HSCT patients, definite NTM infection has been associated with excellent prognosis, while possible and probable infection was associated with increased mortality [12]. This suggests other underlying host factors associated with positive NTM cultures, such as pulmonary disease or GVHD, may have more of an impact on survival than the NTM infection itself. It's also worth noting that there were different pathogens and areas of disease involvement between these two groups, with cutaneous and musculoskeletal *M. haemophilum* in the former vs. pulmonary MAC in the latter, which may have confounded results [12]. Chronic airway colonization with any bacteria in HSCT patients with BOS has been associated with decreased survival, more rapid decline of lung function, and progression of CT findings. A small number of patients with NTM were included in this study [45].

Further study into the impact of positive NTM cultures on the trajectory of GVHD itself is needed.

Data regarding the lung microbiome and its impact on pulmonary health and infection are emerging [46]. Further study into how NTM fit into this niche environment, interact with the usual commensal microbiome, and therefore potentially contribute to pulmonary pathology would be enlightening.

Presentation and Diagnosis

Clinical Presentation

The clinical manifestations of NTM infection in transplant recipients are protean, and constitutional symptoms like fever, weight loss, and night sweats are often absent. Thus, clinicians must maintain a high index of suspicion for these infections and a low threshold to obtain diagnostic specimens for mycobacterial culture.

NTM infections classically cause one of six clinical syndromes: pleuropulmonary disease, skin and soft tissue infection, musculoskeletal infection, catheter-associated disease, lymphadenitis, or disseminated disease (Table 1).

Pleuropulmonary disease is the most common presentation of NTM infection after SOT, and lung transplant recipients are the most commonly affected [8, 30, 42]. Patients typically manifest with cough, dyspnea, sputum production, and occasionally, hemoptysis. Disease can be fibrocavitary, nodular/bronchiectatic, or a combination of both. Computed tomography of the chest may show pulmonary nodules, tree-in-bud opacities, bronchiectasis, and/or consolidations. *M. avium* complex (MAC), *M. kansasii*, *Mycobacterium xenopi* are together with the RGM *M. abscessus*, the most common NTM-causing pulmonary disease [47]. *M. kansasii*, in particular, can cause aggressive disease in lung transplant recipients and often develops into cavitary infiltrates with an upper-lobe predilection, mimicking tuberculosis reactivation [11].

Non-lung SOT recipients most commonly present with cutaneous lesions, tenosynovitis, or septic arthritis [31]. Dissemination to noncontiguous areas is already present at the time of diagnosis in half of these patients. These forms of infection are most frequently caused by RGM (*M. chelonae*, *M. fortuitum*) but also common with *M. haemophilum* and *M. marinum*, owing to their predilection for cooler body sites like the extremities [48]. Skin lesions are typically papules or painful erythematous or violaceous subcutaneous nodules that can form abscesses and/or ulcerate, usually after 2–3 weeks. Severe complications may include osteomyelitis, tenosynovitis, and arthritis of the joints. *M. marinum* rarely disseminates and classically presents with a localized lesion at the inoculation site after exposure to fresh or saltwater ("fish tank granuloma"), spreading in a lymphangitic distribution resembling sporotrichosis (Fig. 1) [1, 9].

Disseminated infections are not uncommon; approximately 50% of patients with pulmonary disease demonstrate dissemination at the time of diagnosis [10]. In these patients, constitutional symptoms are more often described. Sites of

Fig. 1 *M. marinum* infection in a renal transplant recipient following a puncture wound at the tip of the index finger while hunting ducks in a lake

dissemination can include skin, lymph nodes, bone marrow, visceral organs, and musculoskeletal sites. *M. kansasii* and MAC are the most common SGM-causing disseminated disease. Interestingly, the primary involvement of the allograft has been described with MAC infection and has been reported to cause acute interstitial nephritis in renal transplant recipients and hepatic infection in liver transplant recipients [49, 50]. *M. haemophilum* and *M. genavense* less commonly cause disseminated disease in SOT recipients [51, 52]; intestinal involvement may occasionally occur [52, 53].

In recent years, infections with *M. chimaera* have emerged as part of a global outbreak originating in heater-cooler devices used in surgeries requiring

cardiopulmonary bypass. The incubation period of *M. chimaera* can range from 6 weeks to more than 6 years and can present with initial symptoms that may be very subtle. Thus, a high index of suspicion and awareness of the most frequently described manifestations is key to promptly diagnose and treat this infection. Common reported symptoms are prolonged fever, weight loss, generalized malaise, and night sweats. Eventually, patients may develop sternal/wound infections, mediastinitis, or cardiovascular infections (prosthetic valve endocarditis, aortic graft infection, myocarditis, infected pseudoaneurysms, infections of implantable and ventricular-assist devices). Disseminated disease with bacteremia may involve a variety of organs, including the lung (pneumonitis), spleen (splenomegaly), bone marrow (cytopenias), kidney (nephritis), liver (hepatitis), brain (vasculitis, encephalitis), skin, or bone (spine involvement). More importantly, the presence of choroidal lesions and chorioretinitis – which may be asymptomatic – has been found to be a hallmark of disseminated *M. chimaera* infection [19].

In HSCT recipients, catheter-related infections are the most frequent form of NTM infection, although the organisms most typically involved are RGM, followed by *M. haemophilum* [7, 12, 34, 54]. Skin and soft tissue and pulmonary infections are also common [7]; lymphadenitis is less frequent. In a recent study, Beswick et al. reported 30 NTM infections in HSCT recipients, 28 of which were pulmonary and 2 disseminated (pulmonary and an additional site, 1 skin, 1 blood). Twenty-three of these infections were caused by SGM (MAC, *M. xenopi, M. gordonae*) [26].

Diagnosis

Maintaining a high index of clinical suspicion and considering mycobacteria in the differential diagnosis of infection are key to promptly recognize NTM infection in the transplant host.

There is currently no serological test to diagnose SGM infections. However, it is worth mentioning that, while the interferon-gamma release assays (IGRA) have no role in the diagnosis, the antigens used in these assays cross react with *M. marinum*, *M. kansasii,* and *M. szulgai* and possibly other unrecognized NTM.

Patients in whom pleuropulmonary NTM is suspected should be evaluated with a chest computed tomography scan and with at least two mycobacterial sputum (expectorated or induced) cultures collected on separate days. If the latter cannot be obtained or cultures are negative, yet clinical suspicion remains, bronchoscopy for bronchial washings and cultures should be pursued [11, 47]. The diagnosis of pulmonary NTM disease, notably the distinction of invasive infection and colonization, can be particularly challenging, especially in lung transplant recipients. The American Thoracic Society (ATS) jointly with the Infectious Disease of America (IDSA) [11, 36] and more recently the British Thoracic Society (BTS) [36] have formulated in their guidelines specific clinical, radiographic, and bacteriologic criteria that need to be met to establish a diagnosis of pulmonary NTM infection (Table 2). These criteria are not specific to the immunocompromised population and fit best with MAC and *M. kansasii*.

Table 2 Criteria for the diagnosis of NTM lung disease. Adapted from the American Thoracic Society/Infectious Diseases Society of America guidelines [11]

Clinical criteria (1 and 2)
1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules (strong, high)
and
2. Appropriate exclusion of other diagnosis (strong, high)
Microbiological criteria
1. Positive culture results from at least two separate expectorated sputum samples (strong, moderate). If the results are nondiagnostic, consider repeat sputum AFB smears and cultures (weak, low)
or
2. Positive culture result from at least one bronchial wash or lavage (weak, low)
or
3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum samples or bronchial washing that is culture positive for NTM (strong, moderate)
Other considerations (weak, low)
1. Expert consultation when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination 2. Follow patients who are suspected of having NTM lung disease but do not meet the diagnostic criteria until the diagnosis is firmly established or excluded 3. Making the diagnosis of NTM lung disease does not, per se, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients

Nevertheless, they serve as a useful reference for the diagnosis of pulmonary infection in transplant recipients, even though they should be applied with caution.

For extrapulmonary disease, clinical suspicion should be raised whenever a transplant recipient presents with unexplained febrile illness, nonhealing wound (s), or cutaneous lesions, septic arthritis, bone infection, or lymphadenitis, especially when routine bacterial cultures are nondiagnostic. In this situation, clinical specimens and tissue from the involved sites should be sent for mycobacterial staining and cultures, as well as histopathology. Repeated sampling may be required to establish the diagnosis. Recovery of an NTM from a sterile source (e.g., blood, synovial fluid, bone) provides straightforward evidence of invasive disease. If disseminated disease is suspected, mycobacterial blood cultures should be obtained.

On histopathology, the visualization of granuloma is characteristic of NTM infection. However, granulomatous responses may be impaired in the transplanted host and thus may not be present.

The recommended method for microscopy staining of NTM is the auramine-phenol stain, and it is preferred over the Ziehl-Neelsen and Kinyoun methods owing to its improved sensitivity [36, 55].

Table 3 Common slow-growing mycobacteria with special requirements for culture

	Optimal growth temperature	Special media for fastidious NTM	Incubation time
M. haemophilum	28–30 °C	Ferric ammonium citrate, hemin, hemoglobin	
M. marinum	28–30 °C		
M. conspicuum	22–30 °C, 37 °C in liquid media		
M. ulcerans	25–33 °C	Egg yolk	
M. genavense		Mycobactin J, acidic pH (pH 5.5)	8–12 weeks
M. malmoense			8–12 weeks
M. xenopi	42–45 °C		
M. gordonae			3 weeks or longer

NTM: nontuberculous mycobacteria

Mycobacterial cultures should be performed on both solid and liquid (broth) media. Liquid culture systems are more sensitive and rapid. However, solid media allow for the evaluation of colony morphology and quantification, growth rate, and identification of mixed infections and also serve as a backup when liquid media cultures are contaminated [11, 36]. Quantification of colonies in sputum samples is a useful tool to distinguish colonization and infection and can be followed to monitor response to therapy.

Most clinically significant SGM grow well at 35 to 37 °C. However, certain species need lower-than-standard temperatures or special media for optimal recovery [11, 55, 56] (Table 3). *M. marinum* and *M. haemophilum* grow at 28–30 °C, *M. conspicuum* at 22–30 °C, and only at 37 °C in liquid media, and *M. ulcerans* grows at 25–33 °C. As a general rule, all skin, joint fluid, and bone specimens should be cultured at 28–30 °C and at 35–37 °C. In addition, fastidious NTM species like *M. haemophilum*, *M. genavense,* or *M. ulcerans* require special supplementation of the media for growth to occur. *M. haemophilum* needs iron-containing compounds such as ferric ammonium citrate, hemin, or hemoglobin. *M. ulcerans* may be optimally recovered with egg yolk supplementation. *M. genavense* needs mycobactin J and can take 8–12 weeks to grow. Thus, when these pathogens are suspected, the laboratory should be notified so that specimens are incubated appropriately to maximize organism recovery.

In general, NTM should be identified to the species level for the optimization of treatment. For MAC, species-level identification is generally not required with the exception of *M. chimaera*, if suspected.

Rapid molecular methods have replaced conventional phenotypic testing (e.g., growth rate, colony morphology, and pigmentation) for more accurate and rapid mycobacterial identification (Table 4). When these techniques are not available locally, isolates should be referred to a reference laboratory [55].

High-performance liquid chromatography (HPLC) can rapidly identify NTM by species by the analysis of mycolic acids. However, HPLC has a limited library of

Table 4 Advantages and disadvantages of molecular diagnostics for SGM

Diagnostic test	Advantages	Disadvantages	Comments
Direct probe assays	Rapid – 2 h From solid and liquid media	From positive culture Only available for MTB, MAC, *M. gordonae*, and *M kansasii* Sensitivity 85–100% Cross-reactivity	
16 s sequencing, Sequencing ITS, rpoB, secA, hsp65	Rapid – 1.5 h Can be performed from liquid media	From positive culture 16 s: Unable to distinguish *M. avium* subspecies, *M. kansasii* and *M. gastri*, *M. marinum* and *M. ulcerans*, and *M. malmoense* and *M. szulgai*	
MALDI-TOF MS	Rapid – 90 min	From positive culture Mostly from solid media Database dependent	Rapid turnaround but slowed by the need of growth
NGS	Rapid: 2–4 days for plasma cell-free DNA NGS in plasma, can be done noninvasively and without need of growth	Technology needs further investigation Cost	

MTB: Mycobacterium tuberculosis, MAC: *Mycobacterium avium complex*, *MALDI-TOF MS:* matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, *NS:* next-generation sequencing

mycobacteria and thus has also been replaced by the newer molecular methods [11].

Direct probe hybridization assays are commercially available for MAC, *M. gordonae*, and *M kansasii (AccuProbe; Hologic, San Diego, CA)* [55]. This technique is based on the release of target 16S rRNA from the organism, identifying the species from solid or liquid culture media within 2 h, with 100% specificity but a sensitivity of 85–100%. Cross-reactivity (e.g., *M. celatum*) has been described [11].

Sequence-based identification, most commonly with the 16S rRNA gene as the target, is increasingly being used. One advantage of this approach is the ability to perform sequencing directly from a positive liquid media culture and its rapid turnaround time (~1.5 days). However, initial growth of an isolate is still required. In addition, some mycobacteria are indistinguishable by 16S rRNA gene sequencing, like the *M. avium* subspecies, *M. kansasii* and *M. gastri*, *M. marinum* and *M. ulcerans*, and *M. malmoense* and *M. szulgai*. Nevertheless, most of these organisms can be differentiated by sequencing of other genes, such as the internal transcribed spacer (ITS), rpoB, secA, or hsp65 [57–59].

In recent years, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a method to reliably identify NTM [60, 61]. It is relatively inexpensive, and results can be obtained within 90 min. Like

16S rRNA gene sequencing, the accuracy of MALDI-TOF MS also depends on the robustness of the database. One caveat is that SGM are often extracted after 14–21 days of growth. Furthermore, the MALDI-TOF MS identification of mycobacteria has been studied mainly using isolates on solid media, which delays significantly the time of reporting compared to sequencing techniques. Although identification directly from broth media looks promising, more studies are needed to determine the appropriate scores to use for the acceptability of species-level identifications [60, 62].

Finally, whole-genome next-generation sequencing (NGS) technologies have emerged as a promising diagnostic platform, but data in the transplant population are lacking. In a recent study evaluating the performance of NGS for mycobacterial detection in liquid cultured clinical samples compared to that of liquid probe assay, the concordance rate of detection was 99.2% (98.7–99.5%). NGS overall sensitivity and specificity were 94.2% (88.4–97.6%) and 99.4% (99–99.7%), respectively [63]. Plasma microbial cell-free DNA NGS has also been used to noninvasively detect mycobacteria, including *M. chimaera*, with rapid turnaround time (~2–4 days) [64, 65]. Further investigation of this technique and clinical experience are needed to better understand its clinical applications and result interpretation.

Management

General Principles

Individual treatment regimens for SGM vary based on the species, but there are a few principles that are common in management. More than one active agent should be used for the treatment of these infections to avoid development of resistance. Susceptibility testing for a particular antimicrobial is proven to be clinically predictive in few instances. Among SGM species, drug susceptibilities are predictive of clinical response only for clarithromycin and amikacin against MAC and rifamycin against *M. kansasii* [11, 47, 66]. There is otherwise no clear correlation between treatment efficacy and susceptibility of other medications in patients treated for SGM with a multidrug regimen.

Recommendations for species-specific treatment of SGM in the transplant population are the same as in immunocompetent individuals with a few notable exceptions. Interactions between NTM therapy and immunosuppressive medications complicate the treatment of this disease for the transplant patient. Azithromycin is the preferred macrolide for the treatment of SGM over clarithromycin since it is a less potent inhibitor of cytochrome P450 (CYP450) and, thus, has less of an impact on calcineurin inhibitor (CNI) and mammalian target of rapamycin (mTOR) levels. Rifampin is a potent inducer of CYP3A4 resulting in decreased CNI and mTOR levels which can lead to organ rejection. Rifabutin exhibits less of this effect and is thus the preferred rifamycin in transplant recipients. Close monitoring of drug levels, medication side effects, and allograft

function during treatment for NTM infection is critical. Careful consideration should be given to the appropriateness of intermittent or three-times-a-week therapy in this population given the potential for fluctuating levels of immunosuppression if thrice-weekly antibiotics are administered and also the potential absorption issues in this population for various reasons (i.e., complications of lung transplantation or GVHD in HSCT patients).

Whenever possible, a reduction in the intensity of immunosuppression should be employed as part of the treatment of SGM infection. However, similar to *Mycobacterium tuberculosis* in transplant patients, paradoxical immune reconstitution inflammatory syndrome (IRIS) is possible. This has been described in a kidney patient with *M. kansasii* infection [67]. In a patient being treated with appropriate antibiotic therapy and reduction in immunosuppression with initial improvement, a clinical deterioration in the absence of positive culture results should prompt consideration of IRIS. An increase in immunosuppression led to favorable response in the abovementioned case.

In addition to lowering immunosuppression as able and treating with antibiotics, surgical debridement is sometimes utilized for NTM infection and should be considered for localized lesions, such as cutaneous disease.

MAC

Drug susceptibility should be performed for MAC against clarithromycin (susceptible $\leq$8 μg/mL, intermediate 16 μg/mL, and resistant $\geq$32 μg/mL) at baseline, if previously treated and if persistent culture positivity despite 6 months of effective therapy [11]. More recently, amikacin MICs have also been shown to be predictive of clinical response; however, breakpoints have not been adopted by the Clinical Laboratory Standards Institute (CLSI). In vitro data from over 400 MAC isolates showed an amikacin MIC $\leq$16 ug/mL was susceptible, 32 ug/mL intermediate, and $\geq$64 ug/mL resistant [68]. Resistance to both of these agents can be acquired while on therapy. Macrolide resistance results from 23S rRNA mutation. Risk factors for acquired resistance include treatment with macrolide monotherapy or macrolide plus a fluoroquinolone alone; therefore, these suboptimal treatment regimens should be avoided [66]. Resistance to amikacin is associated with mutations in 16SrRNA and similarly can develop if a patient is not on a robust treatment regimen [68].

Guidelines suggest treatment with three active agents including a macrolide, ethambutol, and rifamycin for both pulmonary and extrapulmonary MAC infection [11]. Macrolides are the backbone of therapy targeting MAC. There has not been a head-to-head comparison between clarithromycin and azithromycin, but retrospective data shows no difference in response between the two agents for pulmonary disease [69]. A prospective noncomparative trial also showed azithromycin is an effective agent [70]. As mentioned above, azithromycin is the favored macrolide based on better tolerability and lesser drug interactions; the latter is especially pertinent to the transplant population. Rifabutin is the favored

rifamycin in transplant patients to minimize drug interactions [47]. Previous studies of therapy for disseminated MAC infection in AIDS patients showed success with macrolide plus ethambutol combination therapy [71, 72]. Although the data for addition of rifamycin is not strong, this is generally recommended [11, 71, 72]. Case reports of disseminated MAC in transplant patients have shown good outcomes with daily triple antibiotic therapy as per these recommendations [73, 74]. A small open-label study in immunocompetent patients with pulmonary disease suggested dual therapy with macrolide plus ethambutol is non-inferior to guideline-based triple antibiotic therapy [75]. A larger randomized controlled trial is ongoing to further evaluate this finding (clinicaltrials.gov NCT03672630). Notably this study does not include immunocompromised patients, so results may not be applicable to the transplant population.

Addition of intravenous amikacin upfront should be considered for cavitary pulmonary disease or extensive/severe disease [11]. A recent study evaluating patients with pulmonary cavitary disease suggested that those patients that received over 3 months of intravenous amikacin had better treatment outcomes, although this had no impact on rates of recurrence [76]. The decision to initiate aminoglycoside therapy and the treatment duration should be individualized since nephrotoxicity and ototoxicity can be significant. This is particularly relevant to the transplant population who are often receiving other nephrotoxic medications. Liposomal inhaled amikacin has recently been approved for treatment of refractory pulmonary MAC disease (defined as positive cultures for $\geq$6 months). A randomized trial showed that addition of inhaled liposomal amikacin to failing guideline-based therapy resulted in higher rates of culture clearance compared to continuation of guideline-based therapy alone (30% compared to 9%). Adverse events included dysphonia, dyspnea, and hoarseness which generally were more pronounced in the first month of therapy and then dissipated [77].

Treatment of macrolide-resistant MAC is challenging and requires multidrug therapy with little data to support various agents. There are promising novel agents for consideration in the treatment of resistant disease or in those patients who fail to tolerate first-line treatment. Clofazimine has good in vitro activity against slow-growing NTM and has in vitro synergistic activity in combination with amikacin or clarithromycin against MAC [78]. It has been used as part of a salvage regimen and is reported to be safe for use in SOT patients, although this is based on very small numbers [79]. Data in pulmonary MAC disease in non-transplant patients shows a 40% culture conversion rate when added to standard regimen for treatment refractory disease. The most commonly reported side effects in this cohort were related to the skin including discoloration, photosensitivity, and dryness as well as gastrointestinal upset [80]. Fluoroquinolones are additional agents with activity against MAC. A retrospective study of patients with pulmonary MAC who failed first-line therapy showed a 30% microbiologic clearance rate after addition of moxifloxacin to standard therapy [81]. Oxazolidinones can also be utilized to treat MAC. Tedizolid has been shown to have more favorable in vitro MIC values compared to linezolid and thus appears to be a preferred treatment option [82]. Of note, side effects of long-term use of tedizolid appear to be similar to that of linezolid [83]. Bedaquiline is an

option with low in vitro MICs even in clarithromycin- and amikacin-resistant isolates [84]. Preliminary clinical data for treatment-experienced pulmonary MAC including with macrolide-resistant isolates appear promising. Ten patients (six with MAC and four with *M. abscessus*) with refractory NTM pulmonary disease received bedaquiline as part of a multidrug regimen, and a microbiologic response (defined as improvement in semiquantitative sputum culture) was achieved in 60% of patients. Common side effects included nausea, arthralgias, anorexia, and subjective fever [85]. The novel beta-lactam ceftazidime/avibactam also appears to have some activity against MAC [86]. A combination of ceftazidime/avibactam, tedizolid, moxifloxacin, and rifabutin was found to have similar activity in vitro to standard therapy [87]. Further clinical studies involving these potential therapies are needed.

M. chimaera

Recent guidelines for the treatment of *M. chimaera* infection following cardiac surgery with cardiopulmonary bypass suggest combination therapy with azithromycin, ethambutol, and rifamycin. Intravenous amikacin is recommended for at least the first 6–12 weeks with continuation for as long as tolerated. Resistance is rare, but if confirmed, addition of clofazimine is recommended. Even in the absence of resistance to clarithromycin or amikacin, a fifth agent such as clofazimine is often added due to the severity of these infections. Moxifloxacin or linezolid are alternate agents, although they have limited data supporting their use. Revision surgery with the removal of all cardiovascular prosthetic material is recommended for source control. The duration of therapy is not clearly defined, but some experts treat for 12 months after conversion of blood cultures to negative. For those patients who are not candidates for surgery, long-term suppressive antibiotic therapy should be considered [19].

M. kansasii

For *M. kansasii*, rifampin susceptibility has clinical relevance and should be performed routinely at the onset of therapy [11, 47]. The recommended treatment regimen includes a combination of rifampin, isoniazid, and ethambutol [11]. A case series of *M. kansasii* disease in renal transplant patients, including disseminated, cutaneous, and isolated pulmonary infection, showed a 87.5% cure rate with a rifabutin-containing drug regimen. The remaining agents varied, but all patients were treated with at least two drugs with the majority of cases receiving three or more antibiotics [88]. Recently, a retrospective study compared a macrolide-containing regimen to an isoniazid-containing regimen in immunocompetent individuals with pulmonary disease. There was no difference in outcome found between the two groups, suggesting substitution of a macrolide for isoniazid is a reasonable treatment alternative [89].

Other Slow-Growing NTM

There is little data to guide treatment of other slow-growing NTM species. Treatment is based on expert opinion and case reports and is summarized in Table 5 for the most commonly isolated organisms. Table 6 lists medications used in the treatment of SGM with doses, side effects, and notable interactions with immunosuppression.

Duration of Therapy

The optimal duration of therapy in this patient population is not clear. Guidelines recommend 12 months of therapy from date of culture clearance for pulmonary disease and at least 4–6 months for soft tissue disease or bone disease, respectively. Due to underlying immunosuppression, the duration of therapy may need to be longer in transplant patients. It is reasonable to consider these recommended durations as the minimum in this patient population [3, 47]. Relapse is possible after

Table 5 Initial Antibiotic regimens for treatment of common slow-growing NTM infections[a]

NTM species	Recommended regimen	Alternative agents	Considerations
MAC	Azithromycin Rifabutin Ethambutol +/− Amikacin	Clarithromycin Rifampin Amikacin Moxifloxacin Clofazimine Bedaquiline	Amikacin should be included in initial regimen if pulmonary cavitary disease present or considered in setting of severe or disseminated disease
M. haemophilum	Azithromycin Rifabutin Ciprofloxacin	Rifampin Clarithromycin Amikacin Sulfonamides Doxycycline	Not susceptible to ethambutol
M. kansasii	Rifabutin Ethambutol Isoniazid	Rifampin Clarithromycin Azithromycin Moxifloxacin Amikacin Sulfonamides	Macrolide could be substituted for isoniazid
M. marinum	Azithromycin Ethambutol +/− Rifabutin	Rifampin Clarithromycin Azithromycin Sulfonamides Doxycycline Minocycline	Rifabutin can be considered for extensive disease
M. xenopi	Azithromycin Ethambutol Rifabutin +/− Amikacin	Moxifloxacin Amikacin	Similar to MAC, amikacin can be considered for cavitary pulmonary disease or severe disease

[a]Adapted from ATS/IDSA and AST guidelines for treatment of NTM infections [11]

Table 6 Antibiotics used to treat slow-growing mycobacteria

Drug	Dose	Requires renal adjustment	Side effects	Interactions
Amikacin	10–15 mg/kg IV daily or 20–25 mg/kg IV 3x week	Yes	Vestibular/ ototoxicity, renal toxicity	Possible potentiation of renal failure with CNI
Liposomal amikacin inhaled	590 mg inhaled daily	No	Dysphonia, bronchospasm, ototoxicity	None
Azithromycin	250–500 mg PO daily	No	Ototoxicity, QTc prolongation, hepatotoxicity	Increased CNI, CsA, mTOR, and rifamycin levels
Bedaquiline	Load with 400 mg PO daily for 2 weeks, followed by 200 mg PO 3x week	No	Increased risk of death, QTc prolongation, hepatotoxicity, GI upset, headache, chest pain, arthralgias	Rifamycins Potentiation of QTc prolongation with CNI, co-administration with rifampin decreases bedaquiline levels and should be avoided
Ciprofloxacin	500 mg PO twice daily	Yes	GI upset, tendonitis, insomnia/agitation, QTc prolongation, possible aneurism formation	May potentiate nephrotoxic effect of CsA
Clarithromycin	500 mg PO twice daily	Yes	GI upset, hepatotoxicity, QTc prolongation	Increased CNI, CsA, mTOR, and rifamycin levels
Clofazimine	50–100 mg PO daily	No	Skin hyperpigmentation, enteropathy, nausea, and vomiting, QTc prolongation	Potentiation of QTc prolongation with CNI
Doxycycline	100 mg PO twice daily	No	GI upset, photosensitivity, pill esophagitis	None
Ethambutol	15 mg/kg PO daily	Yes	Optic neuritis, peripheral neuropathy	None
Isoniazid	5 mg/kg PO daily (up to 300 mg daily)	No	Hepatotoxicity, taken with vitamin B6 to avoid neuropathy	None
Levofloxacin	500–750 mg PO daily	Yes	GI upset, tendonitis, insomnia/agitation, QTc prolongation,	Increased CNI, CsA levels

(continued)

Table 6 (continued)

Drug	Dose	Requires renal adjustment	Side effects	Interactions
			possible aneurism formation	
Linezolid	600 mg PO daily	No	GI upset, cytopenias, peripheral neuropathy, optic neuritis, serotonin syndrome	None
Minocycline	100 mg PO twice daily	No	GI upset, photosensitivity, vertigo/dizziness	None
Moxifloxacin	400 mg PO daily	No	GI upset, tendonitis, insomnia/agitation, QTc prolongation, possible aneurism formation	None
Rifabutin	300 mg PO daily. If <50 kg, 150 mg daily	Yes	Arthralgias, myalgias, leukopenia, anterior uveitis	Decreased CNI, mTOR inhibitor levels
Rifampin	600 mg PO daily. If <50 kg, 450 mg PO daily	Yes	Orange discoloration of secretions, GI upset, hepatitis, drug-drug interactions	Decreased CNI, mTOR inhibitor levels
Tedizolid	200 mg PO daily	No	Thrombocytopenia, no serotonin syndrome risk	None
Trimethoprim/ sulfamethoxazole	8–12 mg/kg/d of TMP component PO divided twice daily (max 320 mg/d)	Yes	Nephrotoxicity, cytopenias, rash	Possible potentiation of renal failure with CNI

PO: per os, *CNI:* calcineurin inhibitors, *CsA:* cyclosporine, *GI:* gastrointestinal, *TMP:* trimethoprim

completion of therapy, especially in the setting of ongoing immunosuppression, so patients should have ongoing clinical and, if possible, microbiologic monitoring.

Prevention

Since these organisms are found in the environment, transplant patients should be counseled on lifestyle modifications which may reduce the risk of SGM exposure. They should wear gloves while cleaning fish tanks and during gardening. Hot tubs,

saunas, and ultrasonic humidifiers should be avoided [7, 90]. Sterile water should be used for sinus and respiratory care [90]. Maintaining the house water heater temperature greater than 130 degrees Celsius has been associated with less NTM growth in the water circuit [13]. Monthly shower head cleaning with bleach should be considered [90]. Providers should optimize management of other comorbidities that are associated with pulmonary NTM disease such as gastroesophageal reflux and aspiration.

The management of transplant candidates who are colonized or infected with NTM organisms pretransplant can be challenging. This occurs most commonly in the lung transplant population as many of these transplant candidates have structural lung disease, such as CF or COPD, which is a risk factor for the presence of NTM. NTM infection incidence in the CF population is as high as 20% [24]. The significance of colonization with SGM including MAC pre-lung transplant is unclear. Practices vary from no treatment with monitoring to pretransplant and/or peritransplant anti-mycobacterial therapy. The American Society of Transplantation recommends that lung transplant candidates colonized or infected with MAC should be treated with multidrug therapy prior to transplant [47]. However, others suggest observation without treatment may be reasonable based on the finding that most cases of MAC pulmonary disease occur later posttransplant, suggesting environmental acquisition rather than early recolonization of allograft with host flora [7, 8].

Azithromycin secondary prophylaxis is sometimes considered based on experience with AIDS patients, although there is no data backing this in transplant patients. In support of this practice is the observation that CF patients with their first episode of NTM growth on culture were less likely to have received chronic macrolide therapy within the last year [91]. Routine screening for NTM should be performed in patients who are maintained on azithromycin as secondary prophylaxis due to concerns for the development of macrolide resistance. An additional caveat of long-term azithromycin use is the potential for polypharmacy-related adverse events. Given these disadvantages, close monitoring after completion of therapy is preferred over extended macrolide therapy [90]. Relapse of NTM disease is fairly common and may be more so in the setting of need for ongoing immunosuppression [69]. Therefore, it is important to monitor patients closely for relapsed or recurrent disease.

Conclusion

Slow-growing NTM infection is relatively rare posttransplant, but the incidence is increasing. In transplant recipients, NTM infections can be difficult to recognize, but carry significant associated morbidity and mortality. Clinicians should maintain a high index of suspicion for this disease, so it can be diagnosed and monitored or treated accordingly. Advances in mycobacterial diagnostics, notably in molecular testing, hold promise for rapid and accurate identification of these NTM. Further research is needed to better understand NTM infections in this population, to improve diagnostic and preventive strategies, and to investigate new and more tolerable therapeutic regimens.

Key Points

- Slow-growing NTM infections are relatively rare posttransplant, but the incidence is increasing.
- In the transplanted host, clinical manifestations of NTM infection may be protean, and NTM classically cause one of six clinical syndromes: pleuropulmonary disease, skin and soft tissue infection, musculoskeletal infection, catheter-associated disease, lymphadenitis, or disseminated disease.
- Molecular diagnostic techniques are emerging and replacing traditional methods for rapid and accurate identification of NTM.
- The clinical impact of the presence of slow-growing NTM depends on the site and extent of involvement, host immune status, and comorbidities.
- Treat with a multidrug regimen based on current guidelines. Treatment will often need to be individualized to the patient based on drug-drug interactions and tolerance of therapy.

References

1. Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM. Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. Mayo Clin Proc. 2013;88:38–45.
2. Adjemian J, Olivier KN, Seitz AE, Holland SM, Prevots DR. Prevalence of nontuberculous mycobacterial lung disease in U.S. Medicare beneficiaries. Am J Respir Crit Care Med. 2012;185:881–6.
3. Doucette K, Fishman JA. Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. Clin Infect Dis. 2004;38:1428–39.
4. Song Y, Zhang L, Yang H, Liu G, Huang H, Wu J, Chen J. Nontuberculous mycobacterium infection in renal transplant recipients: a systematic review. Infect Dis. 2018;50:409–16.
5. Yoo J-W, Jo K-W, Kim S-H, et al. Incidence, characteristics, and treatment outcomes of mycobacterial diseases in transplant recipients. Transpl Int. 2016;29:549–58.
6. Patel R, Roberts GD, Keating MR, Paya CV. Infections due to nontuberculous mycobacteria in kidney, heart, and liver transplant recipients. Clin Infect Dis. 1994;19:263–73.
7. Knoll BM. Update on nontuberculous mycobacterial infections in solid organ and hematopoietic stem cell transplant recipients. Curr Infect Dis Rep. 2014;16:421.
8. Knoll BM, Kappagoda S, Gill RR, Goldberg HJ, Boyle K, Baden LR, Fuhlbrigge AL, Marty FM. Non-tuberculous mycobacterial infection among lung transplant recipients: a 15-year cohort study. Transpl Infect Dis. 2012;14:452–60.
9. Pandian TK, Deziel PJ, Otley CC, Eid AJ, Razonable RR. *Mycobacterium marinum* infections in transplant recipients: case report and review of the literature. Transpl Infect Dis. 2008;10:358–63.
10. Piersimoni C. Nontuberculous mycobacteria infection in solid organ transplant recipients. Eur J Clin Microbiol Infect Dis. 2012;31:397–403.
11. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175:367–416.
12. Weinstock DM, Feinstein MB, Sepkowitz KA, Jakubowski A. High rates of infection and colonization by nontuberculous mycobacteria after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2003;31:1015–21.

13. Falkinham JO 3rd. Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. Emerg Infect Dis. 2011;17:419–24.
14. Falkinham JO 3rd. Nontuberculous mycobacteria in the environment. Clin Chest Med. 2002;23:529–51.
15. Falkinham JO 3rd. Hospital water filters as a source of *Mycobacterium avium* complex. J Med Microbiol. 2010;59:1198–202.
16. Gouby A, Branger B, Oules R, Ramuz M. Two cases of *Mycobacterium haemophilum* infection in a renal-dialysis unit. J Med Microbiol. 1988;25:299–300.
17. Struelens MJ, Plachouras D. Mycobacterium chimaera infections associated with heater-cooler units (HCU): closing another loophole in patient safety. Euro Surveill. 2016; https://doi.org/10.2807/1560-7917.ES.2016.21.46.30397
18. Balsam LB, Louie E, Hill F, Levine J, Phillips MS. *Mycobacterium chimaera* left ventricular assist device infections. J Card Surg. 2017;32:402–4.
19. Hasse B, Hannan M, Keller PM, et al. International Society of Cardiovascular Infectious Diseases Guidelines for the diagnosis, treatment and prevention of disseminated *Mycobacterium chimaera* infection following cardiac surgery with cardiopulmonary bypass. J Hosp Infect. 2019. https://doi.org/10.1016/j.jhin.2019.10.009.
20. Nakamura S, Azuma M, Sato M, Fujiwara N, Nishino S, Wada T, Yoshida S. Pseudo-outbreak of *Mycobacterium chimaera* through aerators of hand-washing machines at a hematopoietic stem cell transplantation center. Infect Control Hosp Epidemiol. 2019;40(12):1433–35.
21. Bryant JM, Grogono DM, Greaves D, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. Lancet. 2013;381:1551–60.
22. Bryant JM, Grogono DM, Rodriguez-Rincon D, et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. Science. 2016;354:751–7.
23. Ose N, Minami M, Funaki S, Kanou T, Kanzaki R, Shintani Y. Nontuberculous mycobacterial infection after lung transplantation: a report of four cases. Surg Case Rep. 2019;5:11.
24. Adjemian J, Olivier KN, Rebecca Prevots D. Epidemiology of pulmonary nontuberculous Mycobacterial sputum positivity in patients with cystic fibrosis in the United States, 2010–2014. Ann Am Thorac Soc. 2018;15:817.
25. Hirama T, Brode SK, Beswick J, et al. Characteristics, treatment and outcomes of non-tuberculous mycobacterial pulmonary disease after allogeneic haematopoietic stem cell transplant. Eur Respir J. 2018. https://doi.org/10.1183/13993003.02330-2017.
26. Beswick J, Shin E, Michelis FV, Thyagu S, Viswabandya A, Lipton JH, Messner H, Marras TK, Kim DDH. Incidence and risk factors for nontuberculous mycobacterial infection after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2018;24:366–72.
27. George IA, Santos CAQ, Olsen MA, Bailey TC. Epidemiology and outcomes of non-tuberculous mycobacterial infections in solid organ transplant recipients at a Midwestern Center. Transplantation. 2016;100:1073–8.
28. Higgins R, Kusne S, Reyes J, Yousem S, Gordon R, Van Thiel D, Simmons RL, Starzl T. *Mycobacterium tuberculosis* after liver transplantation: management and guidelines for prevention. Clin Transpl. 1992;6:81–90.
29. Keating MR, Daly JS, AST Infectious Diseases Community of Practice. Nontuberculous mycobacterial infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):77–82.
30. Longworth SA, Vinnard C, Lee I, Sims KD, Barton TD, Blumberg EA. Risk factors for nontuberculous mycobacterial infections in solid organ transplant recipients: a case-control study. Transpl Infect Dis. 2014;16:76–83.
31. Abad CL, Razonable RR. Non-tuberculous mycobacterial infections in solid organ transplant recipients: an update. J Clin Tuberc Other Mycobact Dis. 2016;4:1–8.
32. Mirsaeidi M, Farshidpour M, Allen MB, Ebrahimi G, Falkinham JO. Highlight on advances in nontuberculous mycobacterial disease in North America. Biomed Res Int. 2014;2014:919474.

33. Mirsaeidi M, Vu A, Leitman P, Sharifi A, Wisliceny S, Leitman A, Schmid A, Campos M, Falkinham J, Salathe M. A patient-based analysis of the geographic distribution of *Mycobacterium avium* complex, *Mycobacterium abscessus*, and *Mycobacterium kansasii* infections in the United States. Chest. 2017;151:947–50.
34. Gaviria JM, Garcia PJ, Garrido SM, Corey L, Boeckh M. Nontuberculous mycobacterial infections in hematopoietic stem cell transplant recipients: characteristics of respiratory and catheter-related infections. Biol Blood Marrow Transplant. 2000;6:361–9.
35. Winthrop KL, Mariette X, Silva JT, Benamu E, Calabrese LH, Dumusc A, Smolen JS, Aguado JM, Fernández-Ruiz M. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) consensus document on the safety of targeted and biological therapies: an infectious diseases perspective (Soluble immune effector molecules [II]: agents targeting interleukins, immunoglobulins and complement factors). Clin Microbiol Infect. 2018. https://doi.org/10.1016/j.cmi.2018.02.002.
36. Haworth CS, Banks J, Capstick T, et al. British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax. 2017;72:ii1–64.
37. Andréjak C, Nielsen R, Thomsen VØ, Duhaut P, Sørensen HT, Thomsen RW. Chronic respiratory disease, inhaled corticosteroids and risk of non-tuberculous mycobacteriosis. Thorax. 2013;68:256–62.
38. Lee W-I, Huang J-L, Yeh K-W, Jaing T-H, Lin T-Y, Huang Y-C, Chiu C-H. Immune defects in active mycobacterial diseases in patients with primary immunodeficiency diseases (PIDs). J Formos Med Assoc. 2011;110:750–8.
39. Dirac MA, Horan KL, Doody DR, Meschke JS, Park DR, Jackson LA, Weiss NS, Winthrop KL, Cangelosi GA. Environment or host?: a case-control study of risk factors for *Mycobacterium avium* complex lung disease. Am J Respir Crit Care Med. 2012;186:684–91.
40. Baddley JW, Cantini F, Goletti D, Gómez-Reino JJ, Mylonakis E, San-Juan R, Fernández-Ruiz M, Torre-Cisneros J. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) consensus document on the safety of targeted and biological therapies: an infectious diseases perspective (Soluble immune effector molecules [I]: anti-tumour necrosis factor-α agents). Clin Microbiol Infect. 2018. https://doi.org/10.1016/j.cmi.2017.12.025.
41. Shah SK, McAnally KJ, Seoane L, Lombard GA, LaPlace SG, Lick S, Dhillon GS, Valentine VG. Analysis of pulmonary non-tuberculous mycobacterial infections after lung transplantation. Transpl Infect Dis. 2016;18:585–91.
42. Huang HC, Weigt SS, Derhovanessian A, et al. Non-tuberculous mycobacterium infection after lung transplantation is associated with increased mortality. J Heart Lung Transplant. 2011;30:790–8.
43. Kang JY, Ha JH, Kang HS, Yoon H-K, Kim H-J, Lee S, Lee D-G, Jung JI, Kim SC, Kim YK. Clinical significance of nontuberculous mycobacteria from respiratory specimens in stem cell transplantation recipients. Int J Hematol. 2015;101:505–13.
44. Longworth SA, Blumberg EA, Barton TD, Vinnard C. Non-tuberculous mycobacterial infections after solid organ transplantation: a survival analysis. Clin Microbiol Infect. 2015;21:43–7.
45. Yomota M, Yanagawa N, Sakai F, Yamada Y, Sekiya N, Ohashi K, Okamura T. Association between chronic bacterial airway infection and prognosis of bronchiolitis obliterans syndrome after hematopoietic cell transplantation. Medicine. 2019;98:e13951.
46. Man WH, de Steenhuijsen Piters WAA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol. 2017;15:259–70.
47. Longworth SA, Daly JS, AST Infectious Diseases Community of Practice. Management of Infections due to non-tuberculous mycobacteria in transplant recipients – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019:e13588.
48. Krueger S, Rork J, Lee J, Cornejo K, Gibson L, Daly JS, Levin NA. *Mycobacterium haemophilum* infection in a renal transplant patient with inflammatory bowel disease. Dermatol Online J. 2017;23(9):13030/qt8vs8509k.

49. Rawla MS, Kozak A, Hadley S, LeCates WW. *Mycobacterium avium*-intracellulare-associated acute interstitial nephritis: a rare cause of renal allograft dysfunction. Transpl Infect Dis. 2009;11:529–33.
50. Singhal A, Gates C, Malhotra N, Irwin DA, Chansolme DH, Kohli V. Successful management of primary nontuberculous mycobacterial infection of hepatic allograft following orthotopic liver transplantation for hepatitis C. Transpl Infect Dis. 2011;13:47–51.
51. Brix SR, Iking-Konert C, Stahl RAK, Wenzel U. Disseminated *Mycobacterium haemophilum* infection in a renal transplant recipient. BMJ Case Rep. 2016; https://doi.org/10.1136/bcr-2016-216042.
52. Ombelet S, Van Wijngaerden E, Lagrou K, Tousseyn T, Gheysens O, Droogne W, Doubel P, Kuypers D, Claes KJ. *Mycobacterium genavense* infection in a solid organ recipient: a diagnostic and therapeutic challenge. Transpl Infect Dis. 2016;18:125–31.
53. Santos M, Gil-Brusola A, Escandell A, Blanes M, Gobernado M. *Mycobacterium genavense* infections in a tertiary hospital and reviewed cases in non-HIV patients. Pathol Res Int. 2014;2014:371370.
54. Ward MS, Lam KV, Cannell PK, Herrmann RP. Mycobacterial central venous catheter tunnel infection: a difficult problem. Bone Marrow Transplant. 1999;24:325–9.
55. Forbes BA, Hall GS, Miller MB, Novak SM, Rowlinson M-C, Salfinger M, Somoskövi A, Warshauer DM, Wilson ML. Practice guidelines for clinical microbiology laboratories: mycobacteria. Clin Microbiol Rev. 2018; https://doi.org/10.1128/CMR.00038-17.
56. Richter E, Brown-Elliott BA, Wallace RJ. Mycobacterium: laboratory characteristics of slowly growing mycobacteria *. In: Manual of clinical microbiology. 10th ed: ASM Pub2Web; 2011. p. 503–24.
57. de Zwaan R, van Ingen J, van Soolingen D. Utility of rpoB gene sequencing for identification of nontuberculous mycobacteria in the Netherlands. J Clin Microbiol. 2014;52:2544–51.
58. Lau SKP, Curreem SOT, Ngan AHY, Yeung C-K, Yuen K-Y, Woo PCY. First report of disseminated Mycobacterium skin infections in two liver transplant recipients and rapid diagnosis by hsp65 gene sequencing. J Clin Microbiol. 2011;49:3733–8.
59. Roth A, Fischer M, Hamid ME, Michalke S, Ludwig W, Mauch H. Differentiation of phylogenetically related slowly growing mycobacteria based on 16S-23S rRNA gene internal transcribed spacer sequences. J Clin Microbiol. 1998;36:139–47.
60. Buckwalter SP, Olson SL, Connelly BJ, Lucas BC, Rodning AA, Walchak RC, Deml SM, Wohlfiel SL, Wengenack NL. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of Mycobacterium species, Nocardia species, and other aerobic Actinomycetes. J Clin Microbiol. 2016;54:376–84.
61. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem. 2015;61:100–11.
62. Balada-Llasat JM, Kamboj K, Pancholi P. Identification of mycobacteria from solid and liquid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry in the clinical laboratory. J Clin Microbiol. 2013;51:2875–9.
63. Quan TP, Bawa Z, Foster D, et al. Evaluation of whole-genome sequencing for mycobacterial species identification and drug susceptibility testing in a clinical setting: a large-scale prospective assessment of performance against line probe assays and phenotyping. J Clin Microbiol. 2018. https://doi.org/10.1128/JCM.01480-17.
64. Benamu E, Gajurel K, Anderson JN, et al. 2294. Evaluation of the Karius plasma next-generation sequencing cell-free pathogen DNA test to determine the etiology of infection and impact on anti-microbial management in patients with severe neutropenia and fever. Open Forum Inf Diseases. Oxford University Press US; 2018. p. S680–S680.
65. Nomura J, Rieg G, Bluestone G, Tsai T, Lai A, Terashita D, Bercovici S, Hong DK, Lee BP. Rapid detection of invasive Mycobacterium chimaera disease via a novel plasma-based next-generation sequencing test. BMC Infect Dis. 2019;19:371.
66. Griffith DE. Treatment of *Mycobacterium avium* complex (MAC). Semin Respir Crit Care Med. 2018;39:351–61.

67. Lemoine M, Laurent C, Hanoy M, Leporrier J, François A, Guerrot D, Godin M, Bertrand D. Immune reconstitution inflammatory syndrome secondary to *Mycobacterium kansasii* infection in a kidney transplant recipient. Am J Transplant. 2015;15:3255–8.
68. Brown-Elliott BA, Iakhiaeva E, Griffith DE, Woods GL, Stout JE, Wolfe CR, Turenne CY, Wallace RJ Jr. In vitro activity of amikacin against isolates of *Mycobacterium avium* complex with proposed MIC breakpoints and finding of a 16S rRNA gene mutation in treated isolates. J Clin Microbiol. 2013;51:3389–94.
69. Wallace RJ Jr, Brown-Elliott BA, McNulty S, Philley JV, Killingley J, Wilson RW, York DS, Shepherd S, Griffith DE. Macrolide/Azalide therapy for nodular/bronchiectatic *Mycobacterium avium* complex lung disease. Chest. 2014;146:276–82.
70. Griffith DE, Brown BA, Girard WM, Griffith BE, Couch LA, Wallace RJ Jr. Azithromycin-containing regimens for treatment of *Mycobacterium avium* complex lung disease. Clin Infect Dis. 2001;32:1547–53.
71. Benson CA, Williams PL, Currier JS, et al. A prospective, randomized trial examining the efficacy and safety of clarithromycin in combination with ethambutol, rifabutin, or both for the treatment of disseminated *Mycobacterium avium* complex disease in persons with acquired immunodeficiency syndrome. Clin Infect Dis. 2003;37:1234–43.
72. Gordin FM, Sullam PM, Shafran SD, Cohn DL, Wynne B, Paxton L, Perry K, Horsburgh CR Jr. A randomized, placebo-controlled study of rifabutin added to a regimen of clarithromycin and ethambutol for treatment of disseminated infection with *Mycobacterium avium* complex. Clin Infect Dis. 1999;28:1080–5.
73. Fadlallah J, Rammaert B, Laurent S, Lanternier F, Pol S, Franck N, Mamzer MF, Dupin N, Lortholary O. *Mycobacterium avium* complex disseminated infection in a kidney transplant recipient. Transpl Infect Dis. 2016;18:105–11.
74. Hou R, Nayak R, Pincus SM, Lai J, Omran LM, Alkaade S, Abate G. Esophageal *Mycobacterium avium*-intracellulare infection in a bone marrow transplant patient: case report and literature review. Transpl Infect Dis. 2019;21:e13019.
75. Miwa S, Shirai M, Toyoshima M, et al. Efficacy of clarithromycin and ethambutol for *Mycobacterium avium* complex pulmonary disease. A preliminary study. Ann Am Thorac Soc. 2014;11:23–9.
76. Kim O-H, Kwon BS, Han M, et al. Association between duration of aminoglycoside treatment and outcome of cavitary *Mycobacterium avium* complex lung disease. Clin Infect Dis. 2019;68:1870–6.
77. Griffith DE, Eagle G, Thomson R, et al. Amikacin liposome inhalation suspension for treatment-refractory lung disease caused by *Mycobacterium avium* complex (CONVERT). A prospective, open-label, randomized study. Am J Respir Crit Care Med. 2018;198:1559–69.
78. Ferro BE, Meletiadis J, Wattenberg M, de Jong A, van Soolingen D, Mouton JW, van Ingen J. Clofazimine prevents the regrowth of *Mycobacterium abscessus* and *Mycobacterium avium* type strains exposed to amikacin and clarithromycin. Antimicrob Agents Chemother. 2016;60:1097–105.
79. Cariello PF, Kwak EJ, Abdel-Massih RC, Silveira FP. Safety and tolerability of clofazimine as salvage therapy for atypical mycobacterial infection in solid organ transplant recipients. Transpl Infect Dis. 2015;17:111–8.
80. Martiniano SL, Wagner BD, Levin A, Nick JA, Sagel SD, Daley CL. Safety and effectiveness of Clofazimine for primary and refractory nontuberculous mycobacterial infection. Chest. 2017;152:800–9.
81. Koh W-J, Hong G, Kim S-Y, Jeong B-H, Park HY, Jeon K, Kwon OJ, Lee S-H, Kim CK, Shin SJ. Treatment of refractory *Mycobacterium avium* complex lung disease with a moxifloxacin-containing regimen. Antimicrob Agents Chemother. 2013;57:2281–5.
82. Brown-Elliott BA, Wallace RJ Jr. In vitro susceptibility testing of Tedizolid against non-tuberculous mycobacteria. J Clin Microbiol. 2017;55:1747–54.

83. Kim T, Wills A, Markus A, Prevots DR, Olivier KN. Safety and tolerability of long term use of Tedizolid for treatment of nontuberculous mycobacterial infections. Open Forum Infect Dis. 2016. https://doi.org/10.1093/ofid/ofw172.440.
84. Brown-Elliott BA, Philley JV, Griffith DE, Thakkar F, Wallace RJ Jr. In vitro susceptibility testing of Bedaquiline against *Mycobacterium avium* complex. Antimicrob Agents Chemother. 2017. https://doi.org/10.1128/AAC.01798-16.
85. Philley JV, Wallace RJ Jr, Benwill JL, Taskar V, Brown-Elliott BA, Thakkar F, Aksamit TR, Griffith DE. Preliminary results of Bedaquiline as salvage therapy for patients with nontuberculous mycobacterial lung disease. Chest. 2015;148:499–506.
86. Deshpande D, Srivastava S, Chapagain ML, Lee PS, Cirrincione KN, Pasipanodya JG, Gumbo T. The discovery of ceftazidime/avibactam as an anti-*Mycobacterium avium* agent. J Antimicrob Chemother. 2017;72:i36–42.
87. Deshpande D, Srivastava S, Pasipanodya JG, Lee PS, Gumbo T. A novel ceftazidime/avibactam, rifabutin, tedizolid and moxifloxacin (CARTM) regimen for pulmonary *Mycobacterium avium* disease. J Antimicrob Chemother. 2017;72:i48–53.
88. Cho J-H, Yu C-H, Jin M-K, Kwon O, Hong K-D, Choi J-Y, Yoon S-H, Park S-H, Kim C-D, Kim Y-L. *Mycobacterium kansasii* pericarditis in a kidney transplant recipient: a case report and comprehensive review of the literature. Transpl Infect Dis. 2012;14:E50–5.
89. Moon SM, Choe J, Jhun BW, Jeon K, Kwon OJ, Huh HJ, Lee NY, Daley CL, Koh W-J. Treatment with a macrolide-containing regimen for *Mycobacterium kansasii* pulmonary disease. Respir Med. 2019;148:37–42.
90. Anjan S, Morris MI. Nontuberculous mycobacteria in solid organ transplant. Curr Opin Organ Transplant. 2019;24:476–82.
91. Binder AM, Adjemian J, Olivier KN, Prevots DR. Epidemiology of nontuberculous mycobacterial infections and associated chronic macrolide use among persons with cystic fibrosis. Am J Respir Crit Care Med. 2013;188:807–12.
92. Alexander S, John GT, Jesudason M, Jacob CK. Infections with atypical mycobacteria in renal transplant recipients. Indian J Pathol Microbiol. 2007;50:482–4.
93. Peters EJ, Morice R. Miliary pulmonary infection caused by *Mycobacterium terrae* in an autologous bone marrow transplant patient. Chest. 1991;100:1449–50.
94. Malouf MA, Glanville AR. The spectrum of mycobacterial infection after lung transplantation. Am J Respir Crit Care Med. 1999;160:1611–6.
95. Hoff E, Sholtis M, Procop G, Sabella C, Goldfarb J, Wyllie R, Cunningham R, Stockman L, Hall G. Mycobacterium triplex infection in a liver transplant patient. J Clin Microbiol. 2001;39:2033–4.
96. Perandones CE, Roncoroni AJ, Frega NS, Bianchini HM, Hübscher O. *Mycobacterium gastri* arthritis: septic arthritis due to *Mycobacterium gastri* in a patient with a renal transplant. J Rheumatol. 1991;18:777–8.
97. Neeley SP, Denning DW. Cutaneous Mycobacterium thermoresistibile infection in a heart transplant recipient. Rev Infect Dis. 1989;11:608–11.
98. Christiansen DC, Roberts GD, Patel R. *Mycobacterium celatum*, an emerging pathogen and cause of false positive amplified *Mycobacterium tuberculosis* direct test. Diagn Microbiol Infect Dis. 2004;49:19–24.
99. Qvist T, Katzenstein TL, Lillebaek T, Iversen M, Mared L, Andersen AB. First report of lung transplantation in a patient with active pulmonary *Mycobacterium simiae* infection. Transplant Proc. 2013;45:803–5.

Nocardia Infections in Transplantation: Diagnosis, Management, and Impact on Outcomes

21

Reuben J. Arasaratnam and Alejandro Restrepo

Contents

Introduction 488
Disease Epidemiology of *Nocardia* 488
Routes of Infection 488
Nocardia Species Responsible for Infection in Humans 488
Incidence and Risk Factors for Nocardiosis in Solid Organ Transplant Recipients 489
Incidence and Risk Factors for Nocardiosis in Hematopoietic Stem Cell Transplant Recipients 489
Clinical Impact on Solid and Hematopoietic Stem Cell Transplantation 490
Mortality 490
Treatment-Associated Morbidity 491
Graft Outcomes 491
Disease Diagnostics and Clinical Evaluation of *Nocardia* 491
Pulmonary Nocardiosis 491
Cutaneous Manifestations 492
Central Nervous System Manifestations 492
Other Presentations of Nocardiosis 493
General Approach to Diagnosis of Nocardiosis 493
Management 495
Empiric Therapy 495
Duration of Therapy 498
Drug Toxicity Monitoring 499
The Role of Adjunctive Surgery 499
Immunosuppression Reduction and Immune-Based Therapy 499
Use of Oxazolidinone Antimicrobials in Therapy: Linezolid and Tedizolid 500

R. J. Arasaratnam (✉)
Division of Infectious Diseases and Geographic Medicine, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA
e-mail: Reuben.Arasaratnam@UTSouthwestern.edu

A. Restrepo
Division of Infectious Diseases, Department of Internal Medicine, Baylor College of Medicine, Houston, TX, USA
e-mail: alejorpo75@hotmail.com

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_18

Prevention 500
Conclusion 500
Key Points 501
Cross-References 501
References 501

Introduction

Nocardia species are ubiquitous Gram-positive bacteria belonging to the *Actinomycetaceae* family and are responsible for opportunistic infections in humans. While nocardiosis may affect immunocompetent individuals, the disease is more commonly seen in immunocompromised patients such as recipients of solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients. The most common sites of infection include the lung, skin, and central nervous system. Disseminated disease affecting multiple organs may occur in 40% of transplant recipients with nocardiosis and is associated with a high mortality. In this chapter, we will review the disease epidemiology of *Nocardia* species and clinical impact of infection and give an overview of diagnosis and management strategies, with special consideration given to the solid organ and hematopoietic stem cell transplant recipient.

Disease Epidemiology of *Nocardia*

Routes of Infection

Nocardia spp. are ubiquitous environmental organisms found in stagnant water, decaying plants, and soil. As the organism is readily aerosolized, the main portal of entry for this bacterium is inhalation into the lung, although direct ingestion and inoculation via skin injury are other forms of recognized entry sites for the bacteria [1–3]. Given the potential for this bacterium to be aerosolized, clusters of infections have been reported in immunocompromised patients related to occupational exposures with soil [4] and nosocomial infection from construction work [5].

Nocardia Species Responsible for Infection in Humans

Over 80 species of the genus *Nocardia* have been identified to date, with at least 40 species implicated in human infection. In the last decade, the use of novel molecular technology such as PCR-restriction enzyme analysis and 16S ribosomal RNA (rRNA) sequencing has greatly enhanced our ability to identify *Nocardia* species that are predominantly responsible for human infection [1]. These include *Nocardia asteroides*, *N. nova*, *N. farcinica*, *N. otitidiscaviarum*, *N. transvalensis*, *N. brasiliensis*, *N. brevicatena*, *N. abscessus*, *N. cyriacigeorgica*, *N. veterana*,

N. paucivorans, *N. wallacei*, *N. blacklockiae*, *N. elegans*, *N. concava*, and *N. cassostreae.*

N. farcinica and *N. nova* complex have been identified as the major species responsible for disease in SOT recipients accounting for over 50% of cases in one study in which isolates were identified with sequencing of 16S rRNA or hsp65 gene [6]. Isolates less commonly found responsible for infection in this particular study were *N. cyriacigeorgica*, *N. abscessus*, *N. veterana*, *and N. beijingensis*. *Nocardia* species responsible for infection in the HSCT population are less well-characterized, but in a study from MD Anderson Cancer Center of 132 patients of which 30% received a stem cell transplant, 27 species of *Nocardia* were identified as being responsible for infection with the most prevalent being *N. nova*, *N. cyriacigeorgica*, *N. farcinica*, *and N. abscessus* [7].

Incidence and Risk Factors for Nocardiosis in Solid Organ Transplant Recipients

Given that T cell-mediated immune responses are protective against infection with *Nocardia*, transplant recipients, persons living with HIV, and those on systemic corticosteroid immunosuppression are at higher risk of infection. In SOT recipients, the incidence of nocardiosis is estimated to be between 0.04% and 3.5% [8–10] and highest in heart and lung transplant recipients [9, 10] likely reflecting the differences in the net state of immunosuppression among organ transplant subtypes and respiratory portal of entry for the bacterium. Onset of infection typically occurs within the first 2 years of transplantation, with infection within the first month considered to be very rare [6, 10, 11] and late infection (greater than 2 years posttransplant) also rare, but still possible. In a large retrospective case-control study of solid organ transplant recipients [6], high level of immunosuppression preceding or at the time of diagnosis was identified as the main risk factor for nocardiosis following solid organ transplant. *Cytomegalovirus* disease 6 months prior [10] has also been associated with nocardiosis, but it is not clear whether this finding represents coinfection due to high level of immunosuppression or a causal immunomodulatory factor increasing susceptibility to *Nocardia* infection. Overall risk factors for nocardiosis in SOT recipients are summarized in Table 1.

Incidence and Risk Factors for Nocardiosis in Hematopoietic Stem Cell Transplant Recipients

Fewer studies have characterized the epidemiology of nocardiosis in HSCT recipients. While nocardiosis can occur in autologous stem cell transplant recipients, it is allogeneic stem cell transplant recipients who are at higher risk with a reported incidence of 0.5–1.7% within the first 2 years of transplant [4, 12–14]. Late infections, with a median onset of 508 days HSCT, have been described in a small case series [15], but in these patients, the vast majority were receiving

Table 1 Risk factors for nocardiosis in solid organ transplant recipients

High-dose corticosteroids at the time of diagnosis
Use of tacrolimus
High calcineurin inhibitor trough level 1 month before diagnosis
Prolonged stay in intensive care unit after transplant
Patient age
Coinfections (*Cytomegalovirus*)

high-dose steroids for graft-versus-host disease. The small size of all of these studies in immunocompromised population did not permit a detailed analysis of independent risk factors for infection, but like their solid organ transplant counterparts, an augmented net state of immunosuppression is likely to be a contributory factor in the risk of infection.

Trimethoprim-sulfamethoxazole (TMP-SMX) is frequently used as prophylaxis following SOT and HSCT for the prevention of *Pneumocystis jirovecii* pneumonia. While this drug is active in vitro and in vivo against many *Nocardia* species, breakthrough infections have been reported in transplant recipients receiving TMP-SMX, and thus use of this drug should not be considered a protective factor in the evaluation of a patient with possible *Nocardia* infection [6, 10, 16].

Clinical Impact on Solid and Hematopoietic Stem Cell Transplantation

Mortality

Although infections with *Nocardia* spp. are considered to be rare, the mortality from this infection is high. In SOT recipients, historical mortality rates from small case series have been reported to be between 11% and 70% [10, 17–19]. A more recent, larger, European case-control study of 117 patients demonstrated a tenfold higher mortality in solid organ transplant recipients with nocardiosis compared to a control group without infection, with a 1-year, all-cause mortality rate of 17% [20]. Independent risk factors for mortality identified in this study included history of malignancy, history of invasive fungal infection, and donor age. Interestingly, in this same study, acute rejection in the year proceeding the diagnosis (with resultant intensification of immunosuppression) was associated with *reduction* in mortality. The authors speculated that this paradoxical finding may have been due to these patients receiving more vigilant surveillance for infection leading to earlier detection and treatment of *Nocardia* or possible benefits associated with the immunosuppression-associated modulation of inflammatory response from *Nocardia* infection. Mortality rates in HSCT recipients are less well-characterized, but several small case series have reported mortality rates in the range of 40–50% [4, 13, 15]. Further, larger studies in HSCT recipients are required for better estimation of mortality in this population.

Treatment-Associated Morbidity

Due to the antimicrobial-associated side effects of many first-line choices for nocardiosis, treatment-associated morbidity is important in solid organ transplant and stem cell transplant recipients. For several decades, sulfonamide-based therapy (e.g., trimethoprim-sulfamethoxazole) was established as the drug of choice for nocardiosis due to its widespread activity against many *Nocardia* species and ability to achieve high concentrations in affected tissues (including the brain, skin, and lung). However, gastrointestinal disturbances, bone marrow suppression, and nephrotoxicity have led to cessation of this antibiotic during *Nocardia* treatment in up to 50% of cases [21, 22]. Other antimicrobial-associated toxicities which should be noted are nephrotoxicity and ototoxicity with amikacin, seizures with carbapenem therapy, and bone marrow suppression, peripheral neuropathy, and lactic acidosis with linezolid. A special toxicity consideration in transplant patients is the use of calcineurin inhibitors (cyclosporine and tacrolimus) which may lead to additive renal injury [23] due to the concomitant use of antimicrobials such as TMP-SMX and amikacin. In addition, for any antimicrobial used, it is important to assess the potential impact of drug-drug interactions with immunosuppressive medications and monitor immunosuppression levels accordingly [24].

Graft Outcomes

Allograft rejection and graft-versus-host disease (GvHD) have been reported as outcome measures in studies of nocardiosis in transplant recipients. While the rates reported are typically high, 9–70% in solid organ transplant recipients [11, 19, 25] and 52–83% in HSCT recipients [4, 13, 26], it is unclear if these numbers represent a direct consequence of infection with *Nocardia* or representative of the aberrant immunological state of the host preceding the diagnosis of nocardiosis.

Disease Diagnostics and Clinical Evaluation of *Nocardia*

Pulmonary Nocardiosis

Given that inhalation remains the most frequent portal of entry for *Nocardia*, pulmonary disease is the most frequent clinical presentation of nocardiosis in transplant recipients, occurring at least 80% of the time [6]. The tempo of presentation in transplant recipients can be acute, subacute, or chronic, and symptoms may include dyspnea, cough (productive or nonproductive), chest pain, or hemoptysis [27]. Constitutional symptoms may be present such as fever, chills, and weight loss. A wide variety of radiographic presentations of nocardiosis have been described in the literature including lobar pneumonia (also multi-lobar involvement), diffuse interstitial pneumonia, and pleural effusions. However nodular and cavitary lesions

are most typical [28] and should immediately raise suspicion for infection with *Nocardia*. An analysis of *Nocardia* infection presentations in patients with a wide spectrum of immune competency found that pulmonary cavitation was a distinct clinical presentation of patients receiving immunosuppression compared to those that did not [29]. Isolation of the organism with sputum culture and bronchial alveolar lavage (BAL) are appropriate initial diagnostic steps. If diagnostic uncertainty persists following these initial tests, transbronchial biopsy or open surgical/percutaneous lung biopsy may be necessary.

Cutaneous Manifestations

Cutaneous manifestations of nocardiosis include nodular lesions, ulcers, mycetomas, cellulitis, and skin abscesses [2]. In studies of solid organ transplant recipients with nocardiosis, cutaneous manifestations represent approximately 10% of all presentations [10, 17, 18]. The mechanism of infection may be direct inoculation of the organism secondary to injury or trauma or by dissemination from other organs to the skin. As in pulmonary disease, the time course of presentation can be both acute and subacute. It is important to note that many of the cutaneous manifestations of nocardiosis can mimic other opportunistic infections in transplant recipients, such as fungal and mycobacterial infections [30]. Thus, obtaining a microbiologic diagnosis with tissue biopsy is essential. In addition, given that cutaneous manifestations may represent part of a disseminated process, imaging evaluation of the lung and brain should be performed routinely in patients presenting with skin disease.

Central Nervous System Manifestations

Brain involvement of *Nocardia* infection may occur in up to one third of cases of nocardiosis. In 30 cases of central nervous system (CNS) nocardiosis of solid organ transplant recipients reported by Coussement et al. [6], over 40% of these patients were asymptomatic, illustrating the importance of routine evaluation of the central nervous system (preferably with magnetic resonance imaging (MRI)) in *Nocardia* infection, regardless of symptoms. Infection of the CNS is believed to be a secondary dissemination by the bloodstream although positive blood cultures are rarely detected. Neurological manifestations of central nervous system nocardiosis include asymptomatic presentation, headache, focal neurological signs, seizures, and coma [31]. Radiographic findings of CNS nocardiosis typically include single or multiple brain abscesses that may involve both cerebral hemispheres. An example of nocardiosis presenting as a brain abscess is shown in Fig. 1. Additional CNS radiological presentations that have been reported in solid organ transplant recipients include ventriculitis and spinal cord abscesses. If a microbiologic diagnosis has already been obtained from another organ site (such as the lung or skin), it is reasonable to assume that the radiographic abnormalities seen in the brain are part of the same infective process. However in the absence of the clear microbiologic

Fig. 1 Coronal MRI (T1 post-contrast image) of a patient showing an abscess in the right cerebellar hemisphere. Surgical drainage of the abscess and culture later revealed the etiology of this infection as *Nocardia ignorata*

diagnosis, brain biopsy may be necessary to confirm the diagnosis of central nervous system nocardiosis [32]. Among the *Nocardia* species described, *Nocardia farcinica* has been associated with predilection for CNS infection [6, 33], although, as stated before, evaluation for CNS disease should be routinely performed regardless of *Nocardia* species.

Other Presentations of Nocardiosis

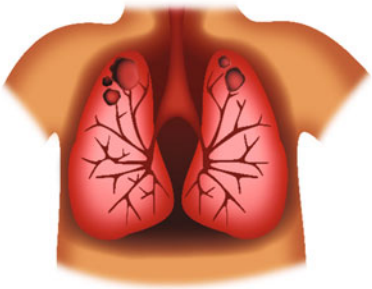
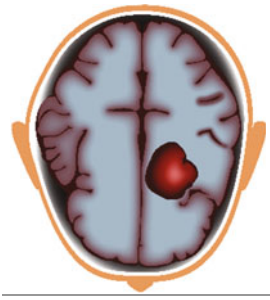
Disseminated nocardiosis is defined as *Nocardia* infection involving two organs that are not contiguous. It represents the most severe spectrum of disease conferring a high mortality and has been reported to occur in up to 40% of solid organ transplant recipients with nocardiosis. Other less common presentations of *Nocardia* infection that have been described included involvement of the eye, liver, kidney, and adrenal gland and spinal abscess.

The presentations and approaches to diagnosis of nocardiosis of the skin, lung, and brain are summarized in Table 2.

General Approach to Diagnosis of Nocardiosis

Isolation of *Nocardia* in culture from a suspected site of infection in a patient with clinically compatible disease is sufficient for the diagnosis of nocardiosis. Often, obtaining such cultures may entail invasive diagnostic and potentially risky

Table 2 Presentation and diagnoses of nocardiosis in transplant recipients

Skin	Presentation	Diagnosis
	Nodule Ulcer Mycetoma Cellulitis Abscess	Skin biopsy
Pulmonary disease		
	Consolidation Nodules Cavitary lesion Pleural effusion	Sputum sample Bronchoscopy with BAL Transbronchial biopsy Surgical or percutaneous biopsy
CNS disease		
	Brain abscess Meningitis Ventriculitis Spinal abscess	Brain biopsy (if no other Lesion can be biopsied) Spinal abscess biopsy Lumbar puncture (if deemed safe)

procedures including biopsy sampling of lung or brain tissue, but these are warranted given the antimicrobial toxicities associated with empiric treatment for *Nocardia* and need to exclude other opportunistic infections. *Nocardia* colonization of the respiratory tract is possible particularly in immunocompetent patients with structural lung disease, but the isolation of *Nocardia* from culture in a transplant recipient should generally be considered to be pathogenic unless there is substantive evidence that points to the contrary.

Nocardia can be detected on both Gram stain and modified acid-fast stain (Fig. 2). On Gram stain, *Nocardia* is seen as a filamentous Gram-positive branching rod. Isolation of the organism for culture, identification, and antimicrobial susceptibility can take anywhere from a few days to several weeks. While the organism can grow on nonselective media, recovery of the bacteria may be enhanced by longer incubation period and use of selective media such a Thayer-Martin agar.

Identification of *Nocardia* down to the species level can be incredibly helpful in guiding empiric antimicrobial therapy given the taxonomic association with

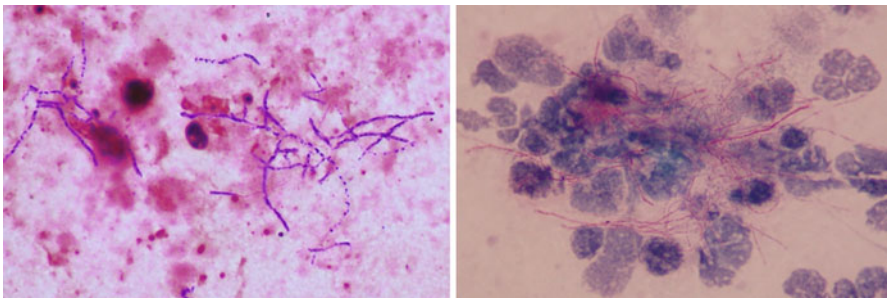


Fig. 2 On the left panel, Gram stain depicting filamentous Gram-positive branching bacteria later identified as *Nocardia* species (original magnification x 100). The right panel shows *Nocardia* species staining positive on modified acid-fast stain (original magnification x 100). (Courtesy of Dr. Dominick Cavuoti, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX)

antimicrobial susceptibilities (discussed later in this chapter). Identification of species is best performed with sequencing of the 16S ribosomal ribonucleic acid, hsp65, secA1, or gyrB genes [34, 35]. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is an alternative method of rapid identification with correct species-level identification reported between 75% and 95% [36–38], depending on the quality of the reference library used.

There has been a recent interest in the use of 16S PCR-based assay to detect *Nocardia* spp. from clinical samples. This may reduce time to diagnosis and may be especially useful in circumstances where the patient has received antibiotic therapy, potentially compromising the yield of traditional culture methods. In a recent study of 68 patients including 14 solid organ transplant patients and 14 allogeneic stem cell transplant recipients, the sensitivity and specificity of the test were 74% and 87%, respectively [39]. In a prospective of study of 21 lung transplant recipients, evaluating *Nocardia* PCR on BAL specimens, *Nocardia* PCR was positive from 5 BAL specimens performed for routine surveillance only. None of these patients developed clinical disease, and thus a major drawback of PCR-based diagnosis, particularly in respiratory samples, is the risk of detecting airway colonization [40].

Management

Empiric Therapy

After isolation of *Nocardia* spp. in the setting of suspected clinical infection, antimicrobial therapy remains the mainstay of therapy. However, there are a number of challenges associated with therapeutic decision-making. Firstly, each species of *Nocardia* has a unique antimicrobial susceptibility profile, and thus "empiric" antimicrobial decisions including the use of combination therapy with two or three

agents in severe disease may be needed until susceptibility testing is available. This may take up to several weeks due to slow-growing strains and technical challenges of performing susceptibility testing. Secondly, special consideration should be given with regard to the site and severity of disease to ensure appropriate penetration of antibiotics into the affected site. Lastly, minimization of drug interactions and antimicrobial toxicities is important given the need for long-term therapy to achieve clinical cure in these patients. When antimicrobial susceptibility testing is still pending, identification of *Nocardia* down to the species level can be helpful in designing empiric therapy given the previously reported taxonomic variances reported of *Nocardia* isolates. Particular attention should be paid to the following resistance patterns associated with *Nocardia* species, and these typical susceptibility patterns are reported in Table 3. For example, *Nocardia farcinica* is usually resistant to ceftriaxone and has variable susceptibility to TMP-SMX. Variable activity of amikacin has been described against *Nocardia transvalensis*, and variable activity of imipenem has been described against *Nocardia brasiliensis*, *Nocardia abscessus*, and *Nocardia otitidiscaviarum*.

In vitro susceptibility testing should be performed at a specialized laboratory experienced at testing *Nocardia* strains. Multiple antimicrobials should be tested up front given the potential need to adjust therapy including switch from intravenous to oral therapy or switch due to antimicrobial-associated toxicities. These include amikacin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline, trimethoprim-sulfamethoxazole, and tobramycin.

There are no randomized trials to guide optimal therapy for *Nocardia* infection including single versus combination antibiotic therapy and duration of therapy. For empiric therapy the following antimicrobials should be considered based on their widespread activity against *Nocardia* species and evidence supporting clinical use. These are TMP-SMX, carbapenems (imipenem and meropenem), amikacin, third-generation cephalosporins (ceftriaxone and cefotaxime), and linezolid. Table 4 gives a suggestion for empiric treatment (in the absence of *Nocardia* spp. identification or antimicrobial susceptibility testing) for each of the main clinical presentations of nocardiosis.

It is reasonable to consider monotherapy with TMP-SMX as a first-line agent for mild skin and pulmonary disease given that this drug has high bioavailability and widespread activity against *Nocardia* isolate. Likewise monotherapy with linezolid could also be considered for the same indications, but current data supporting this clinical use is lacking. The use of combination therapy has been suggested by many authors for severe pulmonary disease, disseminated disease, and central nervous system involvement [2, 9, 42]. Such practice has been based on the following premises: synergy data of combination antimicrobials from murine models (particularly with imipenem and amikacin), improved clearance of high-burden disease with addition of bactericidal drug to bacteriostatic drug (e.g., addition of imipenem to TMP-SMX), and life-threatening disseminated or CNS disease where, in the absence of susceptibility data, probability of effective therapy is increased by the use of multiple antimicrobials.

Table 3 Antimicrobial susceptibility of Nocardia spp.

Antibiotic	*N. abscessus*	*N. farcinica*	*N. nova* complex	*N. brasiliensis*	*N. transvalensis*	*N. otitidiscaviarum*	*N. cyriacigeorgica*
TMP-SMX	S	V	S	S	S	S	S
Imipenem	V	V	S	R	S	R	V
Amikacin	S	S	S	S	V	S	S
Minocycline	V	V	V	V	V	V	V
Ceftriaxone	S	R	S	V	S	R	S
Ciprofloxacin	R	V	R	R	S	V	R
Amoxicillin-clavulanate	S	V	R	S	V	R	V
Linezolid	S	S	S	S	S	S	S
Moxifloxacin	R	S	R	V	S	V	V
Tigecycline	S	R	V	S	X	X	V

Adapted with permission from *Nocardia* infections in solid organ transplantation: Guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation by Restrepo and Clark [41], C*linical Transplantation* published by Wiley and Sons Ltd. © 2019 John Wiley & Sons.
S susceptible, *R* resistant, *V* variable, *X* not enough data for accurate results, *TMP-SMX* trimethoprim-sulfamethoxazole

Table 4 Suggested approach to empiric therapy for *Nocardia* infection in transplant patients

Organ involved	Suggested empiric therapy	Alternative therapy
Skin	TMP-SMX	Linezolid
Lung	**For mild disease**: TMP-SMX **For severe disease**: TMP-SMX + imipenem Imipenem + amikacin	Imipenem + linezolid
Brain	TMP-SMX + imipenem TMP-SMX + imipenem + amikacin	Imipenem + amikacin +/− linezolid TMP-SMX + imipenem +/− linezolid
Severe disseminated disease	TMP-SMX + imipenem TMP-SMX + imipenem + amikacin	Imipenem + amikacin +/− linezolid TMP-SMX + imipenem +/− linezolid

Duration of Therapy

There are still important questions that remain unanswered with regard to ongoing antimicrobial therapy, timing of transition from intravenous to oral therapy, and optimal duration of therapy. The consensus regarding these questions differs and should not supersede clinical judgment and the patient's response to therapy.

Several authors have suggested the use of a minimum of 2 weeks of intravenous antibiotics for severe skin or pulmonary disease and up to 6 weeks for central nervous system disease [41, 42]. While this is a reasonable recommendation, we would add the caveat that assuming there is a suitable oral antibiotic available based on antimicrobial susceptibility testing, a switch from intravenous to oral antibiotic therapy is reasonable if the patient is able to tolerate oral medications without concern for malabsorption, and the antibiotic of choice has high bioavailability with appropriate penetration into the affected tissue site. Use of continuation combination therapy when antimicrobial susceptibility testing has resulted, although not proven to be superior versus single agent therapy, is a reasonable consideration in the following circumstances: high-burden disease and concern regarding pharmacokinetic and/or pharmacodynamic properties of a single antimicrobial.

Given the slow clearance of the organism on antimicrobial therapy and risk of relapse after cessation of therapy, durations of therapy have historically been set to be several months with at least 3 months for skin and soft tissue disease and 12 months recommended for disseminated or CNS disease. A suggested range of duration of therapy based on disease sites is listed in Table 5. It is important to note that shorter durations of therapy have been successful in solid organ transplant recipients including a study in heart transplant recipients where duration of therapy less than 3 months was successful [11]. At this present time, it is unclear which patients may be suitable for shorter-course therapy. After completion of therapy, the risk of relapse has been estimated to be around 5% in solid organ transplant recipients [16], and thus monitoring for recurrence should continue for up to 1 year.

Table 5 Suggested durations of therapy for nocardiosis depending on disease site

	Duration (based on clinical and/or radiographic resolution)
Skin	3 months
Mild pulmonary	6 months
Severe pulmonary	6–12 months
Disseminated and CNS disease	12 months

Drug Toxicity Monitoring

Specific monitoring for myelosuppression and kidney function is required, especially if agents such as TMP-SMX, linezolid, and amikacin are used. For several decades, the use of sulfonamide drug monitoring has been proposed (with a target 2-h post-dose peak level of 100–150 micrograms/ml deemed as safe and therapeutic) for *Nocardia* and other sulfonamide-responsive infections such as *Pneumocystis jirovecii* [43]. A recent study examining sulfamethoxazole use and drug monitoring for a variety of infections (predominantly *Pneumocystis jirovecii* and *Stenotrophomonas maltophilia*, but also including *Nocardia*) has not shown a correlation between the aforementioned drug level and therapeutic efficacy or toxicity [44]. Thus, the role of routine monitoring of sulfonamide levels in therapy of *Nocardia* is unclear. It may be considered in the setting of concern for malabsorption, therapeutic failure, or suspected sulfa drug-related toxicity.

The Role of Adjunctive Surgery

Surgical intervention in conjunction with antimicrobial therapy can be considered for high-burden skin disease and in cerebral abscesses [45, 46]. The optimal approach to management of CNS nocardiosis is not established. Criteria for neurosurgical intervention have been proposed by Mamelak and colleagues [32]. They propose conservative (nonsurgical) management for extra-neurological infection documented with minimal neurological deficit and small abscess (less than 2 cm) and for multiple abscesses where surgery would be technically challenging with poor potential outcome. Mamelak and colleagues suggest surgical intervention for abscesses greater than 2.5 cm, for failure to respond to clinical treatment, or in cases of diagnostic uncertainty.

Immunosuppression Reduction and Immune-Based Therapy

Given the impact of T cell-mediated immunosuppression on the risk of nocardiosis and related mortality, reduction of immunosuppression is extremely important in clearing of disease although this should be carefully weighed against the risk of allograft rejection and GvHD. Although autoantibody production to GM-CSF has been implicated in the disease pathogenesis of nocardiosis [47], therapy with GM-CSF has not been studied as an adjunctive therapy for nocardiosis.

Use of Oxazolidinone Antimicrobials in Therapy: Linezolid and Tedizolid

The oxazolidinone antibiotic class comprising of linezolid and more recently tedizolid represents a promising advance in the therapy for nocardiosis, due to universal in vitro activity of the drugs against all species of *Nocardia* and excellent bioavailability. An 84% cure rate was reported in 19 lung transplant recipients with nocardiosis that were treated with linezolid [48]. However, toxicities including myelosuppression, peripheral neuropathy, and lactic acidosis and serotonin syndrome (when concurrent selective serotonin reuptake inhibitor (SSRI) drugs are used) are documented risks of therapy. Tedizolid is a novel oxazolidinone with a wide spectrum of actions against Gram-positive organisms and nontuberculous mycobacteria. It has been shown to be two- to threefold more potent than linezolid in vitro against common species of *Nocardia* except for *N. nova* and *N. brasiliensis* [49]. Tedizolid has lower rates of myelosuppression and has a lower risk for SSRI-mediated drug-drug interactions and thus holds significant promise in the long-term treatment of nocardiosis. Tedizolid (in combination with TMP-SMX) was successfully used for 6 months to treat CNS nocardiosis in a patient with multiple myeloma without myelotoxicity [50]. CLSI breakpoints for tedizolid have not been established for *Nocardia* spp., and a theoretical concern exists with regard to use of tedizolid for CNS disease given higher protein binding and lower CNS penetration [51].

Prevention

As iterated earlier, breakthrough infections with *Nocardia* spp. in patients on TMP-SMX for opportunistic infection prophylaxis have occurred in SOT recipients. While TMP-SMX may confer partial protection against *Nocardia* or delay onset of infection, it should not be considered an absolute preventative measure for either primary or secondary prevention of *Nocardia* infection in this population. In the HSCT population, the preferential use of atovaquone over TMP-SMX has been reported in one center to lead to increased rates of nocardiosis although this observation is based on small numbers [15]. Like their solid organ transplant counterparts, breakthrough *Nocardia* infections have still been reported in stem cell transplant recipients receiving TMP-SMX as the primary prophylaxis [13]. Thus, the optimal approach for primary or secondary prophylaxis in both solid organ and stem cell transplant recipients thus remains uncertain.

Conclusion

Nocardiosis is a rare opportunistic infection in solid organ and stem cell transplant recipients associated with considerable morbidity and mortality. The most common type of presentation is pulmonary infection, and in all cases central nervous system infection should be ruled out with imaging. While the majority of presentations are within the first 2 years of transplant, late presentations are possible and often related

with a higher level of immunosuppression. Identification of the *Nocardia* down to the species level and antimicrobial susceptibility testing are imperative to guide antimicrobial therapy. Initial therapy may require use of combination of antimicrobials, and durations of therapy are often several months. Relapse of disease is possible after cessation of treatment. There is an ongoing need for novel antimicrobials that can be used against *Nocardia* spp., which are both effective and tolerable in the transplant recipients.

Key Points

- Nocardiosis is a rare but serious opportunistic infection in transplant recipients commonly affecting the lung, brain, and skin.
- Use of primary TMP-SMX prophylaxis for *Pneumocystis jirovecii* pneumonia should not be considered protective for *Nocardia* infection.
- For pulmonary disease, the diagnosis should strongly be considered in transplant recipients with nodular or cavitary lesions of the lung.
- Communication with the microbiology lab is essential in order to maximize the yield of culture specimens. Identification of *Nocardia* down to the species level and antimicrobial susceptibility testing and an experienced laboratory are strongly recommended.
- All transplant recipients should have brain imaging performed to rule out central nervous system disease.
- The use of empiric combination therapy (involving addition of beta-lactam such as imipenem to TMP-SMX or linezolid) can be considered for severe disease in immunocompromised hosts.
- Durations of therapy are variable and can be as short as 3 months for limited skin disease and up to 12 months for central nervous system disease.

Cross-References

- ► Introduction to Immunosuppression, Tolerance, and Infection Risk
- ► Pulmonary Syndromes in Transplantation
- ► Rashes to Recognize in the Immunocompromised Transplant Patient: Focus on the Solid Organ Transplant Recipient
- ► Stem Cell Transplantation: Infection Risk by Transplant Type and Emerging Trends in Infection Management

References

1. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. Clin Microbiol Rev. 2006; 19(2):259–82.
2. Wilson JW. Nocardiosis: updates and clinical overview. Mayo Clin Proc. 2012;87(4):403–7.

3. Beaman BL, Beaman L. Nocardia species: host-parasite relationships. Clin Microbiol Rev. 1994;7(2):213–64.
4. Choucino C, Goodman SA, Greer JP, Stein RS, Wolff SN, Dummer JS. Nocardial infections in bone marrow transplant recipients. Clin Infect Dis. 1996;23(5):1012–9.
5. Sahathevan M, Harvey FA, Forbes G, O'Grady J, Gimson A, Bragman S, et al. Epidemiology, bacteriology and control of an outbreak of Nocardia asteroides infection on a liver unit. J Hosp Infect. 1991;18(Suppl A):473–80.
6. Coussement J, Lebeaux D, van Delden C, Guillot H, Freund R, Marbus S, et al. Nocardia infection in solid organ transplant recipients: a multicenter European case-control study. Clin Infect Dis. 2016;63(3):338–45.
7. Wang HL, Seo YH, LaSala PR, Tarrand JJ, Han XY. Nocardiosis in 132 patients with cancer: microbiological and clinical analyses. Am J Clin Pathol. 2014;142(4):513–23.
8. Minero MV, Marin M, Cercenado E, Rabadan PM, Bouza E, Munoz P. Nocardiosis at the turn of the century. Medicine (Baltimore). 2009;88(4):250–61.
9. Lebeaux D, Morelon E, Suarez F, Lanternier F, Scemla A, Frange P, et al. Nocardiosis in transplant recipients. Eur J Clin Microbiol Infect Dis. 2014;33(5):689–702.
10. Peleg AY, Husain S, Qureshi ZA, Silveira FP, Sarumi M, Shutt KA, et al. Risk factors, clinical characteristics, and outcome of Nocardia infection in organ transplant recipients: a matched case-control study. Clin Infect Dis. 2007;44(10):1307–14.
11. Tripodi MF, Durante-Mangoni E, Fortunato R, Cuccurullo S, Mikami Y, Farina C, et al. In vitro activity of multiple antibiotic combinations against Nocardia: relationship with a short-term treatment strategy in heart transplant recipients with pulmonary nocardiosis. Transpl Infect Dis. 2011;13(4):335–43.
12. Daly AS, McGeer A, Lipton JH. Systemic nocardiosis following allogeneic bone marrow transplantation. Transpl Infect Dis. 2003;5(1):16–20.
13. van Burik JA, Hackman RC, Nadeem SQ, Hiemenz JW, White MH, Flowers ME, et al. Nocardiosis after bone marrow transplantation: a retrospective study. Clin Infect Dis. 1997; 24(6):1154–60.
14. Mansi L, Daguindau E, Saas P, Pouthier F, Ferrand C, Dormoy A, et al. Diagnosis and management of Nocardiosis after bone marrow stem cell transplantation in adults: lack of lymphocyte recovery as a major contributing factor. Pathol Biol (Paris). 2014;62(3):156–61.
15. Molina A, Winston DJ, Pan D, Schiller GJ. Increased incidence of Nocardial infections in an era of Atovaquone Prophylaxis in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2018;24(8):1715–20.
16. Hemmersbach-Miller M, Stout JE, Woodworth MH, Cox GM, Saullo JL. Nocardia infections in the transplanted host. Transpl Infect Dis. 2018;20(4):e12902.
17. Arduino RC, Johnson PC, Miranda AG. Nocardiosis in renal transplant recipients undergoing immunosuppression with cyclosporine. Clin Infect Dis. 1993;16(4):505–12.
18. Santos M, Gil-Brusola A, Morales P. Infection by Nocardia in solid organ transplantation: thirty years of experience. Transplant Proc. 2011;43(6):2141–4.
19. Roberts SA, Franklin JC, Mijch A, Spelman D. Nocardia infection in heart-lung transplant recipients at Alfred Hospital, Melbourne, Australia, 1989–1998. Clin Infect Dis. 2000; 31(4):968–72.
20. Lebeaux D, Freund R, van Delden C, Guillot H, Marbus SD, Matignon M, et al. Outcome and treatment of Nocardiosis after solid organ transplantation: new insights from a European study. Clin Infect Dis. 2017;64(10):1396–405.
21. Smego RA Jr, Moeller MB, Gallis HA. Trimethoprim-sulfamethoxazole therapy for Nocardia infections. Arch Intern Med. 1983;143(4):711–8.
22. Hardak E, Yigla M, Berger G, Sprecher H, Oren I. Clinical spectrum and outcome of Nocardia infection: experience of 15-year period from a single tertiary medical center. Am J Med Sci. 2012;343(4):286–90.
23. Sands M, Brown RB. Interactions of cyclosporine with antimicrobial agents. Rev Infect Dis. 1989;11(5):691–7.

24. Berdaguer S, Bautista J, Brunet M, Cisneros JM. Antimicrobial and immunosuppressive drug interactions in solid organ transplant recipients. Enferm Infecc Microbiol Clin. 2012;30(Suppl 2):86–92.
25. Poonyagariyagorn HK, Gershman A, Avery R, Minai O, Blazey H, Asamoto K, et al. Challenges in the diagnosis and management of Nocardia infections in lung transplant recipients. Transpl Infect Dis. 2008;10(6):403–8.
26. Torres HA, Reddy BT, Raad II, Tarrand J, Bodey GP, Hanna HA, et al. Nocardiosis in cancer patients. Medicine (Baltimore). 2002;81(5):388–97.
27. Kandi V. Human Nocardia infections: a review of pulmonary Nocardiosis. Cureus. 2015;7(8):e304.
28. Kanne JP, Yandow DR, Mohammed TL, Meyer CA. CT findings of pulmonary nocardiosis. AJR Am J Roentgenol. 2011;197(2):W266–72.
29. Steinbrink J, Leavens J, Kauffman CA, Miceli MH. Manifestations and outcomes of nocardia infections: comparison of immunocompromised and nonimmunocompromised adult patients. Medicine (Baltimore). 2018;97(40):e12436.
30. Burke VE, Lopez FA. Approach to skin and soft tissue infections in non-HIV immunocompromised hosts. Curr Opin Infect Dis. 2017;30(4):354–63.
31. Anagnostou T, Arvanitis M, Kourkoumpetis TK, Desalermos A, Carneiro HA, Mylonakis E. Nocardiosis of the central nervous system: experience from a general hospital and review of 84 cases from the literature. Medicine (Baltimore). 2014;93(1):19–32.
32. Mamelak AN, Obana WG, Flaherty JF, Rosenblum ML. Nocardial brain abscess: treatment strategies and factors influencing outcome. Neurosurgery. 1994;35(4):622-31.
33. Kumar VA, Augustine D, Panikar D, Nandakumar A, Dinesh KR, Karim S, et al. Nocardia farcinica brain abscess: epidemiology, pathophysiology, and literature review. Surg Infect. 2014;15(5):640–6.
34. Rodriguez-Nava V, Couble A, Devulder G, Flandrois JP, Boiron P, Laurent F. Use of PCR-restriction enzyme pattern analysis and sequencing database for hsp65 gene-based identification of Nocardia species. J Clin Microbiol. 2006;44(2):536–46.
35. Conville PS, Zelazny AM, Witebsky FG. Analysis of secA1 gene sequences for identification of Nocardia species. J Clin Microbiol. 2006;44(8):2760–6.
36. Marin M, Ruiz A, Iglesias C, Quiroga L, Cercenado E, Martin-Rabadan P, et al. Identification of Nocardia species from clinical isolates using MALDI-TOF mass spectrometry. Clin Microbiol Infect. 2018;24(12):1342.e5–8.
37. Body BA, Beard MA, Slechta ES, Hanson KE, Barker AP, Babady NE, et al. Evaluation of the Vitek MS v3.0 matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of Mycobacterium and Nocardia species. J Clin Microbiol. 2018;56(6): e00237–18.
38. Girard V, Mailler S, Polsinelli S, Jacob D, Saccomani MC, Celliere B, et al. Routine identification of Nocardia species by MALDI-TOF mass spectrometry. Diagn Microbiol Infect Dis. 2017;87(1):7–10.
39. Rouzaud C, Rodriguez-Nava V, Catherinot E, Mechai F, Bergeron E, Farfour E, et al. Clinical assessment of a Nocardia PCR-based assay for diagnosis of Nocardiosis. J Clin Microbiol. 2018;56(6):e00002–18.
40. Coussement J, Lebeaux D, El Bizri N, Claes V, Kohnen M, Steensels D, et al. Nocardia polymerase chain reaction (PCR)-based assay performed on bronchoalveolar lavage fluid after lung transplantation: a prospective pilot study. PLoS One. 2019;14(2):e0211989.
41. Restrepo A, Clark NM, Infectious Diseases Community of Practice of the American Society of T. Nocardia infections in solid organ transplantation: guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation. Clin Transpl. 2019;33(9): e13509.
42. Coussement J, Lebeaux D, Rouzaud C, Lortholary O. Nocardia infections in solid organ and hematopoietic stem cell transplant recipients. Curr Opin Infect Dis. 2017;30(6):545–51.
43. Wallace RJ Jr, Septimus EJ, Williams TW Jr, Conklin RH, Satterwhite TK, Bushby MB, et al. Use of trimethoprim-sulfamethoxazole for treatment of infections due to Nocardia. Rev Infect Dis. 1982;4(2):315–25.

44. Ice LL, Barreto JN, Dao BD, Wolf RC, Dierkhising RA, Jannetto PJ, et al. Relationship of sulfamethoxazole therapeutic drug monitoring to clinical efficacy and toxicity: a retrospective cohort study. Ther Drug Monit. 2016;38(3):319–26.
45. Malincarne L, Marroni M, Farina C, Camanni G, Valente M, Belfiori B, et al. Primary brain abscess with Nocardia farcinica in an immunocompetent patient. Clin Neurol Neurosurg. 2002;104(2):132–5.
46. Hartmann A, Halvorsen CE, Jenssen T, Bjorneklett A, Brekke IB, Bakke SJ, et al. Intracerebral abscess caused by Nocardia otitidiscaviarum in a renal transplant patient–cured by evacuation plus antibiotic therapy. Nephron. 2000;86(1):79–83.
47. Rosen LB, Rocha Pereira N, Figueiredo C, Fiske LC, Ressner RA, Hong JC, et al. Nocardia-induced granulocyte macrophage colony-stimulating factor is neutralized by autoantibodies in disseminated/extrapulmonary nocardiosis. Clin Infect Dis. 2015;60(7):1017–25.
48. De La Cruz O, Minces LR, Silveira FP. Experience with linezolid for the treatment of nocardiosis in organ transplant recipients. J Infect. 2015;70(1):44–51.
49. Brown-Elliott BA, Wallace RJ Jr. In vitro susceptibility testing of tedizolid against isolates of Nocardia. Antimicrob Agents Chemother. 2017;61(12):e01537–17.
50. Matin A, Sharma S, Mathur P, Apewokin SK. Myelosuppression-sparing treatment of central nervous system nocardiosis in a multiple myeloma patient utilizing a tedizolid-based regimen: a case report. Int J Antimicrob Agents. 2017;49(4):488–92.
51. Zhanel GG, Love R, Adam H, Golden A, Zelenitsky S, Schweizer F, et al. Tedizolid: a novel oxazolidinone with potent activity against multidrug-resistant gram-positive pathogens. Drugs. 2015;75(3):253–70.

Mycoplasma and *Ureaplasma* Infections in Transplantation: A Big Impact Despite the Lack of a Cell Wall

22

Peter W. Schreiber and Nicolas J. Mueller

Contents

Disease Epidemiology 505
Clinical Impact in Transplant 506
Lung, Heart, and Combined Heart-Lung Transplantation 506
Liver Transplantation 508
Kidney Transplantation 508
Kidney-Pancreas Transplantation 509
Hematopoietic Stem Cell Transplantation 509
Disease Diagnostics and Clinical Evaluation 510
Management 511
Conclusions 513
Key Points 514
References 514

Disease Epidemiology

Mycoplasma spp. and *Ureaplasma* spp. are cell wall-lacking bacteria that belong to the class of *Mollicutes*. The term genital mycoplasmas encompasses *Mycoplasma hominis*, *M. genitalium*, *Ureaplasma urealyticum*, and *U. parvum*. Genital mycoplasmas are sexually transmitted. *M. genitalium* is considered a true sexually transmitted disease, whereas *M. hominis*, *U. parvum*, and *U. urealyticum* can be found in both healthy and symptomatic individuals [1]. Asymptomatic carriage is common, and the majority of individuals does not develop symptoms. Ureaplasma infection has been associated with chorioamnionitis, preterm labor, and preterm birth [2, 3]. Beyond, vertical transmission during delivery seems frequent, and an association with bronchopulmonary dysplasia of the neonate has been described [4–6]. In

P. W. Schreiber (✉) · N. J. Mueller
Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland
e-mail: peterwerner.schreiber@usz.ch; nicolas.mueller@usz.ch

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_19

immunosuppressed patients, *M. hominis* can be a relevant pathogen in urogenital and extragenital infections [7]. A broad range of manifestations has been described encompassing bacteremia, vascular, wound, central nervous system, respiratory tract, and joint infections [7]. In hypogammaglobulinemic patients, joint infections were attributed to infection with *Ureaplasma urealyticum* [8]. It has been hypothesized that the origin of extragenital infections is frequently the urogenital tract.

M. pneumoniae most frequently affects the respiratory tract in the general population [9]. Extrapulmonary manifestations are present in up to 25% of individuals with *M. pneumoniae* infection and can infect the central nervous system, heart, kidney, gastrointestinal tract, ears, and/or bloodstream. Pneumonia is an uncommon presentation in children below 5 years of age and is most frequent among school-aged children, with a decline in adolescence. In elderly adults suffering from pneumonia, higher age was associated with an increase in *M. pneumoniae* infections [9]. Skin findings associated with *M. pneumoniae* infections are common, being present in up to 25% of infected individuals. The spectrum ranges from mild findings presenting as relatively transient rashes or urticaria to severe manifestations such as bullous exanthems, Stevens-Johnson syndrome, or involvement of mucous membranes [9–11]. Human-to-human transmission occurs via droplet infection. Notably, individuals can remain infectious even after resolution of symptoms. Children are a frequent reservoir due to what can commonly be an asymptomatic or mild course combined with prolonged shedding, even after treatment [12]. In closed or semi-closed settings, outbreaks have been described, such as in a residential facility for adults with developmental disabilities [13].

Clinical Impact in Transplant

Epidemiological data on solid organ and hematopoietic stem cell transplant recipients are scarce and predominantly limited to single-case reports or small case series, which we will review.

Lung, Heart, and Combined Heart-Lung Transplantation

Overall, *M. hominis* seems to be a rare pathogen in surgical site infections after lung and lung/heart transplantation. In a retrospective single center study of deep surgical site infections (SSI) after lung transplantation, a single case out of 31 cases with SSI was caused by *M. hominis* [14] which presented as pericarditis.

M. hominis infections after lung transplantation can be donor-derived. In a cluster of three lung transplant recipients with *M. hominis* infections, whole-genome sequencing indicated clustering of a donor-recipient pair, whereas the two other cases showed genetically unrelated *M. hominis* isolates [15]. At a center in the United States, seven cases of *M. hominis* infections after cardiothoracic transplantation were observed over a time span of 18 years [16]. Clinical presentations encompassed sternal wound infections, pleuritis, mediastinitis, and anastomosis

leaks. In this case series, all patients recovered. All but one patient underwent revision surgery. Antibiotic treatment was given to all affected patients, of which treatment duration varied widely ranging from days to lifelong therapy. Two cases received single lung transplants from the same donor, and multilocus sequence typing also suggested a donor-derived infection.

Isolated pericarditis caused by *M. hominis* was recently reported following lung transplantation [17]. The patient was successfully treated with pericardial drainage, which also enabled pathogen detection, and a 6-week course of doxycycline.

Another manifestation was a periaortic abscess formation caused by *M. hominis* without presence of a sternal wound infection following combined heart-lung transplantation. The authors highlighted the need for considering *M. hominis* as pathogen, once conventional microbiological identification and empiric treatment failed [18]. In this case, a combined approach of surgical revision and prolonged antimicrobial therapy (3 months) was successful.

A single case of empyema due to *M. hominis* after bilateral lung transplantation in a patient with prior HSCT was recently published [19]. The patient developed bilateral pleural effusions and progressive respiratory distress in the early post-transplant course. Interestingly, *M. hominis* was exclusively identified in the left-sided pleural effusion, but not right-sided. The patient received a 6-week course of doxycycline and clindamycin with a complete clinical response.

A study from Australia described a cluster of four cases of *M. hominis* infections within 3 weeks, affecting three cardiac and one lung transplant recipient [20]. In all cases, surgical site infections were observed. A nosocomial transmission seemed likely based on adjacent beds and provision of care for each shift provided by the same nurses. The authors did not identify a single nurse that took care of all affected patients. A donor-derived origin was considered unlikely, as samples gathered at time of organ harvest did not grow *M. hominis*. All affected cases were treated with surgical exploration and debridement combined with antibiotic therapy. The authors suggested that in mild wound infections or local pleuropericarditis a 2- to 3-week course of oral doxycycline and/or ciprofloxacin might suffice. For more severe infections 10 to 14 days of intravenous clindamycin followed by 3–6 weeks of oral doxycycline was proposed.

Several reports of hyperammonemia syndrome have been published, an uncommon but frequently fatal disorder, associated with *Ureaplasma* spp. infections following lung transplantation. In a recent report, an index case of a lung transplant recipient suffering from hyperammonemia syndrome associated with disseminated *U. urealyticum* infection triggered further investigations [21]. In three other lethal cases of hyperammonemia after lung transplant, workup detected *U. urealyticum* in two cases and one case of *U. parvum* and *M. hominis*. The authors described another two cases of lung transplant recipients suffering from hyperammonemia, which were used for prospective validation. Both cases recovered from hyperammonemia syndrome after initiation of quinolone treatment. At time of initiation of quinolone treatment, polymerase chain reaction of blood and airway samples of both cases tested positive for *U. parvum*. One case was retested after 1 week of treatment with negative results for *Ureaplasma* spp. Disseminated *Ureaplasma* spp. infections may

result in an excess of ammonia production due to hydrolysis of urea in carbon dioxide and ammonia.

Liver Transplantation

After liver transplantation, infections caused by *Mycoplasma* spp. or *Ureaplasma* spp. seem to be infrequent. In a small case series, two liver transplant recipients with intra-abdominal wound infections caused by *M. hominis* were described [22]. One patient died due to another infectious complication, whereas the second case was cured by abscess drainage and antibiotic treatment with ciprofloxacin for 12 days. In another case report, *M. hominis* was found in perihepatic hematomas [23]. Recently, following liver transplantation in a child, *M. hominis* was detected in a fluid collection on the cut surface of the allograft [24]. Cure was achieved by treatment with doxycycline and clindamycin for 25 days. A single case of peritonitis caused by *M. hominis* and *U. urealyticum* was published [25]. At 12 days posttransplant revision, laparotomy was performed due to an increase in inflammatory markers and purulent wound secretions. Intraoperatively a retroperitoneal hematoma and fecal peritonitis were detected. Cultures exclusively grew *M. hominis* and *U. urealyticum*. Notably, in this case an increase in ammonia levels was observed along with a mild brain edema. Treatment with ciprofloxacin was administered.

Kidney Transplantation

In a Swiss university hospital, ten kidney transplant recipients with identification of genital mycoplasmas in urine samples were observed over a 3-year period [26]. Five patients were asymptomatic; in four patients sterile pyuria was present, while the remaining patient was screened due to detection of genital mycoplasmas in her partner. Four out of these five asymptomatic patients received treatment with doxycycline. The clinical course was favorable in all asymptomatic patients. Out of the five symptomatic patients, three patients were diagnosed with pyelonephritis, one patient with recurrent dysuria and pain in the graft region along with localized swelling of the upper pole of the kidney, and the remaining patient suffered from dysuria, vaginal discomfort, and pyuria. All symptomatic patients received treatment with doxycycline, in one patient combined with metronidazole. Due to insufficient clinical response, doxycycline was replaced by clindamycin in one case resulting in cure. In one symptomatic case, infection was accompanied by T-cell-mediated rejection, which resulted in end-stage renal disease despite antirejection treatment.

In the early posttransplant course, wound infections can be caused by *U. urealyticum* and *M. hominis* [27–29]. A 49-year-old kidney transplant patient was found to have a large seroma as well as urinary leak due to a severely necrotic transplant ureter, which required a washout and ureteropyeloplasty with a native ureter [30]. Serum and urine PCRs for *M. hominis* and *Ureaplasma* spp. were sent, which returned positive for *M. hominis* from both samples. Although she completed

a course of antimicrobial therapy, neurological complications from hyperammonemia resulted in a prolonged hospital stay, and she eventually succumbed to aspiration pneumonia.

In a 38-year-old kidney transplant recipient, arthritis of the shoulder and the knee was attributed to *M. hominis* and *U. urealyticum* in the early posttransplant course [31]. Despite prolonged antibiotic treatment with doxycycline, the infective arthritis recurred.

A single case of *U. urealyticum* meningitis was reported after kidney transplantation [32]. Following a complicated early posttransplant course with graft explantation due to arterial bleeding, the patient suffered from increasing headaches and showed clinical signs of meningitis. The diagnosis of *U. urealyticum* meningitis was established by a broad-range PCR. Targeted therapy encompassed doxycycline and chloramphenicol. The patient improved rapidly and recovered completely.

Kidney-Pancreas Transplantation

Following combined kidney-pancreas transplantation due to diabetes mellitus type 1, a systemic infection caused by *M. hominis* was reported [33]. Ten days post transplant an intra-abdominal abscess was suspected, while the patient received antimicrobial treatment with imipenem/cilastatin and fluconazole. Due to further deterioration, empirical treatment with ciprofloxacin was added and resulted in rapid clinical improvement. *M. hominis* was repeatedly detected in wound drainage. The patient was treated with a combined approach consisting of revision surgery and antibiotics, resulting in a favorable outcome.

Recently, another case of multiple intra-abdominal abscesses after combined kidney-pancreas transplantation due to diabetes mellitus type 1 was described [34]. Specimens derived from abscess drainage prompted suspicion of *M. hominis* infection, which was confirmed by PCR. After detection of *M. hominis*, treatment with minocycline was initiated.

Hematopoietic Stem Cell Transplantation

Following treatment with rituximab and allogenic hematopoietic stem cell transplantation (HSCT), a patient with prolonged agammaglobulinemia and B-cell aplasia developed septic polyarthritis due to *U. urealyticum* [35]. Treatment consisted of doxycycline and substitution of intravenous immunoglobulins (IVIG). The clinical course was complicated, but the patient finally recovered.

Recently, a case of gross hematuria and scrotal swelling 1 month after haploidentical HSCT was described [36]. At presentation urine culture and urine testing for adenovirus and BK virus were performed with negative results. On day +52, the patient developed fever. Retesting for bacteria, adenovirus and BK were negative again. Testing for *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Neisseria*

gonorrhoeae, and *Mycobacterium* spp. was prompted. PCR tested positive for *U. urealyticum*, and treatment with doxycycline was initiated with rapid clinical improvement. After 14 days of treatment, a repeat PCR for *Ureaplasma* spp. was negative.

Similar to case reports in lung transplant recipients, hyperammonemia in a patient after HSCT has been attributed to *U. parvum* infection [37]. Twelve days after HSCT, the patient developed an alteration in mental status without focal neurological findings. Despite treatment of hyperammonemia, the neurological condition worsened. At day +15, tracheal aspirations and blood samples were obtained prior to initiation of empiric treatment with azithromycin and levofloxacin. PCR of tracheal aspirations tested positive for *U. parvum*, whereas PCR of the blood did not detect *Ureaplasma* spp. An airway sample on day +24 tested negative for *Ureaplasma* spp. Following cessation of azithromycin and levofloxacin after 15 and 18 days, respectively, an increase in ammonia levels was noticed and prompted retreatment with levofloxacin resulting in therapeutic success.

An Italian case series on toxic epidermal necrolysis-like reaction (TEN-LR) after HSCT in children included an 11-year-old child that received treatment with clarithromycin for *M. pneumoniae* infection and *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole at the time of onset [38]. The patient was treated with IVIG and high-dose steroids, while clarithromycin and trimethoprim-sulfamethoxazole were stopped. The authors discussed *M. pneumoniae* as potential etiology of TEN-LR. The patient fully recovered in the latter course. A single case of Steven-Johnson syndrome (SJS) was reported after HSCT in a 21-year-old patient. She presented with fever, throat pain, and dehydration on day +20. Histopathological findings of esophageal biopsies were interpreted as compatible with SJS. Considering the finding of oral and vaginal mucositis, dermatologists suspected SJS most likely caused by *M. pneumoniae*. IVIG was administered, and topical mupirocin was applied to the lesions. Serology confirmed presence of *M. pneumoniae* IgM. A 5-day course of levofloxacin was given with favorable clinical response.

Disease Diagnostics and Clinical Evaluation

In general, culture for *M. pneumoniae* is laborious, time-consuming (up to weeks), and expensive. Due to these limitations, culture techniques are rarely used. In *M. pneumoniae* infections, cold agglutinins can be frequently detected from the first week of illness and may persist for several months after illness. Nowadays, cold agglutinins are rarely used. Serological tests showing a fourfold or greater rise in paired specimens collected 2–3 weeks apart are considered indicative of current or recent infection [9]. In general, nucleic acid amplification tests (NAATs), with PCR being the most common method, may be positive much earlier in the course of infection [39]. NAATs are almost always showing superior sensitivity compared to conventional methods [40]. Correspondingly, these tests are more and more considered the gold standard and are widely applied [40], as they are able to provide timely results. Several in-house as well as commercially available NAATs with a broad

range of different methods have been described in literature [40, 41]. Real-time PCRs offer the advantages of quantification and increased sensitivity compared to conventional PCR [42]. Dumke R. and Jacobs E. tested four commercially available PCRs and compared these to an in-house PCR; all PCRs detected at least 20CFUs/5 μl, whereas the in-house PCR was able to detect 0.2CFUs/5 μl [43]. Commercial multiplex assays detecting "atypical bacteria" and respiratory viruses that might show similar clinical presentation are increasing. However, multiplex assays might be hampered by a lower sensitivity [40]. Recently, assays that combine detection of *M. pneumoniae* with identification of macrolide resistance-associated mutations have been developed [40]. Suitable specimens for detection of *M. pneumoniae* respiratory tract infections include nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates, lung tissue, pleural fluid, and bronchoalveolar lavage fluid. Samples from various extrapulmonary sites such as blood, cerebrospinal fluid, or tissue samples were tested in studies only [39].

Genital mycoplasmas require specialized media for optimal in vitro growth. *M. hominis* can be grown with methods routinely used in clinical laboratories, but sensitivity is poor. The extremely slow growth of *M. genitalium* renders culture methods impractical [42]. PCR methods are considered more sensitive than culture and are less time-consuming. Until recently, no commercially available test for diagnosis of *M. genitalium* infection was cleared by the US Food and Drug Administration (FDA), while several tests were CE approved in Europe [44]. In January 2019, the FDA permitted marketing of the transcription-mediated amplification test Aptima *Mycoplasma genitalium* assay (Hologic, San Diego, CA, USA) [45]. Syndromic multiplex tests for sexually transmitted diseases might also include testing for *M. genitalium* and *U. urealyticum*, such as the STD-Finder 2SMART (PathoFinder, Maastricht, Netherlands). In a study from 2011, sensitivity and specificity of a previous version of this assay were reported each 100% for detection of *M. genitalium*. Notably, a combination assay that detects *M. genitalium* and provides information on macrolide resistance-associated mutations has been described [44]. Considering the frequent detection in healthy individuals, serological tests for *M. hominis*, *M. genitalium*, and *Ureaplasma* spp. are hard to interpret and lack standardization [3, 42]. Specimens can be urogenital tract samples, but also blood, airway samples or biopsies [42].

Ammonia levels should be determined if a transplant recipient develops a neurological deterioration, especially if there is no alternative explanation and suspicion of an infection. Attention needs to be given in pre-analytics of ammonia measurement. EDTA should be used as anticoagulant for plasma samples; samples should be transported on ice and processed ideally within 1 h [46–48].

Management

Depending on the extent of infection, either a conservative, i.e. antibiotic treatment, or a combined approach consisting of antibiotic treatment and drainage or operative debridement should be chosen. In general, with abscess formation or extensive, life-threatening infection, an interventional or operative procedure for reduction of the

pathogen load would be indicated. As *Mycoplasma* spp. and *Ureaplasma* spp. lack a cell wall, these organisms are generally resistant to beta-lactam antibiotics. Empiric treatment for *M. pneumoniae* and genital mycoplasmas is often recommended with macrolides, tetracyclines, or quinolones. Notably, *M. hominis* is resistant against macrolides, but susceptible to clindamycin (Fig. 1) [49]. Resistance of *Mollicutes* against macrolides, quinolones, as well as tetracyclines has been described and should be considered, if treatment response is insufficient [50]. Susceptibility of *Mollicutes* largely varies by local epidemiology (Table 1). For *Ureaplasma* spp., a study from China reported resistance rates for ciprofloxacin, doxycycline, and azithromycin of 94%, 5%, and 39% [51], respectively, whereas Swiss isolates were found to be resistant for ciprofloxacin, doxycycline, and azithromycin in 19%, 0%, and 1%, respectively [52, 53]. A high prevalence of multidrug-resistant *M. genitalium* infections was identified among men who have sex with men in Alabama [54], while a report from the Netherlands published in the same year described one of the first cases of multidrug-resistant *M. genitalium* infections in Europe [55]. Duration of antibiotic treatment widely varied among the reports. Treatment duration should be guided by both general principles in treatment of infections, e.g. prolonged antibiotic treatment in case of affection of poorly perfused structures such as bone or cartilage and overall response (clinical, laboratory, and occasionally repeated imaging).

Respiratory infections caused by *Mycoplasma* spp. should prompt infection control measures, i.e. initiation of droplet precautions in addition to standard precautions for the duration of illness [56]. Nosocomial outbreaks have also been reported outside of transplant wards. In outbreak settings, postexposure prophylaxis

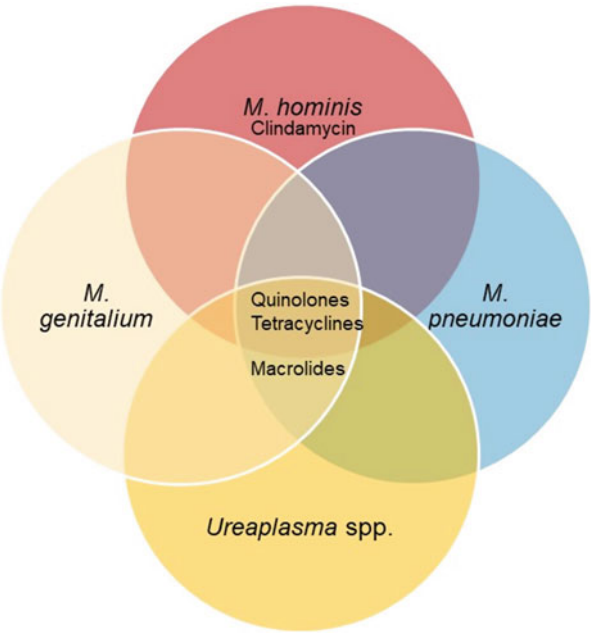


Fig. 1 Venn diagram on expected antibiotic susceptibility of *Mycoplasma* spp. and *Ureaplasma* spp.

Table 1 Examples of highly variable resistance patterns of *Mollicutes* by geography

***Ureaplasma* spp.**						
				Proportion of resistant isolates (%)		
First author (reference)	Country	Year of publication	Sample type (number of samples)	Ciprofloxacin	Doxycycline	Azithromycin
Huang C [51]	China	2016	Semen samples (1951)	94	5	39
Schneider SC [53]	Switzerland	2015	Urogenital samples (103)	19	0	1
Mycoplasma genitalium						
				Proportion of resistant isolates (%)		
First author (reference)	Country	Year of publication	Sample type (number of samples)	Quinolone	Macrolide	Quinolone and Macrolide
Dionne-Odom J [54]	USA	2017	Urogenital and rectal samples (27)	29.6	74.1	24.0
Braam JF [55]	Netherlands	2017	Urogenital sample (1)			100

can be considered, especially in vulnerable populations such as lung transplant recipients. A few reports support an enhanced outbreak control approach through a combined method of infection control measures and azithromycin prophylaxis [13, 57]. In a setting of high macrolide resistance, tetracyclines might be an effective alternative [58, 59]. The role of infection control measures in *Mycoplasma* spp. infections that do not primarily affect the respiratory tract is less clear. However, as highlighted in the study by Hopkins PM et al., a nosocomial clustering of surgical site infections caused by *M. hominis* has been suggested [20]. Measures to reinforce standard precautions seem prudent.

To our knowledge, no approved vaccinations against *Mycoplasma* spp. or *Ureaplasma* spp. are available.

Conclusions

In general, infections caused by *Mycoplasma* spp. and *Ureaplasma* spp. after solid organ transplantation or hematopoietic stem cell transplantation seem to be rare. The most commonly reported manifestation is surgical site infection following solid organ transplantation. Urogenital infections in kidney and hematopoietic stem cell transplant recipients have been also attributed to these pathogens. Beyond, an

association of infections caused by genital mycoplasmas and increased ammonia levels is postulated. *M. pneumoniae* may be associated with severe dermatologic manifestations. For diagnosis, NAAT, most commonly PCR, is considered gold standard. Depending on extent of infections, management is either conservative with antibiotic therapy or combined with a surgical procedure.

Key Points

- Common manifestations encompass surgical site infections after solid organ transplant and urogenital infections after kidney and hematopoietic stem cell transplant.
- An association with hyperammonemia syndrome has been described.
- *M. pneumoniae* might be relevant in severe dermatologic manifestations after HSCT.
- Consider *Mycoplasma* spp. and *Ureaplasma* spp., if routine diagnostics do not identify causative pathogens.
- Insufficient response to empirical antibiotic treatment should prompt testing for mycoplasmas and ureaplasmas with PCR.
- Depending on extent of infection treatment either conservative or combination of interventional and conservative treatment.

References

1. Horner P, Donders G, Cusini M, Gomberg M, Jensen JS, Unemo M. Should we be testing for urogenital *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum* in men and women? – a position statement from the European STI Guidelines Editorial Board. J Eur Acad Dermatol Venereol. 2018;32(11):1845–51.
2. Gerber S, Vial Y, Hohlfeld P, Witkin SS. Detection of *Ureaplasma urealyticum* in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. J Infect Dis. 2003;187(3):518–21.
3. Capoccia R, Greub G, Baud D. *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse pregnancy outcomes. Curr Opin Infect Dis. 2013;26(3):231–40.
4. Kasper DC, Mechtler TP, Bohm J, Petricevic L, Gleiss A, Spergser J, et al. In utero exposure to *Ureaplasma* spp. is associated with increased rate of bronchopulmonary dysplasia and intraventricular hemorrhage in preterm infants. J Perinat Med. 2011;39(3):331–6.
5. Abele-Horn M, Genzel-Boroviczeny O, Uhlig T, Zimmermann A, Peters J, Scholz M. *Ureaplasma urealyticum* colonization and bronchopulmonary dysplasia: a comparative prospective multicentre study. Eur J Pediatr. 1998;157(12):1004–11.
6. Abele-Horn M, Peters J, Genzel-Boroviczeny O, Wolff C, Zimmermann A, Gottschling W. Vaginal *Ureaplasma urealyticum* colonization: influence on pregnancy outcome and neonatal morbidity. Infection. 1997;25(5):286–91.
7. Meyer RD, Clough W. Extragenital *Mycoplasma hominis* infections in adults: emphasis on immunosuppression. Clin Infect Dis. 1993;17(Suppl 1):S243–9.
8. Taylor-Robinson D, Furr PM, Webster AD. *Ureaplasma urealyticum* in the immunocompromised host. Pediatr Infect Dis. 1986;5(6 Suppl):S236–8.
9. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev. 2004;17(4):697–728, table of contents.

10. Foy HM, Grayston JT, Kenny GE, Alexander ER, McMahan R. Epidemiology of *Mycoplasma pneumoniae* infection in families. JAMA. 1966;197(11):859–66.
11. Schalock PC, Dinulos JG. *Mycoplasma pneumoniae*-induced cutaneous disease. Int J Dermatol. 2009;48(7):673–80; quiz 80–1.
12. Alexander ER, Foy HM, Kenny GE, Kronmal RA, McMahan R, Clarke ER, et al. Pneumonia due to *Mycoplasma pneumoniae*. Its incidence in the membership of a co-operative medical group. N Engl J Med. 1966;275(3):131–6.
13. Gdalevich M, Haas EJ, Dukhan L, Katz M, Zelenski V, Moran-Gilad J. Control of a *Mycoplasma pneumoniae* outbreak in an institutional setting using azithromycin prophylaxis. Front Public Health. 2017;5:366.
14. Shields RK, Clancy CJ, Minces LR, Shigemura N, Kwak EJ, Silveira FP, et al. Epidemiology and outcomes of deep surgical site infections following lung transplantation. Am J Transplant. 2013;13(8):2137–45.
15. Smibert OC, Wilson HL, Sohail A, Narayanasamy S, Schultz MB, Ballard SA, et al. Donor-derived *Mycoplasma hominis* and an apparent cluster of *M. hominis* cases in solid organ transplant recipients. Clin Infect Dis. 2017;65(9):1504–8.
16. Sampath R, Patel R, Cunningham SA, Arif S, Daly RC, Badley AD, et al. Cardiothoracic transplant recipient *Mycoplasma hominis*: an uncommon infection with probable donor transmission. EBioMedicine. 2017;19:84–90.
17. Mitsani D, Nguyen MH, Silveira FP, Bermudez C, Toyoda Y, Pasculle AW, et al. *Mycoplasma hominis* pericarditis in a lung transplant recipient: review of the literature about an uncommon but important cardiothoracic pathogen. Transpl Infect Dis. 2010;12(2):146–50.
18. Hagiya H, Yoshida H, Yamamoto N, Kimura K, Ueda A, Nishi I, et al. *Mycoplasma hominis* periaortic abscess following heart-lung transplantation. Transpl Infect Dis. 2017;19(3). https://doi.org/10.1111/tid.12697. Epub 2017 May 3.
19. Dixit A, Alexandrescu S, Boyer D, Graf EH, Vargas SO, Silverman M. *Mycoplasma hominis* empyema in an 18-year-old stem cell and lung transplant recipient: case report and review of the literature. J Pediatric Infect Dis Soc. 2017;6(4):e173–e6.
20. Hopkins PM, Winlaw DS, Chhajed PN, Harkness JL, Horton MD, Keogh AM, et al. *Mycoplasma hominis* infection in heart and lung transplantation. J Heart Lung Transplant. 2002;21(11):1225–9.
21. Bharat A, Cunningham SA, Scott Budinger GR, Kreisel D, DeWet CJ, Gelman AE, et al. Disseminated Ureaplasma infection as a cause of fatal hyperammonemia in humans. Sci Transl Med. 2015;7(284):284re3.
22. Vogel U, Luneberg E, Kuse ER, Neulinger AL, Frosch M. Extragenital *Mycoplasma hominis* infection in two liver transplant recipients. Clin Infect Dis. 1997;24(3):512–3.
23. Jacobs F, Van de Stadt J, Gelin M, Nonhoff C, Gay F, Adler M, et al. *Mycoplasma hominis* infection of perihepatic hematomas in a liver transplant recipient. Surgery. 1992;111(1):98–100.
24. Losada I, Canle D, Moure R, Villanueva R, Bueno J, Sanchez-Galindo A, et al. *Mycoplasma hominis* infection after liver transplantation in a child. Transplantation. 2004;77(10):1624–5.
25. Haller M, Forst H, Ruckdeschel G, Denecke H, Peter K. Peritonitis due to *Mycoplasma hominis* and *Ureaplasma urealyticum* in a liver transplant recipient. Eur J Clin Microbiol Infect Dis. 1991;10(3):172.
26. Gerber L, Gaspert A, Braghetti A, Zwahlen H, Wuthrich R, Zbinden R, et al. Ureaplasma and Mycoplasma in kidney allograft recipients-A case series and review of the literature. Transpl Infect Dis. 2018;20(5):e12937.
27. Loupy A, Join-Lambert OF, Bebear CM, Legendre C, Anglicheau D. Urogenital mycoplasma: an emerging cause of deep wound infection after kidney transplantation? NDT Plus. 2008;1(4):239–40.
28. Pastural M, Audard V, Bralet MP, Remy P, Salomon L, Tankovic J, et al. *Mycoplasma hominis* infection in renal transplantation. Nephrol Dial Transplant. 2002;17(3):495–6.
29. Miranda C, Carazo C, Banon R, Mendoza J, Montes A, de la Rosa M. *Mycoplasma hominis* infection in three renal transplant patients. Diagn Microbiol Infect Dis. 1990;13(4):329–31.

30. Nowbakht C, Edwards AR, Rodriguez-Buritica DF, Luce AM, Doshi PB, De Golovine A, et al. Two cases of fatal hyperammonemia syndrome due to *Mycoplasma hominis* and *Ureaplasma urealyticum* in immunocompromised patients outside lung transplant recipients. Open Forum Infect Dis. 2019;6(3):ofz033.
31. Burdge DR, Reid GD, Reeve CE, Robertson JA, Stemke GW, Bowie WR. Septic arthritis due to dual infection with *Mycoplasma hominis* and *Ureaplasma urealyticum*. J Rheumatol. 1988;15(2):366–8.
32. Geissdorfer W, Sandner G, John S, Gessner A, Schoerner C, Schroppel K. *Ureaplasma urealyticum* meningitis in an adult patient. J Clin Microbiol. 2008;46(3):1141–3.
33. Geissdorfer W, Schorner C, Lohoff M. Systemic *Mycoplasma hominis* infection in a patient immunocompromised due to combined transplantation of kidney and pancreas. Eur J Clin Microbiol Infect Dis. 2001;20(7):511–2.
34. Okumura Y, Kajihara T, Koba Y, Onodera M, Hara T, Tahara H, et al. Multiple intraabdominal abscesses caused by *Mycoplasma hominis* infection following simultaneous pancreas-kidney transplantation. Ann Lab Med. 2018;38(4):381–3.
35. Arber C, Buser A, Heim D, Weisser M, Tyndall A, Tichelli A, et al. Septic polyarthritis with *Ureaplasma urealyticum* in a patient with prolonged agammaglobulinemia and B-cell aplasia after allogeneic HSCT and rituximab pretreatment. Bone Marrow Transplant. 2007;40(6):597–8.
36. Choi GJ, Kim SJ, Kang JM, Choi SR, Yoo KH, Kim YJ. Severe hematuria in a hematopoietic cell transplant recipient caused by *Ureaplasma urealyticum* not by BK virus or adenovirus infection. Transpl Infect Dis. 2018;20(4):e12921.
37. Graetz R, Meyer R, Shehab K, Katsanis E. Successful resolution of hyperammonemia following hematopoietic cell transplantation with directed treatment of *Ureaplasma parvum* infection. Transpl Infect Dis. 2018;20(2):e12839.
38. Faraci M, Giardino S, Lanino E, Morreale G, Ghibaudo E, Francesia Berta M, et al. Toxic epidermal necrolysis-like reaction after hematopoietic stem cell transplantation in children. J Pediatr Hematol Oncol. 2017;39(4):254–8.
39. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. *Mycoplasma pneumoniae* from the respiratory tract and beyond. Clin Microbiol Rev. 2017;30(3):747–809.
40. Loens K, Ieven M. *Mycoplasma pneumoniae*: current knowledge on nucleic acid amplification techniques and serological diagnostics. Front Microbiol. 2016;7:448.
41. Loens K, Goossens H, Ieven M. Acute respiratory infection due to *Mycoplasma pneumoniae*: current status of diagnostic methods. Eur J Clin Microbiol Infect Dis. 2010;29(9):1055–69.
42. Waites KB, Xiao L, Paralanov V, Viscardi RM, Glass JI. Molecular methods for the detection of Mycoplasma and Ureaplasma infections in humans: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. J Mol Diagn. 2012;14(5):437–50.
43. Dumke R, Jacobs E. Evaluation of five real-time PCR assays for detection of *Mycoplasma pneumoniae*. J Clin Microbiol. 2014;52(11):4078–81.
44. Gaydos CA. *Mycoplasma genitalium*: accurate diagnosis is necessary for adequate treatment. J Infect Dis. 2017;216(suppl_2):S406–S11.
45. US Department of Health & Human Services, US Food and Drug Administration. In: Nucleic acid detection system for non-viral microorganism(s) causing sexually transmitted infections. Medical Devices Databases. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?ID=DEN180047. Accessed 3 june 2019.
46. Hashim IA, Cuthbert JA. Elevated ammonia concentrations: potential for pre-analytical and analytical contributing factors. Clin Biochem. 2014;47(16–17):233–6.
47. Nikolac N, Omazic J, Simundic AM. The evidence based practice for optimal sample quality for ammonia measurement. Clin Biochem. 2014;47(12):991–5.
48. Schuff-Werner P, Steiner M. Preanalytical ammonia generation: a race with time but not with temperature. J Clin Pathol. 2015;68(9):757–8.
49. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored butterfly. Clin Microbiol Rev. 2011;24(3):498–514.

50. Wang QY, Li RH, Zheng LQ, Shang XH. Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in female outpatients, 2009–2013. J Microbiol Immunol Infect. 2016;49(3):359–62.
51. Huang C, Long X, Jing S, Fan L, Xu K, Wang S, et al. *Ureaplasma urealyticum* and *Mycoplasma hominis* infections and semen quality in 19,098 infertile men in China. World J Urol. 2016;34(7):1039–44.
52. Beeton ML, Spiller OB. Antibiotic resistance among *Ureaplasma* spp. isolates: cause for concern? J Antimicrob Chemother. 2017;72(2):330–7.
53. Schneider SC, Tinguely R, Droz S, Hilty M, Dona V, Bodmer T, et al. Antibiotic susceptibility and sequence type distribution of Ureaplasma species isolated from genital samples in Switzerland. Antimicrob Agents Chemother. 2015;59(10):6026–31.
54. Dionne-Odom J, Geisler WM, Aaron KJ, Waites KB, Westfall AO, Van Der Pol B, et al. High prevalence of multidrug-resistant *Mycoplasma genitalium* in human immunodeficiency virus-infected men who have sex with men in Alabama. Clin Infect Dis. 2018;66(5):796–8.
55. Braam JF, van Dommelen L, Henquet CJM, van de Bovenkamp JHB, Kusters JG. Multidrug-resistant *Mycoplasma genitalium* infections in Europe. Eur J Clin Microbiol Infect Dis. 2017;36(9):1565–7.
56. Prevention CoDCa. Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings (2007) 09/2018.
57. Klausner JD, Passaro D, Rosenberg J, Thacker WL, Talkington DF, Werner SB, et al. Enhanced control of an outbreak of *Mycoplasma pneumoniae* pneumonia with azithromycin prophylaxis. J Infect Dis. 1998;177(1):161–6.
58. Jensen KJ, Senterfit LB, Scully WE, Conway TJ, West RF, Drummy WW. *Mycoplasma pneumoniae* infections in children. An epidemiologic appraisal in families treated with oxytetracycline. Am J Epidemiol. 1967;86(2):419–32.
59. Schillinger JA, Arnold K, Mokulis EC, et al. Prophylaxis against *Mycoplasma pneumoniae*; a double-blind placebo-controlled trial using doxycycline. In: Microbiology ASf, editor. 35th Interscience Conference on Antimicrobial Agents and Chemotherapy San Francisco: program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy 1995.

Clostridioides Difficile: Where Should the Management Strategies in Transplant Vary? 23

Jennifer C. Pisano and Kathleen M. Mullane

Contents

Introduction 519
Epidemiology of *C. difficile* in Transplant Recipients 520
Pathogenesis of *C. difficile* 524
Diagnosis of *C. difficile* Infection: Clinical Evaluation and Available Emerging Diagnostics 528
Approach to Patient Management Including Emerging Treatment Modalities and Options for Prophylaxis, Prevention, and Prognosis (See Fig. 4) 531
Prophylaxis 534
Probiotics 535
Conclusions 535
Key Points 536
References 536

Introduction

More than 50,000 hematopoietic stem cell transplantation (HSCT) and 100,800 solid organ transplants (SOT) are performed worldwide with 17,000 and 36,530, respectively performed in the United States [1–4]. In these immunocompromised patients, infectious complications are frequently seen posttransplant. *Clostridioides* (formerly *Clostridium*) *difficile* (*C. difficile*), a spore-forming, anaerobic, gram-positive bacillus, is the most common cause of hospital-acquired infectious diarrhea and the most common nosocomial infection in the United States [5, 6]. Clinical manifestations of *C. difficile* may range from a mild diarrheal illness to fulminant colitis [7]. *C. difficile* is ubiquitous in nature and may be isolated from soil, water, food, livestock, and pets.

J. C. Pisano · K. M. Mullane (✉)
Department of Medicine, Section of Infectious Diseases and Global Health, University of Chicago, Chicago, IL, USA
e-mail: jpisano@medicine.bsd.uchicago.edu; kmullane@medicine.bsd.uchicago.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_23

Immunologically vulnerable ICH populations such as HSCT and SOT recipients are disproportionately affected [8–11].

Epidemiology of *C. difficile* in Transplant Recipients

The incidence and severity of CDI increased dramatically from the year 2000 to 2010 and plateaued between 2011 and 2013 at these historically high levels in the United States, while in Western Europe and England, rates have declined [10–12]. This increase was reflected in the frequency of CDI diagnoses captured in hospital discharges from 4.5 cases per 1000 discharges in 2001 to 12.1 cases in 2010 and 14.0 cases in 2014 [13]. These changes in epidemiology of CDI were associated with the emergence of the North American pulsed-field gel electrophoresis type 1 (NAP1)/restriction enzyme analysis (REA) type BI/PCR-ribotype 027 (NAP1/BI/027) strain of *C. difficile* [12]. Currently the annual burden of CDI in the United States is estimated to be nearly 500,000 cases and may be associated with 15,000–30,000 deaths per year [8]. CDI is a more frequently encountered problem in SOT recipients than in other hospitalized populations. While the incidence of CDI has been reported to be as high as 30% in SOT recipients, a meta-analysis evaluating published studies from 1991 to 2014 estimated the prevalence to be approximately 7.4% in the peri-transplant period after SOT [14]. The reported incidence of CDI associated with SOT varies depending upon the time after transplantation and the specific organ transplanted. The highest incidence is reported in lung transplant recipients (7–31%) [14–19]. The incidence of CDI is reported to be lowest in kidney recipients (2.6–16%), followed by liver recipients (2.7–19%), 0.5–7.9% in pancreas recipients, 9% in intestinal recipients, 1.8–15% in heart recipients, and 6.4–20.9% for those undergoing multiorgan transplantation This incidence in SOT recipients is higher than that seen in the general hospitalized patient populations, that typically being <1% [20, 21]. Fulminant *C. difficile*-associated colitis develops in up to 8% of immunocompetent patients with CDI while it occurs in 5.3–13% of SOT recipients [14, 22]. The incidence of CDI in SOT recipients is highest within the first 30–60 days after transplantation, probably because of more frequent antimicrobial exposure, more intense immunosuppression, and increased exposure to the healthcare setting [15, 22, 23]. Late-onset CDI occurs months to years after transplantation and is usually associated with either antimicrobial exposure or intensified immunosuppression to treat an episode of graft rejection [22]. It is not known how the NAP1/BI/027 strain has impacted the incidence and severity of CDI in SOT recipients relative to the general hospital population.

In addition to the most common risk factor for CDI, antibiotic exposure, other established factors in the general population putting individuals at risk for CDI include frequent exposures to healthcare settings, hospitalization especially if prolonged, age older than 65 years, gastrointestinal (GI) surgery or manipulation of the GI tract, uremia, and receipt of chemotherapy [10, 24]. An association between the

use of gastric acid suppressing agents, such as proton pump inhibitors and histamine-2-receptor antagonists, has been identified as a risk for CDI [25]. Both SOT and HSCT recipients share these established risk factors, in an amplified manner, but there are also risk factors specific to each of these populations (Table 1).

The enhanced incidence of CDI in HSCT recipients may be related to the use of myeloablative conditioning chemotherapy and total body irradiation, prolonged neutropenia (attributed both to resultant gut translocation of colonizing organisms and to antimicrobial prophylaxis causing microbiome disruption), mucositis, concomitant herpesvirus reactivation, and the presence of GVHD [26–28]. Individuals undergoing a cord-HSCT are at highest risk of CDI. With an incidence of 10–30% CDI is fivefold to ninefold higher in HSCT recipients when compared to the general hospitalized population [19, 28, 29]. In individuals receiving an autologous HSCT (auto-HSCT), the rates of confirmed CDI range from 5% to 15% while in those undergoing allogeneic HSCT (allo-HSCT) rates of CDI range from 12% to 34% [15, 18–20].

Among SOT recipients, hospital onset CDI has been reported to be five times more common in patients who have undergone as SOT compared to the general inpatient population. In the SOT population, the incidence of CDI ranges from 3.5% to 31% of recipients in the early posttransplant period [14, 17]. Risks for CDI specific to SOT include frequent exposures to healthcare settings, age greater than 55 years, history of CDI prior to transplantation, deceased donor organ transplant, antimicrobial exposure, graft failure/re-transplantation, and the persistent need for enhanced immunosuppression (such as pulsed steroid dosing and antithymocyte globulin for organ rejection) [30]. CDI is most likely to occur within the first 30–60 days after SOT with the risk decreasing during the 60–90 days after transplant and approximating pretransplant rates by 90 days posttransplantation [15, 23, 28]. Those individuals experiencing CDI after transplantation are at an increased risk of mortality, graft failure, and graft-versus-host disease (GVHD) [17, 31–34].

In HSCT recipients, CDI is more likely to occur in the first 100 days after transplant, with most infections occurring in the first 30 days (pre-engraftment); this increased risk of CDI continues later posttransplant as well and is highest in the post-engraftment phase in patients receiving high-risk broad-spectrum antibiotics and in those with acute GVHD [35].

Because *C. difficile* spores are ubiquitous in the environment, both ICH and non-ICH may be asymptomatically colonized [36]. Most commonly, non-toxigenic strains of *C. difficile* as well as toxigenic *C. difficile* and *C. difficile*-associated toxins are carried asymptomatically as part of a normal gut microbiome. Asymptomatic carriers of *C. difficile* have been reported to outnumber true cases of CDI 10:1; however, from an individual patient perspective, *C. difficile* colonization is a risk factor for the development of active CDI [37]. In healthy adults, 0–7% may be asymptomatic carriers of *C. difficile*, but in individuals having contact with the healthcare system, 3–26% have been found to be colonized [38–41] [42, 43]. Among individuals admitted to the hospital, a meta-analysis of 19 studies, with extractable data published between 1925 through 6/2014 that were identified

Table 1 Risk factors for CDI [27, 30, 124, 125]

All patients	Specific to SOT	Specific to HSCT
Any antibiotic therapy Highest risk antibiotics: Clindamycin Fluoroquinolones Beta-lactam and/or beta-lactamase inhibitor combination Cephalosporins Metronidazole	Immunosuppressive agents [corticosteroids > mycophenolate > azathioprine or Calcineurin inhibitors]	Male gender
Gastric ulcer prophylaxis	Male gender	Acute leukemia
Age > 65y (and especially >85y)	Gastrostomy or jejunostomy tube	Antibiotic prophylaxis
Hospitalization	Surgical procedures	Allogeneic (vs. autologous HSCT)
Intra-abdominal surgery	Antibiotic prophylaxis	Umbilical cord blood HSCT
Enteral feeding	Extended hospital stays	Total body irradiation
Chronic kidney disease	Hypogammaglobulinemia	Myeloablative conditioning
Cardiac disease/hypertension	Age > 55	Chemotherapy
Inflammatory bowel disease	History of CDI prior to SOT	Neutropenia
Chemotherapy	ATG	Mucositis
Cirrhosis	Re-transplantation	Reciprocal association with GVHD
Housing in an extended care facility	Deceased donor transplantation	CMV reactivation
Outpatient care	Organ-type affects risk Liver > heart > kidney	HSV or VZV reactivation
Emergency room/urgent care visits		Pre-colonization with toxigenic strain
Outpatient procedure/outpatient surgery center		VRE colonization
Doctor's office		Bacterial infection
Hemodialysis		Mechanical ventilation
Depression/antidepressant use		Hypogammaglobulinemia

from searches of the PUBMED and EMBASE databases, reported a pooled prevalence of toxigenic *C. difficile* colonization to be 8.1% overall and 10% in North America [37]. When hospitalized, these asymptomatic carriers are an important reservoir for transmission of *C. difficile* to previously uncolonized individuals admitted to healthcare settings [44, 45]. It is important to note that ≥50% of individuals colonized with toxigenic *C. difficile* will never develop active CDI [10, 40, 46]. Asymptomatic carriage of *C. difficile* is reported to occur in 19–84% of infants under the age of 6 months [47–50]. Colonized infants may represent a reservoir of *C. difficile* in the community. *C. difficile* has also been found to be a colonizer in community environments such as parks, stores (fresh produce and raw meat) and fast food restaurants, and in homes; all of these sites are possible reservoirs of the organism [36].

The effect of transient asymptomatic carriage of, or colonization with, toxigenic *C. difficile* on the development of CDI in SOT recipients has not been well delineated. Studies comparing the incidence of CDI in SOT recipients colonized with toxigenic *C. difficile* to those not colonized are needed.

Studies evaluating the carriage of *C. difficile* in symptomatic patients admitted to HSCT units for treatment of a hematologic malignancy or for HSCT and who on admission were screened for the presence of toxigenic *C. difficile* have reported carriage rates of 14–26% [51–54]. Those patients admitted for planned HSCT and found to be colonized with *C. difficile* have been shown to develop significantly higher rates of active CDI than those screened and found not to be colonized (56–87% active CDI in colonized individuals compared to 12–17% active CDI in those not colonized) [51, 55, 56]. There have been several studies evaluating the effect of colonization with *C. difficile* on subsequent CDI in hematologic malignancy and transplant recipients. In a large observational cohort performed at Memorial Sloan Kettering Medical Center, once weekly fecal specimens were collected on adult patients admitted for HSCT during their transplant hospitalization [57]. These specimens were tested for the presence of toxigenic *C. difficile*. Of those patients who screened positive for colonization with *C. difficile*, 17% went on to develop clinical CDI compared to 12.2% in the control population. In a prospective cohort study of patients admitted to a hematology/BMT unit for a hospital stay of more than one night, 9.3% were found to be colonized with a toxigenic strain of *C. difficile* [53]. While 3.4% of patients who were not found colonized with *C. difficile* developed CDI during their hospital stay, 13.3% of those colonized with toxigenic *C. difficile* on admission developed CDI which on multivariate analysis gave an adjusted odds ratio for development of symptomatic CDI of 11.6 (95%CI, 2.9–47.1; $P = 0.001$). Similarly, in a prospective cohort study in pediatric oncology and HSCT recipients, baseline stool samples collected within 3 days of admission found colonization with a toxigenic strain of *C. difficile* in 32.8% of patients [58]. Colonization was predictive of CDI with a clinical isolate sharing identical or closely related pulsed-field gel electrophoresis patterns to the baseline isolate (OR = 28.6%; 95% CI, 6.58–124.39; $P < 0.001$).

Hospital preventative strategies to reduce the incidence of hospital onset CDI (HO-CDI) include antimicrobial stewardship programs, contact precautions

Table 2 Strategies to prevent HO-CDI [45, 59–61]

Strategies to prevent hospital onset CDI (HO-CDI)
Antimicrobial stewardship programs
Presence and enforcement of contact precautions/isolation for infected patients
Decontamination of infected areas with sporicidal cleaners (bleach) with oversight on cleaning
Promotion of hand hygiene

(isolation and use of gowns and gloves) for infected patients, sporicidal decontamination of areas/equipment exposed to infected patients, promotion of hand hygiene (hand washing with soap and water along with mechanical drying), and identification and isolation of asymptomatic carriers [45, 59–61] (Table 2). *C. difficile* spores can remain in the hospital environment on floors, commodes, toilets, bedpans, and high-touch surfaces (call bells, over-bed tables, phones) for months or years surviving heat, desiccation, and most disinfectants [62, 63]. Daily cleaning in the rooms of CDI cases with attention to high-touch surfaces regardless of the presence of visible soiling is recommended using a hypochlorite-based disinfectant in sporicidal concentrations (at least 1000 ppm, but ideally 5000 ppm, available chlorine) after mechanical cleaning with a germicide to reduce spore colonization and organic material [10, 64].

Pathogenesis of *C. difficile*

In the environment and colon lumen, C. difficile may remain in its spore form for months. In a healthy state, the gastrointestinal microbiota provides resistance to colonization with pathogenic organisms through various mechanisms including: prevention of adherence; metabolism of primary bile acids to secondary bile acids and short chain fatty acids (SCFAs), as well as metabolism of bilirubin, heterocyclic amines, and polysaccharides; production of inhibitory substances; depletion of nutrients; and stimulation of the host's immune system [65, 66] (Fig. 1). Exposure to antimicrobials or other therapies that alter the gut microbiome (i.e., cancer chemotherapy, proton pump inhibitors, or H2 blockers) result in increased concentrations of primary bile acids along with pressure of the bacterial metabolic pathway to secondary bile acids over SCFAs in the colonic environment thereby directly leading to germination of *C. difficile* spores and overgrowth of vegetative toxin producing organisms leading to active CDI [10, 67, 68]. Any antimicrobial agent may predispose to CDI; however, third−/fourth-generation cephalosporins, fluoroquinolones, carbapenems, and clindamycin have been found to be the highest risk antibiotics and are frequently implicated in cases of active CDI.

Toxigenic strains of *C. difficil*e present in the colonic environment in the vegetative form produce one or both toxins A and B. These toxins trigger a cytotoxic response inducing host cell death, neutrophilic infiltration with profound inflammation, and cytokine release [10, 68] (Figs 2 and 3). The resulting inflammatory response leads to the development of pseudomembranes which are characteristic

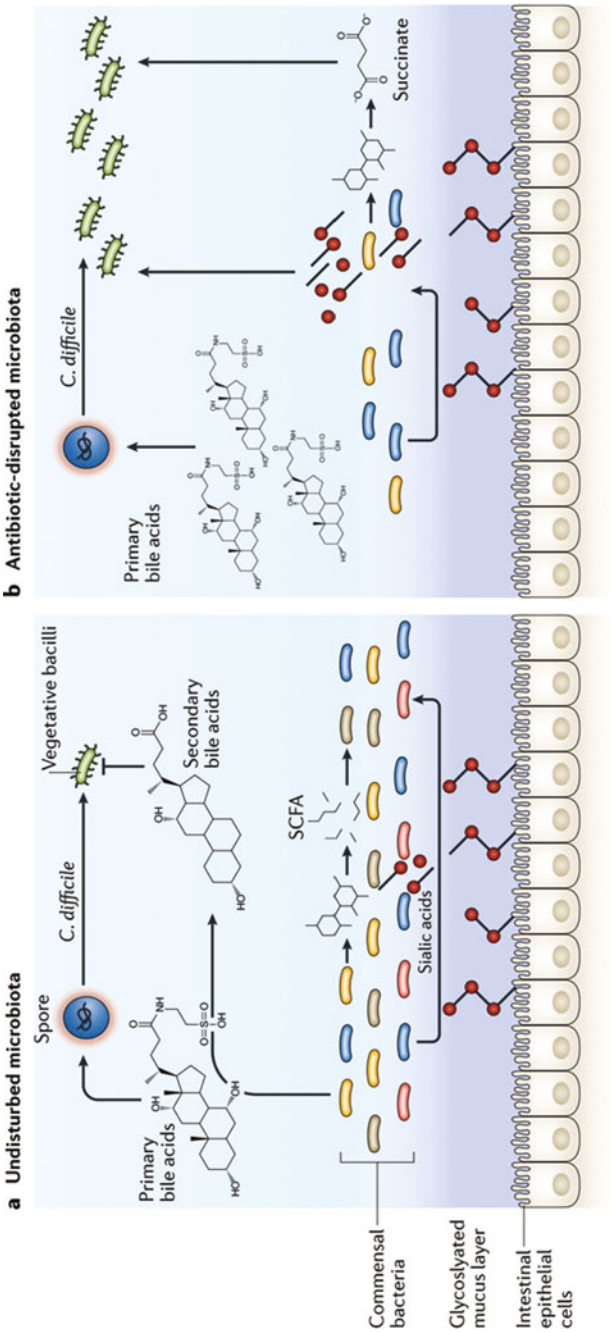


Fig. 1 Microbiota-mediated defenses against *C. difficile*: effect of antibiotics on colonic microbiome. (**a**) The intact microbiota converts primary bile acids into secondary bile acids, several derivatives of which inhibit the growth of *Clostridium difficile* through detergent-induced toxicity to vegetative bacilli. Commensal bacteria that express sialidases cleave sugars that are attached to epithelial cells and release sialic acid into the intestinal lumen. Fermenting commensal bacterial species convert carbohydrates into short-chain fatty acids (SCFAs), such as succinate. Bystander commensal bacterial populations can consume these metabolites as energy sources. (**b**)| Antibiotic-mediated disruption of the microbiota depletes primary bile acid converters, which enables *C. difficile* sporulation and growth. Antibiotics can also deplete competing sialic acid and succinate consumers, liberating an energy source for *C. difficile*. (Nat Rev. Microbiol 2016; 14: 609–620. (Copyright obtained and sent to publisher))

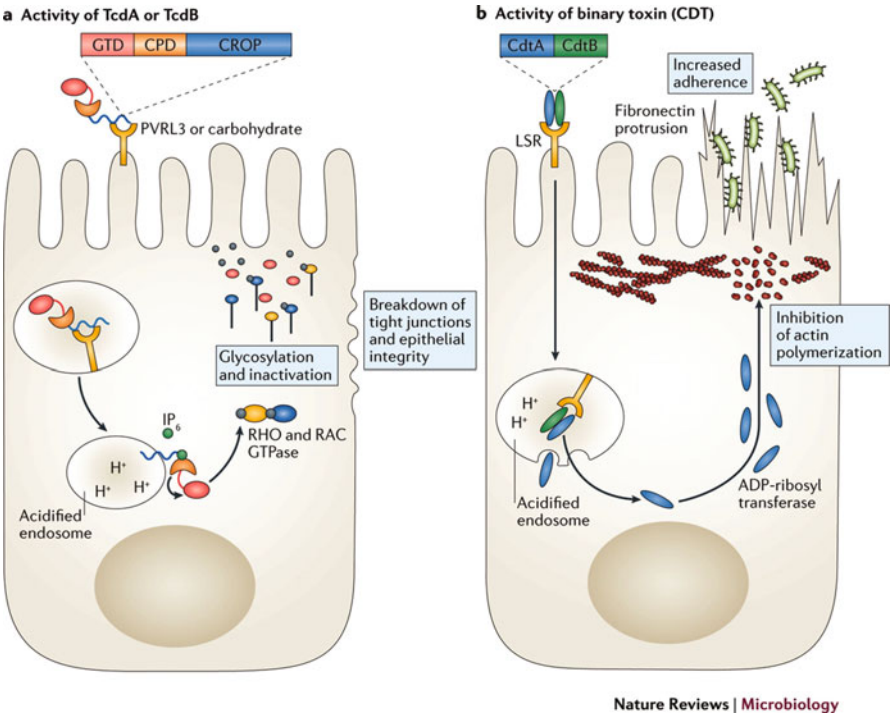


Fig. 2 Effect of *C. difficile* toxins on colonic epithelial cells. (**a**) The combined repetitive oligopeptide repeat (CROP) domain of TcdA binds to carbohydrates on the apical surface of epithelial cells, whereas TcdB binds to poliovirus receptor-like 3 (PVRL3) expressed on colonic epithelial cells. Toxin is internalized and acidification of the endosome enables the CROP domain to embed into the endosomal membrane and the subsequent transport of the cysteine protease domain (CPD) and the glucosyl transferase domain (GTD) into the cytosol. Inositol hexakisphosphate (IP_6) activates the cysteine protease to cleave and release the toxin glycotransferase. Glycosylation and thereby inactivation of RHO or RAC GTPases ultimately results in the breakdown of tight junctions and epithelial integrity. (**b**)| Binary toxin (also known as *Clostridium difficile* transferase (CDT)) binds to the lipolysis-stimulated lipoprotein receptor (LSR) and is internalized. The CdtB subunit creates pores in the acidified endosome that enable the release of the CdtA subunit into the cytosol. The ADP-ribosyl transferase activity of CdtA inhibits actin polymerization near the cell membrane, enabling fibronectin microtubules to elongate and protrude through microvilli, which increases *C. difficile* adherence to the epithelium through type IV pili. (Nat Rev. Microbiol 2016; 14: 609–620. (Needs Copyright / requested but Nat wants to charge?))

visible yellow plaques throughout the colon. Some *C. difficile* strains also produce a third toxin, binary toxin or *C. difficile* transferase (CDT), an adenosine diphosphate (ADP)-ribosyltransferase that causes actin cytoskeletal disruption and may lead to a more fulminant clinical course [68–70]. CDT is typically produced by the major, hypervirulent strains and has been associated with more severe disease, an elevated risk of recurrence, and higher mortality [71–73]. Because of blunted inflammatory responses and/or the presence of neutropenia in immunocompromised patients, the finding of pseudomembranes is less commonly seen in those being treated with

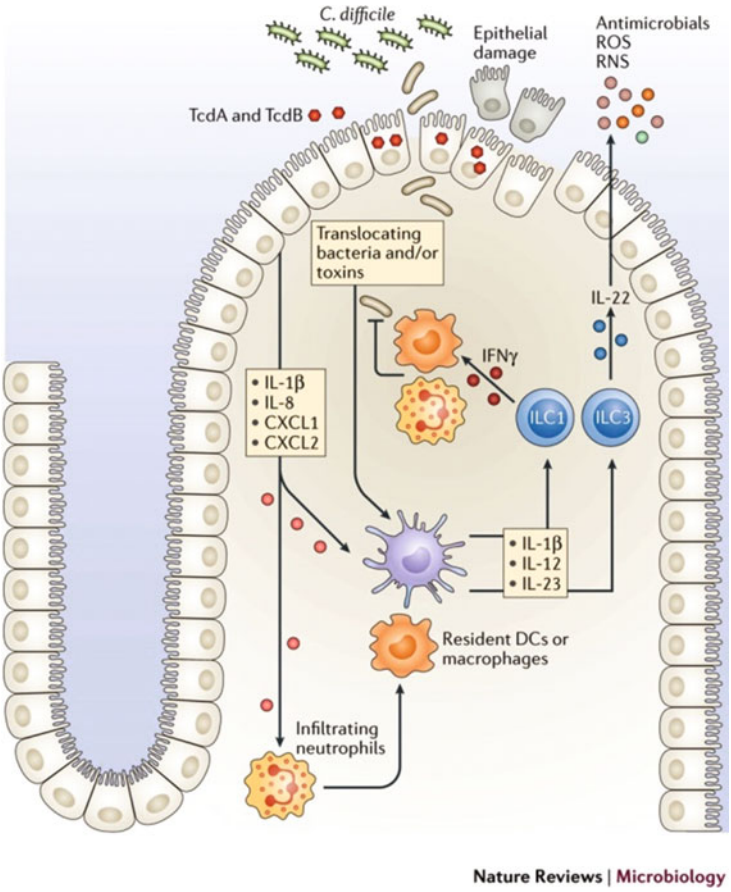


Fig. 3 Innate immune-mediated defenses against *C. difficile.* The acute host response to *Clostridium difficile* is initiated by toxin-mediated damage, loss of epithelial integrity, and the detection of translocating bacteria. Intestinal epithelial cells and resident innate immune cells secrete pro-inflammatory chemokines (such as chemokine C-X-C motif ligand 1 (CXCL1), CXCL2, and interleukin-8 (IL-8)) and pro-inflammatory cytokines (such as IL-1β, IL-12, and IL-23), which leads to the recruitment of neutrophils and the activation of innate lymphoid cells (ILCs). IL-12 signaling drives the expression of interferon-γ (IFNγ), whereas IL-1β and IL-23 signaling induces the production of IL-22. The effector cytokines IFNγ and IL-22 induce defense mechanisms such as increased phagocytic activity of macrophages and neutrophils, and the production of antimicrobial peptides and enzymes that synthesize reactive oxygen species (ROS) and reactive nitrogen species (RNS). These defense mechanisms limit bacterial dissemination, attenuate toxin activity, and repair epithelial damage. DCs, dendritic cells. (Nat Rev. Microbiol 2016; 14: 609–20. (Needs Copyright / requested but Nat wants to charge?))

immunosuppressive agents and those who previously received myeloablative regimens [74].

CDI Differential Diagnosis and Clinical Impact on Transplant Outcomes (SOT and HSCT).

As in the non-immunocompromised host population, detection of *C. difficile* or its toxins should not be assumed to be the cause of diarrhea. Etiologies of diarrhea other than *C. difficile* in transplant recipients include: drug-induced diarrhea (i.e., concomitant antibiotics, laxative use, mycophenolate) or other common etiologies of infectious diarrhea such as adenovirus, cytomegalovirus, or norovirus. In both SOT and HSCT populations, the highest rates of CDI occur within the first 30 days posttransplantation when patients have received intensive immunosuppression [28]. It should be remembered, however, that CDI in neutropenic patients may lack classic findings of inflammation such as pseudomembranes and leukocytosis [29, 33, 75].

Consequences of active CDI reported in transplant populations include dehydration, inanition, increased rates of other infections (such as cytomegalovirus (CMV) and pneumonia), longer hospital stays resulting in higher healthcare costs, organ allograft dysfunction in the SOT population, and the development of GVHD in the HSCT population [7, 17, 32, 33, 75–78]. The GVHD association may be restricted to late complications of HSCT in select hosts; two studies that included recipients of T-cell depleted grafts reported no association between CDI and GVHD, especially if CDI occurred early post-HSCT [47, 77, 79]. The pathogenesis of GI tract GVHD may result from the initiating effects of gut epithelial damage from high-intensity conditioning chemotherapy compounded by alteration of gut microflora enhancing susceptibility to infection and initiating an immune attack from graft-derived T-lymphocytes driving a destructive cascade of inflammation. The need for colectomy is relatively low in HSCT patients compared to other inpatient populations with CDI, possibly the result of reduced inflammatory responses with neutropenia and immunosuppressive therapy [33]; however, CDI-related mortality is high independent of mortality associated with relapsed malignancy [75, 78]. In the SOT population, CDI is also an independent risk factor for mortality (OR: 2.48; 2.22–2.76) with reported mortality rates between 2.3% and 8.5% [32].

Diagnosis of *C. difficile* Infection: Clinical Evaluation and Available Emerging Diagnostics

The approach and use of diagnostic testing methods to identify a patient with active CDI continues to evolve as testing methods emerge with varying sensitivities and specificities for the presence of *C. difficile* toxins or of the ability of the organism to produce toxin. After the use of expensive, complex, and time-consuming toxigenic culture (TC) and cell cytotoxicity neutralization assays (CCNAs), newer enzyme-linked fluorescent immunoassays (EIA) and nucleic acid amplification tests (NAAT) provided less cumbersome and expedient testing methods [10, 11, 80, 81]. Current guidelines recommend using these newer tests in combination, simultaneously, or in an algorithmic approach to increase the specificity of testing. Also, NAAT testing alone may be used in the diagnosis of CDI but, in an effort to increase the specificity of testing, should be reserved for use in patient specimens screened to be liquid in consistency after other common etiologies of diarrhea (e.g., laxative use) have been ruled out [10, 11]. In the future, diagnostics may include the use of fecal biomarkers,

including lactoferrin, calprotectin, and cytokines IL8 and IL1B. These modalities have been used in the diagnosis of inflammatory bowel diseases, but at the present time, there is insufficient evidence to recommend their use for CDI diagnosis in clinical practice [10].

In general, diagnostic testing for *C. difficile* has targeted the presence of the organism, the production of the toxin, or both. Toxigenic culture (TC) detects the presence of the *C. difficile* organisms in culture using selective media followed by identification using biochemical testing or MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) and is currently considered a reference method to which other testing modalities are compared. With culture however, a separate testing modality is needed to detect the presence of toxin. The cell cytotoxicity neutralization assay (CCNA) detects toxin directly in the stool by observing for cytopathic effect on an appropriate cell line (i.e.,: human fibroblasts) after a stool filtrate has been applied to the cell culture. The filtrate is also inoculated in parallel to a second set of tissue culture cells to which *C. difficile*-specific antitoxin has been added to verify that the cytopathic effect is caused by a *C. difficile*-associated toxin and not by some other toxic component or viral infection that may be harbored in the specimen. Duplicate testing with neutralizing antibodies to *C. difficile* or cross-reactive *C. sordellii* is often performed to ensure that the cytopathic effect is due to *C. difficile* toxins. CCNA has historically been considered the gold standard in the diagnosis of active CDI; however, recently it has been questioned if this test's sensitivity of 65–90% is high enough to be appropriate for assay-comparison studies [82–86]. Further, sensitivities are approximately 75–85% when compared to TC, and the level of agreement can vary between labs [82]. These points are important to consider as these two tests are the measures to which most recently available diagnostic tests are compared. Both TC and CCNA are labor and time-intensive compared to more recent testing modalities.

Glutamate dehydrogenase (GDH) is a constitutive metabolic enzyme that converts glutamate to alpha-ketoglutarate and ammonia and is produced by both toxigenic and non-toxigenic *C. difficile* strains [80, 87]. GDH immunoassays have emerged as a screening test for the presence of *C. difficile* organisms in fecal samples obtained from individuals with a diarrheal illness. The sensitivity of GDH immunoassays are high (80–100%), and these assays have a favorable negative predictive value, a rapid turnaround time, and low cost compared to other testing modalities. These characteristics allow the GDH test be used widely as the screening step in the 2- or 3-step algorithmic approaches for the diagnosis of active CDI when it is followed by a toxin-specific test such as an enzyme-linked fluorescent immunoassay (EIA) or molecular testing.

Enzyme-linked fluorescent immunoassays (EIA) utilize monoclonal or polyclonal antibodies that are directed at *C. difficile* toxins [73, 82, 88–90]. These assays initially targeted toxin A alone but now target both toxin A and B as toxin A-negative/toxin B-positive strains have been documented. EIA testing is low cost and has a fast turnaround time. The sensitivity and specificity of EIA tests vary widely from approximately 40–100%. Heterogeneity exists between testing methods due to interlaboratory operator technical variance, geographic circulating strain

types, host antibody binding to toxin in the intestine, institutional practice, and the type of stool sample analyzed (i.e., fresh vs. frozen; cup vs. swab), as well as the gold standard used for assessment of the assay [82]. Like the GDH, EIAs are not recommended for diagnostic testing alone but may be considered to be part of an algorithmic approach [10, 11]. When compared to TC, low sensitivity of EIA immunoassays has been reported in diagnosing active CDI in immunocompromised patients who are on high-dose steroids, have leukocytopenia, and have non-severe CDI [91].

C. difficile nucleic acid amplification testing (NAAT) targets include the tcdA, tcdB, and binary toxin genes. Some assays also test for a deletion at nucleotide 117 on tcdC as surrogate markers for presumptive identification of 027/NAP1/NI strains separately or in combination. Sensitivities of NAAT testing modalities for the presence of these genes which regulate toxin production range from 77% to 100% with specificities of 87–100% depending on the individual test. Negative predictive values exceed 95%; however, positive predictive values range from 42% to 98% [82]. NAAT testing does not test for the presence of *C. difficile* toxins but for the genes responsible for the production of toxin. Because of high sensitivity, NAAT testing modalities may be overly sensitive and may identify patients with *C. difficile* carriage tested for a diarrheal illness but who do not have active CDI.

Because none of these available tests can differentiate between colonization with *C. difficile* and active infection, when considering diagnostic testing, it should be understood that active CDI is a clinical diagnosis that is supported by laboratory testing confirming the presence of toxin or toxigenic C. *difficile* bacteria in the stool of a patient with clinically significant diarrhea [10, 92]. A case of active CDI requires the presence of diarrhea or evidence of megacolon or severe ileus as well as either a positive laboratory diagnostic test result or evidence of pseudomembranes demonstrated by endoscopy or histopathology. Testing relies on submission of and should only be performed on liquid stool specimens from an individual who has had more than three unformed bowel movements within a 24-h period. It is important that other etiologies of diarrhea including other causes of infectious diarrhea, use of drugs which cause diarrhea (i.e.,: laxatives, cancer chemotherapy, or mycophenolate), the presence of inflammatory bowel disease, or use of enteral tube feedings be considered prior to submitting specimens for testing.

Encouraged by recommendations in guidelines, institutions are increasingly developing initiatives to enhance the specificity of diagnostics such as rejecting nonliquid/soft samples (those not taking the shape of the container) and screening for laxative use within the 48 h prior to submission. In a study evaluating appropriateness of testing for CDI, it was found that 19% of patients being tested for *C. difficile* had received laxatives within 48 h prior to sample submission and 36% did not have clinically significant diarrhea [92].

Repeat testing for *C. difficile* within 7 days during the same episode of diarrhea or as test-of-cure is not recommended. Repeat *C. difficile* testing in patients initially negative but having a clinical syndrome consistent with CDI and a worsening status or patients with resolution of symptoms following treatment with suspected

recurrence can be considered. In these instances, toxin-directed assays should be considered over NAAT testing as patients have been shown to continue to carry toxigenic *C. difficile* long after treatment of infection. Additionally, bowel habits may not return to normal for a long period of time after an episode of CDI, and diarrhea alone may not necessarily represent a recurrence of infection.

Approach to Patient Management Including Emerging Treatment Modalities and Options for Prophylaxis, Prevention, and Prognosis (See Fig. 4)

The most recent *C. difficile* guidelines [10, 11, 81, 93, 94] recommend oral vancomycin 125 mg administered orally at 6-h intervals for 10–14 days or fidaxomicin 200 mg by mouth twice daily for 10 days for the treatment of primary CDI. In addition to specific anti-CDI-related therapy, interventions recommended include the reduction of modifiable risk factors (avoiding unnecessary antibiotics, avoiding exposure to fluoroquinolones, clindamycin, and cephalosporins when antibiotic therapy is required) and avoidance of unnecessary proton pump inhibitors when possible.

Therapeutic guideline recommendation changes were based on the findings of clinical trials not focused on transplant recipients which compared vancomycin and metronidazole and reported an advantage to the use of oral vancomycin [10]. Zar et al. compared vancomycin and metronidazole for the treatment in *C. difficile* in a 2007 study which found that metronidazole and vancomycin were equally effective for treatment of mild CDI; however, vancomycin was superior for treating patients with severe CDAD; in this study, 17% of the patient population had underlying malignancy [95]. In a retrospective analysis, Parmer et al. reviewed the incidence and severity of *C. difficile* infection in patients with hematologic malignancies, including those undergoing HSCT and reported the response to metronidazole was 53.7%, vancomycin 50%, and in combination 38.5%, thus representing no significant difference between treatment modalities [96]. In a randomized, controlled clinical trial comparing vancomycin, metronidazole, and tolevamer for the treatment of CDI, clinical success (defined as resolution of diarrhea and absence of severe abdominal discomfort) at end of therapy in patients receiving metronidazole or vancomycin was 72.7% and 81.1%, respectively while in those who received tolevamer, it was 44.2% [89]. Clinical success in patients with severe CDI who received metronidazole was 66.3% compared to 78.5% in those who received vancomycin ($P = 0.059$). In a retrospective, propensity-matched, cohort study, Stevens et al. evaluated the risk of recurrent CDI and 30-day mortality in 10,137 veterans stratified by mild to moderate and severe CDI [97]. In this cohort, 8% received immunosuppression and 11% received chemotherapy within 30 days prior to the CDI episode. They found no difference in risk of CDI recurrence between patients treated with vancomycin versus those treated with metronidazole. However, among patients in the severe CDI cohort, those treated with vancomycin were less likely to die.

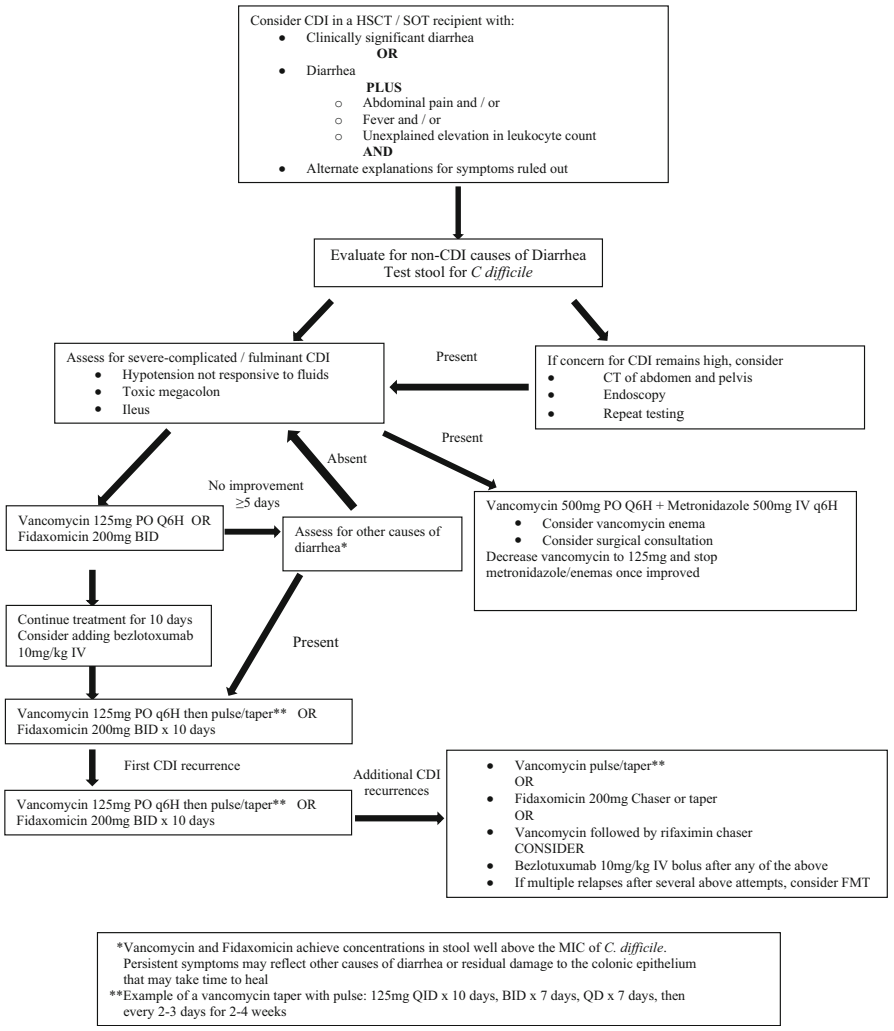


Fig. 4 Approach to diagnosis and management of C. difficile in transplant patients including emerging treatment modalities and approach to recurrences

Fidaxomicin is a macrocyclic narrow-spectrum antibiotic with activity, as is also true with vancomycin, only against vegetative (actively replicating) *C. difficile* organisms. Unlike vancomycin however, fidaxomicin also prevents new sporulation by the vegetative *C. difficile* organisms present and halts continued toxin production. Because of its narrow spectrum of antimicrobial activity, the potential advantage of fidaxomicin is sparing of healthy gut flora allowing for more rapid reconstitution of a normal gut microbiome thereby maintaining the colonization barrier to pathogenic organisms, including *C. difficile,* as well as enhancing metabolism of toxic bile salts on presentation to the colon microbiome. In two large, randomized, double-blinded

fidaxomicin registry clinical trials, fidaxomicin was found to be non-inferior in the initial clinical cure of CDI but was found to be significantly better in sustained clinical cure when compared to vancomycin [98, 99]. Additionally, in a sub-analysis, patients receiving concomitant antibiotics for other infections had a higher cure rate (90% vs. 73%, $p = 0.03$) and 12.3% lower incidence of recurrence when treated with fidaxomicin compared to those treated with vancomycin [100]. In those patients who were enrolled with the presence of a concomitant ongoing cancer, cure was more likely with fidaxomicin than vancomycin (odds ratio [OR] 2.0; $P = 0.065$), recurrence was less likely (OR = 0.37; $P = 0.018$), and sustained response more frequent (OR = 2.56; $P = 0.003$) [101]. Time to resolution of diarrhea was longer in patients with cancer treated with vancomycin compared to those who received fidaxomicin (123 vs. 74 h). No difference in clinical cure was found in a subsequent clinical trial where HSCT patients received either oral vancomycin or fidaxomicin therapy (28% vs. 27%), with no difference observed in global cure (66% vs. 67%), treatment failure, or recurrent disease (7% vs. 5%) in the first 100 days following allogeneic transplant [102]. However, patients who developed acute graft-versus-host disease had a longer mean time to onset in the fidaxomicin group when compared to the vancomycin-treated group (32 vs. 21 days; $p = 0.003$).

Lack of ability to mount an immune response to *C. difficile* toxins has been shown to be a risk factor for recurrence [103, 104]. Bezlotoxumab is a monoclonal antibody against *C. difficile* toxin B. A single infusion of 10 mg/kg bezlotoxumab administered during or at the end of therapy for an initial or first recurrence of CDI was reported to significantly lower the incidence of CDI recurrence compared with placebo (16–17% vs. 26–28%) [105]. A post-hoc analysis which evaluated the 20% of patients enrolled in the study who were immunocompromised showed a recurrence rate of 36% in patients receiving placebo versus 19% in the bezlotoxumab infusion arm [106]. Like fidaxomicin, no controlled trials have been reported comparing standard of care therapy with or without the addition of bezlotoxumab specifically in transplant patient populations.

FMT has increasing support in the literature for patients in the treatment of recurrent CDI, but concerns of safety in immunocompromised hosts, especially when donor samples are considered, exist. A recent report of two immunocompromised adults who received investigational FMT and developed invasive infections with one death due to infection with an extended-spectrum-beta-lactamase producing *Escherichia coli* highlights these concerns [107–109]. FMT has been reported as beneficial in the treatment of both acute and recurrent CDI. Available data on the use of FMT in the ICH population is limited to case reports and small series showing benefit with treatment and a decrease in recurrence with no reported adverse events, but no randomized control trials in these populations have been undertaken [110–112]. One group has reported safe use in 94 SOT patients requiring support for recurrent (78%) or severe/fulminant *C. difficile* (22%), with no FMT-associated bacteremia reported when fresh stool from a patient-directed donor or frozen stool bank samples were used [111]. Other groups have examined the utility of stool collected prior to antibiotic exposure/transplant for use as FMT post-allogeneic HSCT in an effort to restore normal gastrointestinal microbiota [113]. Taur et al.

report reconstitution of the gut microbiome of stem cell transplants through the use of auto-FMT samples collected pretransplant that when administered after engraftment increased bacterial diversity compared to patients not undergoing auto-FMT and may theoretically decrease the risk of *C. difficile* development posttransplant [113]. One small series reported use of FMT in an effort to decolonize carriage of MDR bacteria in after HSCT in an effort to restore the microbiome and potentially impact the development of GVHD [114].

Prophylaxis

There is likely a limited role for *C. difficile* prophylaxis in a general inpatient population who screen positive for *C. difficile* carriage without evidence of ongoing CDI as even exposure to vancomycin used as an agent of prophylaxis may provoke microbiome changes and result in CDI in the absence of other risk factors, including broad-spectrum antibiotic therapy. Several studies evaluating the role of prophylaxis against *C. difficile* in patients undergoing HSCT and SOT have been reported [66, 115–118].

In a large, double-blinded, randomized, placebo controlled trial comparing the use of fidaxomicin to placebo as prophylaxis against CDI in individuals undergoing autologous or allogeneic HSCT who were receiving fluoroquinolone prophylaxis for neutropenia, no difference was found between the two groups in the intention to treat analysis based on a composite outcome of "prophylaxis failure" which included confirmed CDI, receipt of CDI-effective medications (for any indication), and/or missing CDI assessment (for any reason, including death) [56, 115]. However, in the prespecified sensitivity analysis, which counted only confirmed CDI (by toxin immunoassay or nucleic acid amplification test) as failure, cases of confirmed CDAD were found to be significantly lower in those who received fidaxomicin versus the placebo recipients (4.3% vs. 10%; $p = 0.0014$). Ganetsky et al. reported no cases of CDI in patients that received vancomycin prophylaxis (0/90, 0%) compared to 11/55 (20%) who did not receive vancomycin prophylaxis during admission for allogeneic stem cell transplantation. As well, there was no association with higher risk of GVHD, relapse, or survival in these groups [119]. A decreased incidence of recurrent CDI was reported by Morrisette et al. in a retrospective cohort of adult hematologic malignancy patients, 60% of whom had undergone HSCT (of which 77% had received allogeneic HSCT) who received oral vancomycin prophylaxis after a case of primary CDI until the cessation of broad-spectrum antibiotic therapy when compared to patients who did not receive prophylaxis (5% vs. 35%; 0.016). No differences were seen in subsequent VRE infection or 60-day mortality [118].

While prophylaxis may play a role in specific circumstances such as in individuals with known CDI risk factors presenting for transplantation and found to be colonized with toxigenic *C. difficile* or in those patients with a history of recent/recurrent CDI), data are lacking. However, in individuals colonized with *C. difficile* having the expectation of neutropenia and treatment with antibiotic prophylaxis or in

ICH patients colonized with *C. difficile* and given broad-spectrum antibiotics for treatment of febrile neutropenia or other serious infections, it may be considered.

Probiotics

Approaches to support the colonization barrier that the microbiome provides to prevent *C. difficile* colonization and resultant infection have been studied in the form of probiotics, prebiotics, and FMT. The safety in immunocompromised hosts, however, has been questioned. Probiotics are "live microorganisms" which when administered in adequate amounts confer health benefit on the host [120, 121]. Timely use of probiotics in hospitalized adults has variably been shown to prevent CDI in a systematic review with meta-regression analysis [122]. Of 6,261 inpatients in 19 randomized controlled trials who received broad-spectrum antibiotics and various probiotics (*Saccharomyces boulardii*, Lactobacillus spp., Bifidobacterium spp., and Streptococcus spp., alone or in combination), a 0.42 [95% CI 0.3–0.57] relative risk of CDI was reported in the probiotic group. This review highlighted the variability in probiotics used and how cases of CDI were defined depending on study-specific diagnostic criteria.

Finally, a preparation of non-toxigenic *C. difficile* spore administered orally in an effort to compete with toxigenic *C. difficile* present in the colon was successful in reducing recurrent CDI when taken by patients with clinical cure at the end of a course of active treatment for CDI [123].

Conclusions

Clostridioides difficile infection in both SOT and HSCT patients is common and can incur high healthcare associated costs; treatment approaches are evolving. Past studies have focused on treatment of the *C. difficile* bacteria and its spores, but current studies focus on improved immune response to toxin and on the importance of restoration of healthy gut microbiota. Treatment approaches in immunocompromised hosts, including in those after HSCT or SOT, are similar to the general inpatient population for acute infection. However, depending upon the presence and type of ongoing risk factors, the use of tapering doses of antimicrobial therapy or secondary prophylaxis may need to be considered, as this group is at higher risk of developing not only primary but also recurrent CDI with worse associated outcomes when compared to immunocompetent hosts. More work is needed to develop evidence-based methods to prevent transmission and acquisition of *C. difficile* spores, to develop preventive strategies (such as the appropriate use of anti-CDI prophylaxis, bezlotoxumab, prebiotics, FMT, and non-toxigenic C. difficile spores), to prevent the development of CDI in pretransplant patients already colonized with *C. difficile*, and finally, in determining how best to reconstitute the intestinal microbiome after an expected peri-transplantation insult from broad-spectrum antibiotics, chemotherapy, and immunosuppressive agents.

Key Points

- The incidence of CDI is higher in transplant recipients than in the general hospitalized patient population and contributes to significant morbidity and mortality.
- Transplant recipients receiving more aggressive immunosuppression (hematopoietic, heart, lung, and in setting of enhanced immunosuppression due to graft failure/rejection) have a greater incidence of CDI compared to those receiving lower levels of immunosuppression (kidney and liver transplant).
- In addition to the common risk factors associated with the development of CDI, transplant recipients have additional risk factors including exposure to chemotherapy, immunosuppression, radiation therapy, and increased need for antiinfective therapy in the treatment and prevention of concomitant infections.
- Hospital-based strategies to prevent C. difficile in all patient populations, including transplant recipients, include an active antimicrobial stewardship program, use of contact precautions, effective cleaning of contaminated surfaces, and hand hygiene. Specificity of diagnostic testing may be increased through algorithmic approach or restriction programs (laboratory stewardship).
- While treatment algorithms for transplant recipients with CDI do not vary from the general population, risk of relapsing CDI is higher in immunocompromised hosts and use of prolonged courses of vancomycin and fidaxomicin, including tapers, as well as bezlotoxumab and FMT may need to be considered with initial episode or early recurrences depending on clinical context.

References

1. Haematopoietic Stem Cell Transplantation HSCtx. https://www.who.int/transplantation/hsctx/en/: WHO; 2019.
2. hrsa; 2019. Available from: https://bloodcell.transplant.hrsa.gov/about/general_faqs/index.html
3. WHO | GKT1 Activity and Practices: Who.int; 2019. Available from: https://www.who.int/transplantation/gkt/statistics/en/.
4. Transplant trends - UNOS: UNOS; 2019. Available from: www.unos.org/data/transplant-trends/
5. Miller BA, Chen LF, Sexton DJ, Anderson DJ. Comparison of the burdens of hospital-onset, healthcare facility-associated Clostridium difficile infection and of healthcare-associated infection due to methicillin-resistant Staphylococcus aureus in community hospitals. Infect Control Hosp Epidemiol. 2011;32(4):387–90.
6. Zilberberg MD, Shorr AF, Kollef MH. Growth and geographic variation in hospitalizations with resistant infections, United States, 2000-2005. Emerg Infect Dis. 2008;14(11):1756–8.
7. Guh AY, Adkins SH, Li Q, Bulens SN, Farley MM, Smith Z, et al. Risk factors for community-associated Clostridium difficile infection in adults: a case-control study. Open Forum Infect Dis. 2017;4(4):ofx171.
8. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of Clostridium difficile infection in the United States. N Engl J Med. 2015;372(9):825–34.
9. Davies KA, Longshaw CM, Davis GL, Bouza E, Barbut F, Barna Z, et al. Underdiagnosis of Clostridium difficile across Europe: the European, multicentre, prospective, biannual, point-

prevalence study of Clostridium difficile infection in hospitalised patients with diarrhoea (EUCLID). Lancet Infect Dis. 2014;14(12):1208–19.
10. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):e1–e48.
11. Ooijevaar RE, van Beurden YH, Terveer EM, Goorhuis A, Bauer MP, Keller JJ, et al. Update of treatment algorithms for Clostridium difficile infection. Clin Microbiol Infect. 2018;24(5):452–62.
12. Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, et al. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N Engl J Med. 2005;353(23):2442–9.
13. Olsen MA, Young-Xu Y, Stwalley D, Kelly CP, Gerding DN, Saeed MJ, et al. The burden of clostridium difficile infection: estimates of the incidence of CDI from U.S. Administrative databases. BMC Infect Dis. 2016;16:177.
14. Paudel S, Zacharioudakis IM, Zervou FN, Ziakas PD, Mylonakis E. Prevalence of Clostridium difficile infection among solid organ transplant recipients: a meta-analysis of published studies. PLoS One. 2015;10(4):e0124483.
15. Boutros M, Al-Shaibi M, Chan G, Cantarovich M, Rahme E, Paraskevas S, et al. Clostridium difficile colitis: increasing incidence, risk factors, and outcomes in solid organ transplant recipients. Transplantation. 2012;93(10):1051–7.
16. Riddle DJ, Dubberke ER. Clostridium difficile infection in solid organ transplant recipients. Curr Opin Organ Transplant. 2008;13(6):592–600.
17. Cusini A, Beguelin C, Stampf S, Boggian K, Garzoni C, Koller M, et al. Clostridium difficile infection is associated with graft loss in solid organ transplant recipients. Am J Transplant. 2018;18(7):1745–54.
18. Tsapepas DS, Martin ST, Miao J, Shah SA, Scheffert J, Fester K, et al. Clostridium difficile infection, a descriptive analysis of solid organ transplant recipients at a single center. Diagn Microbiol Infect Dis. 2015;81(4):299–304.
19. Dubberke ER, Reske KA, Olsen MA, Bommarito K, Cleveland AA, Silveira FP, et al. Epidemiology and outcomes of Clostridium difficile infection in allogeneic hematopoietic cell and lung transplant recipients. Transpl Infect Dis. 2018;20(2):e12855.
20. Zerey M, Paton BL, Lincourt AE, Gersin KS, Kercher KW, Heniford BT. The burden of Clostridium difficile in surgical patients in the United States. Surg Infect. 2007;8(6):557–66.
21. Dubberke ER, Butler AM, Yokoe DS, Mayer J, Hota B, Mangino JE, et al. Multicenter study of Clostridium difficile infection rates from 2000 to 2006. Infect Control Hosp Epidemiol. 2010;31(10):1030–7.
22. Dallal RM, Harbrecht BG, Boujoukas AJ, Sirio CA, Farkas LM, Lee KK, et al. Fulminant Clostridium difficile: an underappreciated and increasing cause of death and complications. Ann Surg. 2002;235(3):363–72.
23. Schluger A, Rosenblatt R, Knotts R, Verna EC, Pereira MR. Clostridioides difficile infection and recurrence among 2622 solid organ transplant recipients. Transpl Infect Dis. 2019;21(6):e13184.
24. Ananthakrishnan AN. Clostridium difficile infection: epidemiology, risk factors and management. Nat Rev Gastroenterol Hepatol. 2011;8(1):17–26.
25. Medication Guide fda.gov 2019. Available from: www.fda.gov/Drugs/DrugSafety/ucm290510.html.
26. Guddati AK, Kumar G, Ahmed S, Ali M, Kumar N, Hari P, et al. Incidence and outcomes of Clostridium difficile-associated disease in hematopoietic cell transplant recipients. Int J Hematol. 2014;99(6):758–65.
27. Misch EA, Safdar N. Clostridioides difficile infection in the stem cell transplant and hematologic malignancy population. Infect Dis Clin N Am. 2019;33(2):447–66.

28. Ilett EE, Helleberg M, Reekie J, Murray DD, Wulff SM, Khurana MP, et al. Incidence rates and risk factors of Clostridioides difficile infection in solid organ and hematopoietic stem cell transplant recipients. Open Forum Infect Dis. 2019;6(4):ofz086.
29. Zacharioudakis IM, Ziakas PD, Mylonakis E. Clostridium difficile infection in the hematopoietic unit: a meta-analysis of published studies. Biol Blood Marrow Transplant. 2014;20(10):1650–4.
30. Mullane KM, Dubberke ER, Practice AICo. Management of Clostridioides (formerly Clostridium) difficile infection (CDI) in solid organ transplant recipients: guidelines from the American Society of Transplantation Community of Practice. Clin Transpl. 2019;33(9):e13564.
31. Sullivan T, Weinberg A, Rana M, Patel G, Huprikar S. The epidemiology and clinical features of Clostridium difficile infection in liver transplant recipients. Transplantation. 2016;100(9):1939–43.
32. Pant C, Anderson MP, O'Connor JA, Marshall CM, Deshpande A, Sferra TJ. Association of Clostridium difficile infection with outcomes of hospitalized solid organ transplant recipients: results from the 2009 nationwide inpatient sample database. Transpl Infect Dis. 2012;14(5):540–7.
33. Alonso CD, Treadway SB, Hanna DB, Huff CA, Neofytos D, Carroll KC, et al. Epidemiology and outcomes of Clostridium difficile infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2012;54(8):1053–63.
34. Li GJ, Trac J, Husain S, Famure O, Li Y, Kim SJ. Incidence, risk factors, and outcomes of Clostridium difficile infections in kidney transplant recipients. Transplantation. 2018;102(9):1576–81.
35. Dubberke ER, Reske KA, Olsen MA, Bommarito KM, Seiler S, Silveira FP, et al. Risk for Clostridium difficile infection after allogeneic hematopoietic cell transplant remains elevated in the postengraftment period. Transplant Direct. 2017;3(4):e145.
36. Alam MJ, Walk ST, Endres BT, Basseres E, Khaleduzzaman M, Amadio J, et al. Community environmental contamination of toxigenic Clostridium difficile. Open Forum Infect Dis. 2017;4(1):ofx018.
37. Zacharioudakis IM, Zervou FN, Pliakos EE, Ziakas PD, Mylonakis E. Colonization with toxinogenic C. difficile upon hospital admission, and risk of infection: a systematic review and meta-analysis. Am J Gastroenterol. 2015;110(3):381–90. quiz 91
38. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, et al. Host and pathogen factors for Clostridium difficile infection and colonization. N Engl J Med. 2011;365(18):1693–703.
39. Vaughn JL, Balada-Llasat JM, Lamprecht M, Huang Y, Anghelina M, El Boghdadly Z, et al. Detection of toxigenic Clostridium difficile colonization in patients admitted to the hospital for chemotherapy or haematopoietic cell transplantation. J Med Microbiol. 2018;67(7):976–81.
40. Terveer EM, Crobach MJ, Sanders IM, Vos MC, Verduin CM, Kuijper EJ. Detection of Clostridium difficile in feces of asymptomatic patients admitted to the hospital. J Clin Microbiol. 2017;55(2):403–11.
41. Alasmari F, Seiler SM, Hink T, Burnham CA, Dubberke ER. Prevalence and risk factors for asymptomatic Clostridium difficile carriage. Clin Infect Dis. 2014;59(2):216–22.
42. Curry SR, Muto CA, Schlackman JL, Pasculle AW, Shutt KA, Marsh JW, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in Clostridium difficile transmission. Clin Infect Dis. 2013;57(8):1094–102.
43. Guerrero DM, Becker JC, Eckstein EC, Kundrapu S, Deshpande A, Sethi AK, et al. Asymptomatic carriage of toxigenic Clostridium difficile by hospitalized patients. J Hosp Infect. 2013;85(2):155–8.
44. Lanzas C, Dubberke ER, Lu Z, Reske KA, Grohn YT. Epidemiological model for Clostridium difficile transmission in healthcare settings. Infect Control Hosp Epidemiol. 2011; 32(6):553–61.
45. Longtin Y, Paquet-Bolduc B, Gilca R, Garenc C, Fortin E, Longtin J, et al. Effect of detecting and isolating Clostridium difficile carriers at hospital admission on the incidence of c difficile infections: a quasi-experimental controlled study. JAMA Intern Med. 2016;176(6):796–804.

46. Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of Clostridium difficile by hospitalized patients: evidence for colonized new admissions as a source of infection. J Infect Dis. 1992;166(3):561–7.
47. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by Clostridium difficile and decreased risk of subsequent diarrhoea. Lancet. 1998;351(9103):633–6.
48. Rousseau C, Poilane I, De Pontual L, Maherault AC, Le Monnier A, Collignon A. Clostridium difficile carriage in healthy infants in the community: a potential reservoir for pathogenic strains. Clin Infect Dis. 2012;55(9):1209–15.
49. Tullus K, Aronsson B, Marcus S, Mollby R. Intestinal colonization with Clostridium difficile in infants up to 18 months of age. Eur J Clin Microbiol Infect Dis. 1989;8(5):390–3.
50. Matsuki S, Ozaki E, Shozu M, Inoue M, Shimizu S, Yamaguchi N, et al. Colonization by Clostridium difficile of neonates in a hospital, and infants and children in three day-care facilities of Kanazawa. Japan Int Microbiol. 2005;8(1):43–8.
51. Jain T, Croswell C, Urday-Cornejo V, Awali R, Cutright J, Salimnia H, et al. Clostridium difficile colonization in hematopoietic stem cell transplant recipients: a prospective study of the epidemiology and outcomes involving toxigenic and nontoxigenic strains. Biol Blood Marrow Transplant. 2016;22(1):157–63.
52. Kamboj M, Sheahan A, Sun J, Taur Y, Robilotti E, Babady E, et al. Transmission of Clostridium difficile during hospitalization for allogeneic stem cell transplant. Infect Control Hosp Epidemiol. 2016;37(1):8–15.
53. Cannon CM, Musuuza JS, Barker AK, Duster M, Juckett MB, Pop-Vicas AE, et al. Risk of Clostridium difficile infection in hematology-oncology patients colonized with toxigenic C. difficile. Infect Control Hosp Epidemiol. 2017;38(6):718–20.
54. Cho J, Seville MT, Khanna S, Pardi DS, Sampathkumar P, Kashyap PC. Screening for Clostridium difficile colonization on admission to a hematopoietic stem cell transplant unit may reduce hospital-acquired C difficile infection. Am J Infect Control. 2018;46(4):459–61.
55. Bruminhent J, Wang ZX, Hu C, Wagner J, Sunday R, Bobik B, et al. Clostridium difficile colonization and disease in patients undergoing hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20(9):1329–34.
56. Mullane KM, Winston DJ, Nooka A, Morris MI, Stiff P, Dugan MJ, et al. A randomized, placebo-controlled trial of fidaxomicin for prophylaxis of Clostridium difficile-associated diarrhea in adults undergoing hematopoietic stem cell transplantation. Clin Infect Dis. 2019;68(2):196–203.
57. Kinnebrew MA, Lee YJ, Jenq RR, Lipuma L, Littmann ER, Gobourne A, et al. Early Clostridium difficile infection during allogeneic hematopoietic stem cell transplantation. PLoS One. 2014;9(3):e90158.
58. Al-Rawahi GN, Al-Najjar A, McDonald R, Deyell RJ, Golding GR, Brant R, et al. Pediatric oncology and stem cell transplant patients with healthcare-associated Clostridium difficile infection were already colonized on admission. Pediatr Blood Cancer. 2019;66(5):e27604.
59. Grigoras CA, Zervou FN, Zacharioudakis IM, Siettos CI, Mylonakis E. Isolation of C. difficile carriers alone and as part of a bundle approach for the prevention of Clostridium difficile Infection (CDI): a mathematical model based on clinical study data. PLoS One. 2016;11(6):e0156577.
60. Feazel LM, Malhotra A, Perencevich EN, Kaboli P, Diekema DJ, Schweizer ML. Effect of antibiotic stewardship programmes on Clostridium difficile incidence: a systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(7):1748–54.
61. Loo VG. Environmental interventions to control Clostridium difficile. Infect Dis Clin N Am. 2015;29(1):83–91.
62. McFarland LV, Mulligan ME, Kwok RY, Stamm WE. Nosocomial acquisition of Clostridium difficile infection. N Engl J Med. 1989;320(4):204–10.
63. Kim KH, Fekety R, Batts DH, Brown D, Cudmore M, Silva J Jr, et al. Isolation of Clostridium difficile from the environment and contacts of patients with antibiotic-associated colitis. J Infect Dis. 1981;143(1):42–50.

64. Mayfield JL, Leet T, Miller J, Mundy LM. Environmental control to reduce transmission of Clostridium difficile. Clin Infect Dis. 2000;31(4):995–1000.
65. Rosa R, Donskey CJ, Munoz-Price LS. The intersection between colonization resistance, antimicrobial stewardship, and Clostridium difficile. Curr Infect Dis Rep. 2018;20(8):27.
66. Zeng H, Umar S, Rust B, Lazarova D, Bordonaro M. Secondary bile acids and short chain fatty acids in the colon: a focus on colonic microbiome, cell proliferation, inflammation, and cancer. Int J Mol Sci. 2019;20(5):1214.
67. Gil F, Lagos-Moraga S, Calderon-Romero P, Pizarro-Guajardo M, Paredes-Sabja D. Updates on Clostridium difficile spore biology. Anaerobe. 2017;45:3–9.
68. Abt MC, McKenney PT, Pamer EG. Clostridium difficile colitis: pathogenesis and host defence. Nat Rev Microbiol. 2016;14(10):609–20.
69. Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, et al. Clostridium difficile infection in Europe: a hospital-based survey. Lancet. 2011;377 (9759):63–73.
70. Cowardin CA, Buonomo EL, Saleh MM, Wilson MG, Burgess SL, Kuehne SA, et al. The binary toxin CDT enhances Clostridium difficile virulence by suppressing protective colonic eosinophilia. Nat Microbiol. 2016;1(8):16108.
71. Barbut F, Gariazzo B, Bonne L, Lalande V, Burghoffer B, Luiuz R, et al. Clinical features of Clostridium difficile-associated infections and molecular characterization of strains: results of a retrospective study, 2000-2004. Infect Control Hosp Epidemiol. 2007;28(2):131–9.
72. Stewart DB, Berg A, Hegarty J. Predicting recurrence of C. difficile colitis using bacterial virulence factors: binary toxin is the key. J Gastrointest Surg. 2013;17(1):118–24; discussion p 24-5
73. Bacci S, Molbak K, Kjeldsen MK, Olsen KE. Binary toxin and death after Clostridium difficile infection. Emerg Infect Dis. 2011;17(6):976–82.
74. Nomura K, Fujimoto Y, Yamashita M, Morimoto Y, Ohshiro M, Sato K, et al. Absence of pseudomembranes in Clostridium difficile-associated diarrhea in patients using immunosuppression agents. Scand J Gastroenterol. 2009;44(1):74–8.
75. Trifilio SM, Pi J, Mehta J. Changing epidemiology of Clostridium difficile-associated disease during stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(3):405–9.
76. Dubberke ER, Burdette SD, Practice ASTIDCo. Clostridium difficile infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):42–9.
77. Luo R, Weinberg JM, Barlam TF. The impact of clostridium difficile infection on future outcomes of solid organ transplant recipients. Infect Control Hosp Epidemiol. 2018;39(5):563–70.
78. Chakrabarti S, Lees A, Jones SG, Milligan DW. Clostridium difficile infection in allogeneic stem cell transplant recipients is associated with severe graft-versus-host disease and non-relapse mortality. Bone Marrow Transplant. 2000;26(8):871–6.
79. Kong LY, Eyre DW, Corbeil J, Raymond F, Walker AS, Wilcox MH, et al. Clostridium difficile: investigating transmission patterns between infected and colonized patients using whole genome sequencing. Clin Infect Dis. 2019;68(2):204–9.
80. Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. Clin Microbiol Infect. 2016;22(Suppl 4):S63–81.
81. Sartelli M, Di Bella S, McFarland LV, Khanna S, Furuya-Kanamori L, Abuzeid N, et al. 2019 update of the WSES guidelines for management of Clostridioides (Clostridium) difficile infection in surgical patients. World J Emerg Surg. 2019;14:8.
82. Burnham CA, Carroll KC. Diagnosis of Clostridium difficile infection: an ongoing conundrum for clinicians and for clinical laboratories. Clin Microbiol Rev. 2013;26(3):604–30.
83. Pechine S, Collignon A. Immune responses induced by Clostridium difficile. Anaerobe. 2016;41:68–78.
84. Deshpande A, Pant C, Pasupuleti V, Rolston DD, Jain A, Deshpande N, et al. Association between proton pump inhibitor therapy and Clostridium difficile infection in a meta-analysis. Clin Gastroenterol Hepatol. 2012;10(3):225–33.

85. Pepin J, Saheb N, Coulombe MA, Alary ME, Corriveau MP, Authier S, et al. Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile-associated diarrhea: a cohort study during an epidemic in Quebec. Clin Infect Dis. 2005;41(9):1254–60.
86. Mishiro T, Oka K, Kuroki Y, Takahashi M, Tatsumi K, Saitoh T, et al. Oral microbiome alterations of healthy volunteers with proton pump inhibitor. J Gastroenterol Hepatol. 2018;33(5):1059–66.
87. Fradrich C, Beer LA, Gerhard R. Reactive oxygen species as additional determinants for cytotoxicity of Clostridium difficile toxins A and B. Toxins (Basel). 2016;8(1):25.
88. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013;108(4):478–98. quiz 99
89. Johnson S, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, et al. Vancomycin, metronidazole, or tolevamer for Clostridium difficile infection: results from two multinational, randomized, controlled trials. Clin Infect Dis. 2014;59(3):345–54.
90. Sartelli M, Malangoni MA, Abu-Zidan FM, Griffiths EA, Di Bella S, McFarland LV, et al. WSES guidelines for management of Clostridium difficile infection in surgical patients. World J Emerg Surg. 2015;10:38.
91. Erb S, Frei R, Stranden AM, Dangel M, Tschudin-Sutter S, Widmer AF. Low sensitivity of fecal toxin A/B enzyme immunoassay for diagnosis of Clostridium difficile infection in immunocompromised patients. Clin Microbiol Infect. 2015;21(11):998e9–998e15.
92. Dubberke ER, Han Z, Bobo L, Hink T, Lawrence B, Copper S, et al. Impact of clinical symptoms on interpretation of diagnostic assays for Clostridium difficile infections. J Clin Microbiol. 2011;49(8):2887–93.
93. Trubiano JA, Cheng AC, Korman TM, Roder C, Campbell A, May ML, et al. Australasian Society of Infectious Diseases updated guidelines for the management of Clostridium difficile infection in adults and children in Australia and New Zealand. Intern Med J. 2016;46(4):479–93.
94. Feher C, Mensa J. A comparison of current guidelines of five international societies on Clostridium difficile infection management. Infect Dis Ther. 2016;5(3):207–30.
95. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. Clin Infect Dis. 2007;45(3):302–7.
96. Parmar SR, Bhatt V, Yang J, Zhang Q, Schuster M. A retrospective review of metronidazole and vancomycin in the management of Clostridium difficile infection in patients with hematologic malignancies. J Oncol Pharm Pract. 2014;20(3):172–82.
97. Stevens VW, Nelson RE, Schwab-Daugherty EM, Khader K, Jones MM, Brown KA, et al. Comparative effectiveness of vancomycin and metronidazole for the prevention of recurrence and death in patients with Clostridium difficile infection. JAMA Intern Med. 2017;177(4):546–53.
98. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, et al. Fidaxomicin versus vancomycin for Clostridium difficile infection. N Engl J Med. 2011;364(5):422–31.
99. Cornely OA, Crook DW, Esposito R, Poirier A, Somero MS, Weiss K, et al. Fidaxomicin versus vancomycin for infection with Clostridium difficile in Europe, Canada, and the USA: a double-blind, non-inferiority, randomised controlled trial. Lancet Infect Dis. 2012;12(4):281–9.
100. Mullane KM, Miller MA, Weiss K, Lentnek A, Golan Y, Sears PS, et al. Efficacy of fidaxomicin versus vancomycin as therapy for Clostridium difficile infection in individuals taking concomitant antibiotics for other concurrent infections. Clin Infect Dis. 2011;53(5):440–7.
101. Cornely OA, Miller MA, Fantin B, Mullane K, Kean Y, Gorbach S. Resolution of Clostridium difficile-associated diarrhea in patients with cancer treated with fidaxomicin or vancomycin. J Clin Oncol. 2013;31(19):2493–9.
102. Prohaska L, Mahmoudjafari Z, Shune L, Singh A, Lin T, Abhyankar S, et al. Retrospective evaluation of fidaxomicin versus oral vancomycin for treatment of Clostridium difficile infections in allogeneic stem cell transplant. Hematol Oncol Stem Cell Ther. 2018;11(4):233–40.

103. Leav BA, Blair B, Leney M, Knauber M, Reilly C, Lowy I, et al. Serum anti-toxin B antibody correlates with protection from recurrent Clostridium difficile infection (CDI). Vaccine. 2010;28(4):965–9.
104. Kelly CP, Poxton IR, Shen J, Wilcox MH, Gerding DN, Zhao X, et al. Effect of endogenous clostridioides difficile toxin antibodies on recurrence of C. difficile infection. Clin Infect Dis. 2019;71(1):81–6.
105. Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, et al. Bezlotoxumab for prevention of recurrent clostridium difficile infection. N Engl J Med. 2017;376(4):305–17.
106. Gerding DN, Kelly CP, Rahav G, Lee C, Dubberke ER, Kumar PN, et al. Bezlotoxumab for prevention of recurrent clostridium difficile infection in patients at increased risk for recurrence. Clin Infect Dis. 2018;67(5):649–56.
107. Information Pertaining to Additional Safety Protections Regarding Use of Fecal Microbiota for Transplantation – Screening and Testing of Stool Donors for Multi-drug Resistant Organisms: FDA; 2019 [updated 6/18/2019. 6/18/2019]: Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/information-pertaining-additional-safety-protections-regarding-use-fecal-microbiota-transplantation.
108. Important Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Reactions Due to Transmission of Multi-Drug Resistant Organisms: FDA; 2019. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse.
109. Use of Fecal Microbiota for Transplantation (FMT) to Treat Clostridium difficile Infection Not Responsive to Standard Therapies 2019 [cited 2019 11/22]. Available from: https://www.fda.gov/news-events/fda-meetings-conferences-and-workshops/use-fecal-microbiota-transplantation-fmt-treat-clostridium-difficile-infection-not-responsive.
110. Lin SC, Alonso CD, Moss AC. Fecal microbiota transplantation for recurrent Clostridium difficile infection in patients with solid organ transplants: an institutional experience and review of the literature. Transpl Infect Dis. 2018;20(6):e12967.
111. Cheng YW, Phelps E, Ganapini V, Khan N, Ouyang F, Xu H, et al. Fecal microbiota transplantation for the treatment of recurrent and severe Clostridium difficile infection in solid organ transplant recipients: a multicenter experience. Am J Transplant. 2019;19(2):501–11.
112. Wardill HR, Secombe KR, Bryant RV, Hazenberg MD, Costello SP. Adjunctive fecal microbiota transplantation in supportive oncology: emerging indications and considerations in immunocompromised patients. EBioMedicine. 2019;44:730–40.
113. Taur Y, Coyte K, Schluter J, Robilotti E, Figueroa C, Gjonbalaj M, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci Transl Med. 2018;10(460):1–18. https://doi.org/10.1126/scitranslmed.aap9489. PMID: 30257956.
114. Battipaglia G, Malard F, Rubio MT, Ruggeri A, Mamez AC, Brissot E, et al. Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematologic malignancies carrying multidrug-resistance bacteria. Haematologica. 2019;104(8):1682–8.
115. Mullane KM, Adachi J, Dubberke E, Alexander B, Broyde N, Sears P. Outcomes of deflect-1: a multicenter, blinded, randomized clinical trial of Fidaxomicin (FDX) vs. Placebo (PLC) for prophylaxis of Clostridium Difficile Associated Diarrhea (CDAD) in subjects undergoing Hematopoietic Stem Cell Transplantation (HSCT). Biol Blood Marrow Tr. 2016;22(3):S171.
116. Splinter LE, Kerstenetzky L, Jorgenson MR, Descourouez JL, Leverson GE, Saddler CM, et al. Vancomycin prophylaxis for prevention of clostridium difficile infection recurrence in renal transplant patients. Ann Pharmacother. 2018;52(2):113–9.
117. Ganetsky A, Han JH, Hughes ME, Babushok DV, Frey NV, Gill SI, et al. Oral vancomycin is highly effective in preventing clostridium difficile infection in allogeneic hematopoietic stem cell transplant recipients. Blood. 2016;128(22):2225.
118. Morrisette T, Van Matre AG, Miller MA, Mueller SW, Bajrovic V, Abidi MZ, et al. Oral vancomycin prophylaxis as secondary prevention against clostridioides difficile infection in

the hematopoietic stem cell transplantation and hematologic malignancy population. Biol Blood Marrow Transplant. 2019;25(10):2091–7.
119. Ganetsky A, Han JH, Hughes ME, Babushok DV, Frey NV, Gill SI, et al. Oral vancomycin prophylaxis is highly effective in preventing clostridium difficile infection in allogeneic hematopoietic cell transplant recipients. Clin Infect Dis. 2019;68(12):2003–9.
120. Mills JP, Rao K, Young VB. Probiotics for prevention of Clostridium difficile infection. Curr Opin Gastroenterol. 2018;34(1):3–10.
121. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506–14.
122. Shen NT, Maw A, Tmanova LL, Pino A, Ancy K, Crawford CV, et al. Timely use of probiotics in hospitalized adults prevents clostridium difficile infection: a systematic review with meta-regression analysis. Gastroenterology. 2017;152(8):1889–900. e9
123. Gerding DN, Meyer T, Lee C, Cohen SH, Murthy UK, Poirier A, et al. Administration of spores of nontoxigenic Clostridium difficile strain M3 for prevention of recurrent C. difficile infection: a randomized clinical trial. JAMA. 2015;313(17):1719–27.
124. Ramos A, Ortiz J, Asensio A, Martinez-Ruiz R, Munez E, Cantero M, et al. Risk factors for clostridium difficile diarrhea in patients with solid organ transplantation. Prog Transplant. 2016;26(3):231–7.
125. Ochfeld E, Balmert LC, Patel SJ, Muller WJ, Kociolek LK. Risk factors for Clostridioides (Clostridium) difficile infection following solid organ transplantation in children. Transpl Infect Dis. 2019;21(5):e13149.

Part III

Viral Infections Introduction

Cytomegalovirus in Solid Organ Transplant Recipients: Prevention, Diagnosis, and Treatment

24

Camille Nelson Kotton

Contents

Disease Epidemiology 547
Clinical Impact in Solid Organ Transplant 549
Disease Clinical Evaluation and Diagnostics 550
CMV Serology 551
CMV Nucleic Acid Testing 552
CMV Culture and Pathology 553
CMV Immune Assays 554
Management (Both Clinical Management and Preventative Care) 555
Prevention 555
Vaccines 559
Immunoglobulins 559
Treatment of Active Infection 560
Treatment Resistant and Refractory Disease 562
Key Points, Research Needs, and Future Trends 565
References 566

Disease Epidemiology

In the era before effective methods of prevention, up to 75% of organ transplant recipients developed CMV. In the modern era, that number is greatly reduced. Rates of infection after transplant depend primarily on pretransplant serology. CMV seronegative organ transplant recipients (R−) who receive an organ from seropositive donors (D+) are at highest risk for developing CMV infection after transplant. Seropositive recipients (R+) are generally at moderate risk for developing CMV infection. R+ recipients who receive organs from seropositive donors (D+)

C. N. Kotton (✉)
Transplant and Immunocompromised Host Infectious Diseases, Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
e-mail: ckotton@mgh.harvard.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_24

are at higher risk for developing CMV infection compared with those who have seronegative donors, suggesting that superinfection with different CMV genotypes is clinically important [2]. Pairings where both the donor and recipient are seronegative are at very low risk for developing CMV, unless there was an error in the testing, or they were in the "window period" prior to serologic conversion, or the recipient developed CMV from transfusion of blood products at the time of transplant.

Given that the rates of CMV infection after solid organ transplant (SOT) are highest in nonimmune recipients from previously infected donors (D+/R−) and that this accounts for the majority of disease after transplant, overall rates of disease correlate with the general rates of CMV seropositivity in the community. This varies throughout the world. For example, about 50% of adults in the United States [3] and Canada are CMV seropositive, while rates are over 90% in Brazil, South Africa, India, and Italy.

In the United States, the national Scientific Registry of Transplant Recipients (SRTR) data reveal some interesting trends (Table 1) [4–9]. Among living donor adult kidney and liver transplants, there are lower rates of CMV seropositivity among both donors and recipients, which could be potentially explained by the lower average age of living kidney transplant recipients. Table 1 provides details across most organ transplants. The lower rates of CMV exposure after living donor transplantations may contribute to the generally improved outcomes after such transplants, compared with deceased donor transplantation. Furthermore, there were almost twice as many D−/R− pairs among the living donor kidney and liver transplant recipients when compared with deceased donor pairs, rendering a substantial cohort of recipients not at risk for CMV from their organ transplant. There were augmented rates of high-risk D+/R− pairs among pancreas, heart, and lung transplant recipients (25–28%) compared with deceased donor kidney and liver

Table 1 Donor-recipient cytomegalovirus serology pairs, 2016–2018. Donor serology is reported on the OPTN Donor Registration Form and recipient serology on the OPTN Transplant Recipient Registration Form. Data for intestinal recipients was not reported. Most do not add up to 100% due to lack of complete reporting or unknown status. (Data published annually in the *American Journal of Transplantation* supplement 1 (January) [5–9])

		2016–2018			
		Recipient negative		Recipient positive	
Adult	Donor type	Donor negative	Donor positive	Donor negative	Donor positive
Kidney	Deceased	13.6	17.9	25.8	41.2
	Living	25.4	17.7	21.2	32.8
Pancreas	Deceased	19.9	25.9	21.9	31.3
Liver	Deceased	14.5	22.2	22.0	39.8
	Living	32.2	14.6	25.1	26.7
Heart	Deceased	17.5	24.9	20.2	35.0
Lung >=12yo	Deceased	18.1	27.7	19.8	33.0

recipients (17–22%), which might explain some of the higher rates of CMV seen after those transplants.

Pediatric organ transplant recipients are far more likely to be naïve to CMV, and to acquire CMV at the time of transplant, especially for those undergoing organ transplants from adults. Infants may have maternal antibodies and/or passive transfer of antibodies from transfusion of blood products and require careful examination to confirm the precise risk status (i.e., to avoid interpreting maternal antibodies as a true positive, suggesting protection from infection) [10]. Nucleic acid testing for CMV from urine and oropharynx can be quite useful in confirming positive CMV status; cellular immune assays, such as CMV-specific CD4+ T cells, are sensitive and specific in children >18 months but less useful in younger children [11].

For pediatric kidney transplant recipients from deceased donors in 2016–2018, 20.3% were D+R+, 15.2% were D−R+, 39.0% were D+/R−, and 23.4% were D−/R− [6]. Similar to adult transplants, pediatric recipients and donors undergoing living donor kidney transplants were less likely to be CMV seropositive before transplantation; 19.4% were D+R+, 8.3% were D−R+, 28.9% were D+/R−, and 39.5% were D−/R− [6].

CMV infections tend to occur in settings of more intense immunosuppression. This is especially true in R+, who otherwise are at lower risk for infection after transplant given their prior immunity. Use of potent immunosuppressive agents at the time of transplant, such as antithymocyte globulin or alemtuzumab, often conveys a higher risk of CMV. At present, the majority of kidney transplants get induction at the time of transplant; almost 75% of kidney transplant recipients and just over 50% of heart and lung transplant recipients underwent immunosuppression induction with T-cell depleting agents in 2016 [12–14]. The choice of maintenance immunosuppression may impact the risk of developing CMV; for example, a reduced risk of CMV in those on sirolimus and everolimus has been seen across numerous studies [15–17]. As we are more able to effectively suppress the immune system and achieve lower rates of organ rejection with better long-term outcomes, there is often a trade-off resulting in higher rates of infection, including CMV, necessitating greater recognition of the risk and better optimization of prevention methods.

Another significant risk factor for CMV infection is low lymphocyte count, which can be a readily available marker of risk [18, 19]. There is some variation among reported risk factors for developing CMV. One recent report in a largely seropositive population of kidney transplant patients in Brazil who underwent induction with antithymocyte globulin found that reduced kidney graft function, but not acute rejection, was a risk factor for CMV infection [18].

Clinical Impact in Solid Organ Transplant

CMV infection has a significant impact on organ transplant outcomes. The "direct effects of CMV range from asymptomatic to viral syndrome to end organ disease (colitis, pneumonitis, hepatitis, retinitis). In the modern era, much CMV replication

is detected early as either asymptomatic but with a positive CMV DNA PCR test or as viral syndrome; in prior times, this was often detected late, as end organ disease. The clinical presentation of CMV infection and disease can be protean, and clinicians should think of this possibility frequently, especially within the first year after transplant, or after augmentation of immunosuppression. CMV can certainly be "the great masquerader" (akin to syphilis in internal medicine) after organ transplant. The "indirect effects" of CMV infection also have a significant impact on transplant outcomes; these include higher rates of other infections (bacterial, viral, and fungal), posttransplant lymphoproliferative disorder, cardiovascular events, new-onset diabetes mellitus after transplantation, immunosenescence, acute rejection, and mortality, as well as higher rates of transplant-specific effects including chronic allograft nephropathy and/or allograft loss after renal transplant, hepatic artery thrombosis after liver transplant, allograft vasculopathy after cardiac transplant, and bronchiolitis obliterans after lung transplant [20].

The clinical impact of CMV manifests itself in every aspect of transplant care. Prevention of CMV results in significant cost, diagnostic testing, drug toxicity (especially leukopenia, which may necessitate use of white blood cell growth factors), logistical challenges, and antiviral drug resistance. Diagnosis of CMV is straightforward the majority of the time, but can involve additional invasive studies such as endoscopy and bronchoscopy, urgent ophthalmologic examinations, and additional radiographic studies. Treatment of CMV is usually with oral therapy, although more complex cases may require intravenous antiviral therapy, hospitalization, use of CMV immunoglobulin, reduction of immunosuppression, and management of leukopenia. Ganciclovir resistant CMV infection often requires more toxic therapies such as high dose ganciclovir or foscarnet, with a greater frequency of hospitalization (sometimes for weeks) and significant cost as well as morbidity and mortality.

Obviating the risk of CMV infection after SOT through approaches such as vaccination or pathogen inactivation would revolutionize the field of SOT and would likely result in simpler clinical care, fewer diagnostics, less frequent hospitalizations, and overall better outcomes. Thus far, such approaches remain elusive.

Disease Clinical Evaluation and Diagnostics

CMV infection is primarily seen in the first year after transplant, or with subsequent intensification of immunosuppression (i.e., treatment of rejection). It generally occurs within the first three to four months either after transplant (in the setting of preemptive therapy, without prophylaxis) or after the end of prophylaxis [21, 22]. CMV infection represents a continuum of disease, ranging from asymptomatic to viral infection to end organ disease. Like other members of the human herpes virus family, CMV enters a latent phase after primary infection. Humans thus harbor the virus during their lifetime. CMV infection and disease occur during either primary infection or reactivation of latent infection. For clarity between CMV infection

and disease, the following definitions may be useful and are consistent with the American Society of Transplantation and the CMV Drug Development Forum recommendations for use in clinical trials [23, 24]. CMV infection is when there is evidence of CMV replication regardless of symptoms (differs from latent CMV), "defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen" [24]. CMV disease is defined as evidence of CMV infection with attributable symptoms. CMV disease can be further categorized as a viral syndrome (i.e., fever, malaise, leukopenia, and/or thrombocytopenia), or as tissue invasive ("end organ") disease, such as with colitis, gastritis, pneumonitis, retinitis, nephritis, and other sites.

Having a low threshold to consider the diagnosis of CMV in the first year after SOT is generally prudent. Although guidelines suggest that monitoring for an infection does not need to occur while on prophylaxis [10], if there are clinical signs or symptoms of infection, it is prudent to check for CMV, which might occur with inappropriately low dosing of prophylaxis, development of antiviral resistance, excessive immunosuppression, or medication noncompliance. Initial steps include screening for symptoms of viral infection and end organ disease. Physical examination may demonstrate evidence of oral lesions, visual changes, pneumonitis, abdominal tenderness, and rarely neurologic changes. Knowledge of donor and recipient serology to better categorize the level of risk (high/moderate/low) is generally useful. CMV viral load testing of whole blood or plasma are generally readily available screening tests in most transplant centers. A chest x-ray may show findings of pneumonitis. Abdominal and or chest CT scans may be helping in demonstrating evidence of organ involvement. Clinicians should be aware that sometimes CMV viral load testing can be negative even in the setting of gastrointestinal disease, and even less commonly, with pulmonary disease after lung transplant. In that setting, clinicians may wish to pursue endoscopy or bronchoscopy for further diagnostics. Biopsy of intestine or lung may prove useful, especially with immunohistochemistry stains specific for CMV. Intestinal biopsy sent for CMV culture has been shown to enhance the diagnostic sensitivity of endoscopy [25].

Diagnostics primarily focus on the use of serology, viral load testing, CMV specific cellular immune assays, viral culture, and pathology along with immunohistochemistry. As per the CMV guidelines, the term DNAemia is preferred over viremia, to reflect the detection of CMV DNA in either blood or plasma (i.e., DNA is detected, whether actively replicating virus or not) [10]. To enhance accuracy, the phrases "viral load," "nucleic acid testing," or "quantitative nucleic acid amplification testing" are used instead of "polymerase chain reaction (PCR)."

CMV Serology

Serology for CMV is based primarily on IgG testing. IgM testing has limited diagnostic sensitivity in the setting of CMV and SOT, and its use is generally not recommended [10]. Care should be taken when using serology, as passive transfer of

antibody through transfusion of blood products could cause diagnostic confusion, especially given the high rates of seropositivity in the general population. For example, someone who was seronegative could be transiently seropositive for CMV IgG due to recent transfusion of blood products, although this would not convey significant protection against CMV infection, and they might be triaged to the wrong risk group for prophylaxis. A pretransfusion sample should be tested when feasible. Cell-mediated immunity assays may be useful in assisting in establishing true immunologic status in children and adults who may have passive antibody [26, 27]. Although CMV IgG was always perceived to be the gold standard for evidence of prior CMV exposure, levels may be diminished in the setting of hypogammaglobulinemia, immunosuppression, and other causes. Interestingly, the use of CMV specific cellular immune assays has demonstrated that some people are CMV seronegative but clearly have CMV specific cellular immune responses, suggesting that CMV IgG is less of a gold standard test than previously thought.

CMV Nucleic Acid Testing

CMV DNAemia assays are usually done by quantitative nucleic acid amplification testing. There are a variety of commercially developed and laboratory developed assays in active use. Although there has been an international standard for CMV quantitative nucleic acid amplification testing for over half a decade, which dramatically improved testing, there are still significant interassay variations noted [28, 29]. A critical analysis of both clinical specimens and international standard dilutions was disappointing; while 59% of all clinical sample results fell within $\pm$ 0.5 log10 International Units (IU)/mL of the mean viral load of each sample, the remaining 41% had more significant variation, preventing meaningful interassay result comparison. This means that comparison across different testing laboratories and diagnostic platforms should not be done, as it can be highly erroneous [10, 29]. Nuances of the assays impact results; those assays with amplicon sizes $\leq$86 base pairs had significantly higher quantitative CMV DNA results compared to tests with larger amplicon sizes ($\geq$105 base pair) ($P<0.001$) [29].

Whole blood generally has higher results by quantitative nucleic acid amplification testing when compared with plasma, sometimes by one log10 or more. While CMV DNA exists predominantly as small fragments in plasma [30], this seems to be less true for whole blood in which both intracellular and extracellular DNA are detected. Of note, persistent plasma DNAemia was demonstrated to be a better predictor of relapse at day 21 of antiviral treatment when compared with persistent whole blood DNAemia [31].

Thus, quantitative nucleic acid amplification testing provides significant and excessive interassay and interspecimen (whole blood versus plasma) variability, such that transplant programs should be prepared to pick one diagnostic type (plasma or whole blood) and testing platform for monitoring and trending an individual patient with CMV infection. Switching or alternating specimen types and diagnostics laboratories may result in clinical errors. A common example would be the suggestion that a

patient may have antiviral resistance because the viral load increased while on therapy when instead, it was a diagnostic error due to lack of comparability of the testing.

Genotyping for CMV with antiviral resistance is done at a limited number of laboratories internationally. Prior resistance testing required a positive CMV culture and phenotypic testing, which often involved months of laboratory work. Modern testing is done by genotyping and generally entails a rapid turnaround time, sometimes within a few days. The CMV population within an individual patient is often somewhat heterogeneous, and genotyping may not always reflect the extent of the genetic mutations. Knowledge of specific mutations allows for interpretation of likely antiviral resistance, facilitating changes in antiviral therapy to those agents that would be more likely to be efficacious (Table 2) [32–34].

CMV Culture and Pathology

CMV culture is performed less frequently in microbiology laboratories, but may be useful on certain specimens such as intestinal biopsies, where it may enhance diagnostic yield [25]. Sending certain specimens, such as saliva, sputum, bronchoscopy fluid, stool, urine, and others, may not allow determination between innocuous shedding of virus and infection/disease and would generally be discouraged.

Pathology may show the classic findings of CMV infection, with giant cells and "owl eyes" (used to describe the nuclei of cells infected by CMV). Characteristic findings include dark pink nuclear inclusion body surrounded by a clear halo. CMV also causes the development of cytoplasmic inclusions, seen as granules throughout the cytoplasm of the infected cells. Immunohistochemistry specifically for CMV can

Table 2 Antiviral agents with efficacy (at least *in vitro*) against CMV and other transplant related viruses. (Modified from Kotton [86])

Antiviral agent	CMV	HSV	Varicella	BK	Adenovirus	EBV
Commercially available						
Ganciclovir IV/ valganciclovir PO	x	x	x			
Acyclovir/valacyclovir/ famciclovir[a]	High dose +/−	x	x			
Foscarnet[b]	x	x	x			
Cidofovir[b]	x	x	x	Poor	+/− (IC50 dependent)	
Novel/investigational antiviral agents (SOT)						
Brincidofovir	x	x	x	x	x	x
Letermovir	x					
Maribavir	x					x

[a]acyclovir/valacyclovir/famciclovir for prophylaxis only
[b]foscarnet, cidofovir not usually used for prophylaxis

add to the diagnostic sensitivity and should be requested in settings where there is concern for CMV infection.

CMV Immune Assays

CMV specific cellular immune assays have blossomed over the past decade, initially as research assays, and now as commercially available tests. Conceptually, it would be excellent if they could predict the risk of CMV either at the time of SOT or at the end of prophylaxis, or of recurrent disease at the end of treatment. In general, they tend to be more predictive in R+, likely due to prior immunity. The more common approaches include detection of IFN-γ after stimulating whole blood or peripheral blood mononuclear cells with overlapping peptides or CMV specific antigens; other markers that have been used to correlate CMV-specific T-cell responses with the risk of CMV infection include IL-2, CD107, TNF-α, programmed death-1, and CD154. Several commercial assays are now available, usually based on interferon gamma release assays (QuantiFERON- CMV (QIAGEN), T-SPOT.CMV (Oxford Immunotec), T-Track CMV assay (Lophius Biosciences), and the CMV T Cell immunity panel (Viracor), which helps standardize the field, as much of the prior work had been with laboratory developed tests which were not necessarily reproducible across different centers.

Late CMV, which usually develops within several months after the end of prophylaxis, remains an Achilles heel of SOT. Current CMV diagnostics such as viral load and posttransplant serology do not allow accurate prediction of those at risk for infection. Focusing methods of prevention on those at higher need would likely decrease diagnostic hassles, medication related toxicities, and associated costs, as well as attenuate the associated indirect effects of CMV infection that negatively impact transplant outcomes. These CMV-specific cell mediated immunity assays may be able to help us focus on the at-risk population.

Numerous studies of CMV immunity assays exist, many of them single center; some of the more recent ones will be highlighted here. Work with D+/R+ patients who had induction with either anti-interleukin 2-receptor antibody (basilixumab) or rabbit antithymoglobulin found that those with late CMV had much lower rates of cellular mediated immunity as measured by ELISpot for the CMV antigens IE-1 and pp65 (T-spot.CMV, Oxford Immunotec) [35]. Using receiver operating characteristic curve analyses, they demonstrated that determining CMV-specific ELISpot thresholds (25 and 130 IFN-γ spots for IE-1 and pp65, respectively) allowed for classification of patients into high risk, intermediate risk, or low risk, with the antigen IE-1 being the strongest predictor. A multicenter ($n = 43$) trial was recently published, where kidney transplant recipients at the end of antiviral prophylaxis underwent CMV-specific ELISpot testing to enumerate interferon gamma (IFN-γ) binding spot-forming units after stimulation of cells with an overlapping peptide pool of CMV phosphoprotein 65 and immediate early-1 protein. The goal was to see whether the test results were predictive of subsequent CMV events. While the assay was predictive in moderate risk (R+) recipients, it

was not predictive in high risk recipients when used over the months after prophylaxis was stopped [36].

Two interventional trials using CMV immunity assays have been published recently. Kumar et al. used the Quantiferon-CMV assay to guide subsequent management at the end of treatment for CMV viremia necessitating antiviral therapy, demonstrating the efficacy of CMV-specific cellular mediated immunity testing to guide changes in CMV infection management [37]. A total of 27 patients with CMV viremia were treated until viral load negative (median initial viral load 10,900 International Units/mL). At end of treatment, a positive Quantiferon-CMV response was seen in 14/27 (51.9%), in whom antivirals were discontinued, and the remaining 13/27 (48.1%) had a negative CMV-CMI response, and were then given 2 months of secondary antiviral prophylaxis. In those with a positive Quantiferon-CMV and early discontinuation of antivirals, only a single patient experienced a low-level asymptomatic recurrence, while recurrence of infection was seen in 69.2% of Quantiferon-CMV negative patients despite more prolonged antivirals ($p = 0.001$). Although it is still not clear what to do with the Quantiferon-CMV negative patients, it would be helpful to know which population clinicians should focus their attention upon. Westall et al. did a randomized study of Quantiferon-CMV-directed versus fixed duration (5 months) valganciclovir prophylaxis to reduce late CMV after lung transplant, where those with a negative test underwent prolonged valganciclovir prophylaxis for up to 11 months after transplant [38]. The incidence of CMV infection in the lung allograft within 18 months transplant, the primary end-point, was significantly reduced in the Quantiferon-CMV directed arm compared with fixed duration (37% vs. 58%, $p = 0.03$), although CMV viral loads in blood, acute rejection, and chronic lung allograft dysfunction did not differ. Those with a positive Quantiferon-CMV had a lower incidence of CMV viral loads, as well as less severe CMV.

Management (Both Clinical Management and Preventative Care)

Prevention

Prevention of CMV is considered standard of care, as suggested by international guidelines [10, 39]. A recent survey of organ transplant programs in Europe found that almost all use prevention methods, either prophylaxis or preemptive therapy [40].

Prophylaxis involves administration of antiviral medications, often at a half of the treatment dose, for a set period of time, often 3–6 months after SOT [10]. The only readily available antiviral is ganciclovir, either in the form of the intravenous preparation ganciclovir or the oral prodrug, valganciclovir. For ease of administration, most programs use valganciclovir for prophylaxis (and treatment), unless there are concerns about malabsorption. Oral ganciclovir was the precursor to valganciclovir and is now off the market internationally; it was far less bioavailable (<10%) than valganciclovir (~60%). High dose valacyclovir has been used, although it is generally less well tolerated, and recent data suggests that when

compared with valacyclovir, valganciclovir prophylaxis was associated with a reduced risk of moderate-to-severe interstitial fibrosis and tubular atrophy in kidney transplant recipients [41]. A trial of letermovir versus valganciclovir for prophylaxis in 600 kidney transplant patients is underway (ClinicalTrials.gov NCT03443869). Maribavir was studied in the past for high risk D+/R− liver transplant recipients, but found to be inferior to oral ganciclovir [42]. Brincidofovir has not been studied in SOT for prophylaxis.

Duration of prophylaxis often ranges from 3 to 6 months, on the shorter end for those who are R+ and/or otherwise at lower risk, and 6 months for those at higher risk (D+/R−). Longer durations of prophylaxis are sometimes given to those who have undergone higher risk SOTs, such as lung, intestinal, composite tissue, and others. Some programs have even gone to lifelong prophylaxis, primarily after lung transplant, although the cost and toxicities (primarily leukopenia) are often prohibitive, and mortality remains high in those that eventually develop CMV disease [43, 44]. The Improved Protection Against Cytomegalovirus in Transplantation (IMPACT) study demonstrated a decreased risk of CMV disease in D+/R− kidney recipients given 200 days of prophylaxis (21.3%) compared to those given 100 days of prophylaxis (36.8%), $p<0.0001$ [45]. Similar large trials are lacking with other SOT types. An example of suggested prophylaxis for human herpes viruses (including CMV) appears in Table 3.

Preemptive therapy involves monitoring for a period of time during the high risk period, that is, weekly (or twice a week) monitoring for 3–4 months after transplant or during periods of more intense immunosuppression. Those SOT recipients managed with the preemptive approach should be given oral acyclovir (or similar antiherpes nucleoside analogues) to prevent disseminated varicella and herpes simplex infections [46]. While generally effective at preventing CMV disease when done rigorously, it may not prevent the "indirect effects" of CMV from occurring, and it may be less effective especially in previously nonimmune subjects (D+/R−) at preventing very rapid expansion of virus, when rapid doubling times can occur. One study demonstrated that in D+/R−, there was a median viral load doubling time of 1.54 days (range 0.55–5.5) compared with 2.67 days (range 0.27–26.7) in the D+/R+ recipients ($p<0.0001$) [21]. Monitoring patients on therapy should be done at least weekly, given the half-life of viral loads [47]. Less frequent screening (i.e., less than weekly) has been shown to result in higher rates of CMV disease and inferior long-term graft survival when compared to prophylaxis [48, 49].

Preemptive therapy is a recommended alternative to prophylaxis for most R+ SOT recipients, although it is not recommended in higher risk situations such as lung, heart, vascularized composite, pancreas, islet, and intestinal transplant, where prophylaxis may be preferable [10]. Similarly, prophylaxis may be preferable in donor and/or recipient seropositive patients at increased risk for CMV, including those on recent antilymphocyte therapy, potent immunosuppression including desensitization or ABO incompatible protocols (including those on plasmapheresis/immunoadsorption, rituximab, bortezomib, and eculizumab), and those with HIV. A comparison of prophylaxis with preemptive therapy appears in Table 4.

Table 3 Example of human herpes virus prophylaxis after kidney, liver, heart, or pancreas transplant [10]

Induction agent	Donor CMV antibody	Recipient CMV antibody	Prophylaxis	Monitoring with CMV viral load
Antithymocyte globulin, alemtuzumab	Positive	Positive	Valganciclovir × 3 months	Monitoring while on prophylaxis only if clinically indicated by symptoms; consider weekly monitoring after prophylaxis × 8–12 weeks in higher risk patients, including D+/R− and those on more potent immunosuppression
	Negative	Positive		
	Positive	Negative	Valganciclovir × 6 months (plus consider weekly monitoring afterwards × 8–12 weeks in higher risk D+/R− on more potent IS)	
	Negative	Negative	Acyclovir, famciclovir, or valacyclovir × 3 months	
Corticosteroids, basiliximab, or none	Positive	Positive	Valganciclovir × 3 months	
	Negative	Positive		
	Positive	Negative		
	Negative	Negative	Acyclovir, famciclovir, or valacyclovir × 3 months	

Notes on viral prophylaxis:
• Dosages of all antiviral agents need to be adjusted for renal function. The eGFR or creatinine clearance should be used (not simply the serum creatinine).
• For prophylaxis, the first doses may be oral valganciclovir or intravenous ganciclovir, converting IV to oral as soon as patient tolerating oral medications. There is recent data supporting the safety and efficacy of either approach in the treatment (not prophylaxis) setting [87, 88].
• While most kidney transplant recipients (given lower GFR, often 40–60) will need valganciclovir 450 mg a day (or less), some may have GFR>60 and need valganciclovir 900 mg a day. Mini-dosing not recommended [10].
• Lung, intestinal, composite tissue transplant prophylaxis would be similar, although generally with longer courses of prophylaxis, and sometimes with the use of CMV immunoglobulin.

Table 4 Comparison of prophylaxis versus preemptive therapy. (Revised from Kotton et al. [10])

	Prophylaxis	Preemptive therapy
Prevention of CMV disease	Good efficacy	Good efficacy
Late CMV (infection/ disease)	Common (usually after end of prophylaxis)	Rare
Early CMV DNAemia/ infection	Rare	Common
Ease of implementation	Relatively easy; cost/ insurance coverage can be an issue	More difficult, requires diligent testing and follow-up plus intervention if positive testing
Resistance	Uncommon	Uncommon (with weekly testing, higher with irregular testing)
Prevention of other herpes viruses	Prevents HSV, VZV Unlikely to impact EBV, HHV-6, -7, -8	Does not prevent; best if acyclovir (or similar) is given
Costs	Drug costs	Monitoring costs
Safety	Drug side effects	Less drug toxicity, unless treatment dose antiviral therapy is needed
Prevention of rejection	May prevent	Unknown
Graft survival	May improve	May improve

Developing optimal thresholds for initiation of antiviral treatment varies across different institutions, test platforms, and sample types [10]. In general, higher thresholds are acceptable for R+, as their prior immunity may be effective in containing CMV infection, whereas nonimmune subjects need a lower threshold in order to avoid significant infection. The lower levels of positive CMV viral loads, as seen with the ultrasensitive assays, can be hard to interpret with preemptive therapy. Some groups suggest that understanding viral kinetics may predict the risk of disease, which could be done by increasing the frequency of testing, perhaps to twice a week, and measuring the rate of change between samples; rapid increases suggest a need to initiate antiviral therapy at any level.

Surveillance after prophylaxis is an approach that can be considered for those SOT recipients at increased risk for postprophylaxis CMV disease, in order to capture infection in the early stages. This is a hybrid approach, combining prophylaxis followed by preemptive therapy. This may work best when used during the high risk period, done weekly for 8–12 weeks after the end of prophylaxis. Bi-weekly or monthly monitoring is considered to be insufficient for preemptive interventions, as it will likely miss the period of rapid viral expansion. Determining which individuals are at risk for CMV infection remains challenging. Serology alone is not predictive on an individual basis. Recent diagnostic developments have focused on CMV specific cellular mediated community as a marker of risk of primary infection, reactivation infection, recurrent

infection, and for optimizing duration of prophylaxis; see above for further discussion.

Vaccines

Vaccines to prevent CMV have remained elusive. In the 1970s, Dr. Stanley Plotkin and collaborators developed the live, attenuated Towne strain as the first CMV vaccine candidate, with partial but insufficient efficacy. There have been many subsequent attempts at CMV vaccine development, which would be useful for both maternal-fetal and transplant related CMV prevention. One of the more recent trials involved the DNA-based vaccine ASP0113, which had looked promising in earlier trials, but no statistically significant difference was found in D+/R− kidney transplant recipients with respect to the primary endpoint (the proportion of transplant recipients found to have CMV viremia $\geq$1000 IU/mL from Day 100 through to 1 year after the first vaccine injection) between the ASP0113 and placebo groups [50]. There are several studies of CMV vaccines for SOT recipients underway, using a variety of approaches.

Immunoglobulins

Intravenous immunoglobulins (IVIG) are immunoglobulins removed from donated human blood products and processed so as to be safe for intravenous infusion. To provide higher titer CMV IgG in intravenous immunoglobulins, donors who have high CMV IgG antibody levels are included to make "CMV immunoglobulin." There is batch-to-batch variation; generally CMV IgG levels are no more than five- and tenfold greater in CMV immunoglobulin compared with IVIG. CMV immunoglobulin was originally developed for prevention of CMV prior to the advent of directly acting antiviral therapy; this is the only indication approved by the United States Food and Drug Administration. Modern use includes prophylaxis in lung transplant and other high-risk SOT recipients (i.e., small bowel), often with concomitant antiviral prophylaxis, as well as use in settings of CMV antiviral resistance [10]. Data on lung transplant recipients suggest reduced rates of CMV disease and bronchiolitis obliterans syndrome and improved patient survival, although it is not clear if this is due to the anti-inflammatory effects of CMV immunoglobulin or the result of a reduction in CMV infection [51] or perhaps due to the immune modulation of activated CD4+ lymphocyte and of naïve B-cell subsets [52].

There tends to be heterogeneous use of CMV immunoglobulin, likely reflecting the paucity of clinical data supporting its use in the modern era. One trial in abdominal transplant recipients who were given either VGCV for 200 days versus VGCV for 100 days followed by 3 doses of monthly CMV immunoglobulin found more overall CMV infections in the CMV immunoglobulin group (45 vs. 20%), but

no statistically significant differences in overall CMV infections or late CMV disease were seen between groups [53].

Given that IVIGs are human-derived products and thus difficult to standardize, monoclonal antibodies specifically targeting key epitopes would be expected to provide an advantage in efficacy. Recent approach have included multiple monoclonal antibodies used at once, for presumed enhanced efficacy and decreased development of viral resistance. CMV is a complex virus with multiple antigens, including glycoprotein B (gB) and the gHp entameric complex; most of the naturally generated antibodies target the gB antigen, although they may not have adequate neutralizing ability to control reactivation and infection. The pentameric gH complex has been shown to be the primary target for neutralizing antiviral antibodies, so this has been a focus of recent monoclonal antibody development. For example, CSJ148 (Novartis) is a combination of two monoclonal antibodies against CMV, LJP538, which binds to the viral gB protein and LJP539, which binds to the viral gH pentameric complex. A phase 1 trial in healthy volunteers showed good safety and tolerability. Phase 2 clinical trials of CSJ148 in hematopoietic stem cell transplant (HSCT) patients have been completed (ClinicalTrials.gov NCT02268526), data not yet published. A combination of two monoclonal antibodies, RG7667 (Genentech), that bind two distinct antigens (the neutralizing epitope on CMV glycoprotein H (gH) and the neutralizing epitope on the gH/gL/UL128/UL130/UL131 complex) on the surface of CMV and inhibit its entry into relevant host cells is under development. A randomized, double-blind, placebo-controlled trial of RG7667 to prevent CMV infection in high-risk (D+/R−) kidney transplant recipients without prophylaxis demonstrated some efficacy [54]. CMV viremia occurred in 46% (27/59) of those receiving RG7667 and 61% (35 of 57) in the placebo cohort within the first 12 weeks after transplant and in 51% (30 of 59) of those receiving RG7667 and in 70% (40 of 57) on placebo within 24 weeks after transplant. The median time to CMV viremia was 139 days in the cohort on RG7667 compared with 46 days in those on placebo, and CMV disease was less common in the RG7667 (3%) than placebo (16%) groups. Thus, the monoclonal antibody RG7667 was well tolerated, significantly reduced the incidence of CMV infection within 12 and 24 weeks after transplant in high risk kidney transplant recipients (although rates were still high), delayed the onset of CMV viremia, and was associated with less CMV disease than the placebo, yet was not significantly able to prevent CMV infection. Such therapies, if developed further, may have an interesting niche in combination with other treatments (i.e., antivirals, vaccines, etc.).

Treatment of Active Infection

Once active infection has been diagnosed, treatment involves antiviral therapy, most commonly with oral valganciclovir 900 mg twice a day, renally adjusted if needed. The VICTOR trial demonstrated noninferiority of oral therapy when compared with intravenous ganciclovir [55]. Guidelines suggest that for

convenience, oral therapy is generally preferred over intravenous therapy, except when there are concerns for life or sight threatening disease, malabsorption, or economic considerations [10]. CMV viral load should be monitored at least weekly to determine the duration of therapy. Treatment is generally continued until resolution of all clinical signs of CMV disease and eradication of CMV DNAemia is achieved (defined in guidelines as below LLOQ on one highly sensitive assay (LLOQ <200 IU/ml), or lack of detection on two consecutive less sensitive assays [10]. Failure to eradicate plasma DNAemia by the end of treatment is a major predictor of virologic recurrence [55]. One the other hand, a completely undetectable viral load may not always be achievable with use of highly sensitive assays. Consideration of the net state of immunosuppression, clinical setting, time from transplant, diagnostic testing, and other factors should help determine the end of therapy in a given patient.

Appropriate antiviral dosing is crucial in the management of CMV disease. Suboptimal dosing augments the risk for clinical treatment failure and the development of resistance [56], while supratherapeutic doses increase toxicity [57]. Careful monitoring of serum creatinine and kidney function is useful. (Val)ganciclovir dosing should be adjusted as per the Cockcroft-Gault formula, which was used in the VICTOR study; other estimates of renal function, such as the Modified Diet in Renal Disease (MDRD) formula, may lead to inadequate dosing. Caution should be used with automated calculators of renal function, as they are more likely to be erroneous with malnourished, debilitated patients and patients at the extremes of size, where nuclear medicine studies or measured urine creatinine over 24 h may be useful.

Treatment outcomes associated with CMV disease are impacted by the intensity of immunosuppressive therapy [58, 59]. Numerous studies incorporate a reduction in immunosuppression as a therapeutic modality. Dual (versus triple) immunosuppressive therapy and lower blood concentrations of calcineurin inhibitors significantly correlate with eradication of CMV DNA at 21 days [58]. Unfortunately, while a reduction in immunosuppression is encouraged and seems to be effective in helping clear viral infection, there have not been formal studies regarding the relative efficacy of cessation of one agent preferentially over another. In general, clinicians are encouraged to review all immunosuppressive medications and decrease the doses of those that seem to be higher. For cases of severe or resistant CMV, where immunosuppression has been significantly reduced, clinicians may consider adding back immunosuppression after successfully gaining good control of an infection, carefully balancing the risk of rejection with the risk of worsening infection. Given the reduced risk of CMV in those on sirolimus and everolimus, some switch to these agents to diminish further CMV infections.

A significant number of patients experience leukopenia well on treatment dose (val)ganciclovir (and even on prophylaxis dose). This is often multifactorial in nature, attributable to commonly used posttransplant medications (mycophenolate mofetil, trimethoprim/sulfamethoxazole, and others) and induction therapies (antithymocyte globulin, alemtuzumab). With leukopenia, the addition of granulocyte colony stimulating factor and/or discontinuation of other myelosuppressive therapies

are recommended before changing (val)ganciclovir to another agent. Reducing the dose of (val)ganciclovir because of leukopenia is strongly discouraged, as it significantly increases the risk of antiviral resistance.

Secondary prophylaxis after treatment of infection involves administration of antiviral prophylaxis for a period of time, previously for one to 3 months. Although this was thought to likely be an effective therapy, retrospective analyses conducted in diverse transplant populations have not demonstrated a benefit to secondary prophylaxis [60–62]. While there was evidence of some delay of the onset of recurrent infection [63], most have shown no benefit. Although a good number of patients may experience recurrent DNAemia upon completion of treatment, especially in high-risk D+/R− transplant patients [64, 65], viral load replication may be slower and the subsequent peak viral load significantly lower compared with the initial episode [21], potentially due to an developing immune response. Some of these episodes of CMV DNAemia may resolve spontaneously [65]. Guidelines do not generally encourage secondary prophylaxis, although they do encourage monitoring (akin to preemptive therapy) after the end of therapy in higher risk patients [10].

Treatment Resistant and Refractory Disease

Risk factors for resistance include exposure to inadequate doses of antiviral therapy (i.e., underdosing, noncompliance), high levels of immunosuppression, prolonged prophylaxis, multiple serial transplants, and receipt of lung and intestinal transplants [66]. Multiple studies have shown significantly higher rates of morbidity and mortality with resistant CMV [66–69], although not all [70]. Rates are generally less than 5% in recent studies (range, 1% [66] to 6% (lung transplant recipients [69]) and tend to be higher in D+/R− [66], as well as lung transplant recipients and other more immunocompromised SOTs. Although there had been the perception that antiviral prophylaxis conveyed a higher risk than preemptive therapy, this has not been the case in all studies, especially when preemptive therapy is done irregularly (less than weekly blood checks) with resultant high viral loads at the time when therapy is started [71, 72].

Resistant CMV occurs infrequently, such that it is challenging to accumulate sufficient data. Unfortunately, data of this nature is not captured in national databases such as SRTR [4]. A retrospective, case-control (1:3) study of SOT recipients at University of Washington (Seattle) with genotypically confirmed ganciclovir-resistant ($n = 37$) and ganciclovir-susceptible CMV infection ($n = 109$), matched by clinical manifestation(s), donor/recipient CMV serostatus, and year and organ transplanted, found that ganciclovir resistance was associated with a longer prior of exposure to ganciclovir (median, 153 vs. 91 days, $P<0.001$) [66]. Interestingly, 3 of 17 lung transplant recipients with ganciclovir-resistant CMV had received <6 weeks of ganciclovir, while all nonlung SOT recipients had received ≥ 90 days (median, 160 days) prior to the diagnosis of ganciclovir-resistant CMV. They found that ganciclovir-resistant CMV was associated with higher mortality (11% vs. 1%, $P = 0.004$), decreased renal function (42% vs. 19%, $P = 0.008$), and fewer days

alive and nonhospitalized (73 vs. 81, $P = 0.039$) at 3 months after diagnosis. Rates of ganciclovir-resistant CMV were higher in the D+R− recipients, with rates as follows: lung (11.9%), heart (5.8%), kidney (2.4%), pancreas (7.8%), and liver (0.4%). In another series from a single institution, University of Nebraska Medical Center, those who developed antiviral resistance had higher prednisone and tacrolimus levels, had a higher rate of fungal infections, and were more likely to have undergone heart, small bowel, and mutivisceral recipients (not liver or kidney) [70]. They found no difference in duration of antiviral prophylaxis, viremia while on antiviral prophylaxis, rates of end organ CMV disease, graft loss, and overall survival. Persistent viremia and clinical disease despite antiviral therapy, perhaps due to a high degree of immunosuppression, was the most important risk factor for development of CMV resistance.

Guidelines suggest that when antiviral resistance/refractory CMV is suspected, genotypic resistance testing should be requested [10]. If there is life threatening or sight threatening disease, and the patient has been on (val)ganciclovir, a switch to foscarnet is recommended (with or without the continued use of (val)ganciclovir). If the clinical disease is only mild to moderate, high-dose ganciclovir (10 mg/kg every 12 h, renally adjusted) may be used, with the caveat that this may not overcome many resistance mutations, and is likely to cause significant leukopenia. Subsequent management should be based on genotypic resistance test results, which should help guide antiviral choices. Some clinicians may wish to use CMV immunoglobulin, especially in patients with hypogammaglobulinemia. In general, with resistance to valganciclovir, subsequent prophylaxis is very challenging, as there are no readily available oral alternatives. Some clinicians use letermovir for prophylaxis after treatment of resistant virus, although there are not yet clinical studies to support this practice. Drug availability, cost, and insurance coverage can be problematic. Recent definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients, primarily for use in clinical trials, may be helpful for clinicians diagnosing and managing infection [33].

Foscarnet is the most commonly used medication for treatment of resistant/refractory CMV infection. Although it has excellent efficacy in eradicating CMV, it does have significant potential for nephrotoxicity, as well as electrolyte wasting. In general, foscarnet is given to hospitalized patients, given the need for very careful monitoring of kidney function and electrolytes. The best descriptive review of foscarnet is in a single center study at Johns Hopkins University, in SOT and HSCT recipients over a 10-year period who were given foscarnet for treatment of resistant/refractory disease [67]. Of the 39 patients (22 SOT, 17 HSCT), 15 had documented genotypic mutations for ganciclovir resistance, and 11 (28%) of 39 had tissue-invasive CMV. Ganciclovir resistance was more common in SOT than HSCT. The median duration of foscarnet was 32 days; virologic failure occurred in 13 (33%), while relapses of viremia occurred in 31%. Mortality was noted in 12 (31%) of 39 within a year and was higher in HSCT than SOT ($P = 0.001$). Looking back, doses of ganciclovir or valganciclovir had been low in 10 (26%) of 39 at some time before converting to foscarnet. Renal dysfunction was frequent, occurring in 20 (51%) of 39 while on treatment, and was persistent in about a quarter

after 6 months. This series is helpful in framing decision making about management of resistant/refractory CMV and highlights our need for better alternative therapies.

Cidofovir is another agent that may be useful with resistant/refractory CMV, although there is some overlap between the resistance mutations for (val)ganciclovir and cidofovir, such that it may be less effective, and the nephrotoxicity and ophthalmologic toxicities of cidofovir are significant. Nonetheless, due to the long half-life, with susceptible CMV, this agent could be given as prophylaxis intravenously every two weeks, which makes it one of the few options after successful treatment of resistant/refractory CMV. Data on its use for treatment of resistant/refractory CMV is limited. A series from Innsbruck Medical University described 9 SOT recipients with complicated CMV infection or disease (0.4% of all SOTs there between 1 June 1999 and 31 December 2007), 4 of whom had undergone lung transplant, and 2 of which had undergone composite tissue transplant [68]. Most had a treatment response, yet 5 of 9 had relapse of CMV infection, and the majority had renal compromise, some of which persisted after therapy.

Maribavir is a newer agent that inhibits viral DNA assembly and inhibits egress of viral capsids from the nucleus of infected cells, with efficacy against CMV and EBV, although not against varicella and herpes. It was studied in high risk liver transplant recipients in a randomized, double-blind, multicenter controlled trial, with a primary endpoint of confirmed CMV disease within 6 months of transplantation. In a modified intent-to-treat analysis, the noninferiority of maribavir 100 mg twice a day compared to oral ganciclovir for prevention of CMV disease was not established (12% rate of CMV disease with maribavir vs. 8% with ganciclovir). There was no significant difference in graft rejection, patient survival, and non-CMV infections, and while maribavir was safe, it was not adequate for prevention of CMV disease at the dose studied [42]. More recent work with resistant/refractory CMV in a dose escalation (400, 800, and 1200 mg twice daily) phase 2 study in 120 SOT and HSCT recipients showed that 80/120 (67%) patients achieved undetectable CMV DNA within 6 weeks of treatment, with similar rates across various doses. Recurrent CMV infections while on treatment occurred in 25 patients; of those, 13 had developed mutations that conferred maribavir resistance [73]. Further phase 3 studies are underway to assess the efficacy and safety of maribavir treatment compared to standard, investigator-assigned treatment in transplant recipients with refractory or resistant CMV infections (ClinicalTrials.gov NCT02931539).

Letermovir is a CMV-specific antiviral agent that inhibits CMV replication by binding to components of the terminase complex (UL51, UL56, or both), resulting in the formation of concatemers of CMV. It has been shown in a phase 3 trial to be effective in preventing CMV infections when given through 14 weeks after HSCT when compared with placebo, with decreased rates of CMV infection at 24 weeks (38%) versus placebo (61%), and less mortality, with all-cause mortality at week 48 after transplantation 20.9% in letermovir recipients and 25.5% among placebo recipients [74]. Studies are underway for letermovir in SOT (ClinicalTrials.gov NCT03443869). Letermovir is not likely to be useful for

treatment of active infection and will probably be reserved for prophylaxis, as it has a low barrier to resistance [75].

Brincidofovir is a prodrug of cidofovir, conjugated to a lipid. It releases cidofovir intracellularly, allowing for higher intracellular and lower plasma concentrations of cidofovir, effectively increasing antiviral activity while minimizing toxicity. A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for CMV prophylaxis in allogeneic HSCT recipients demonstrated that although it had good anti-CMV activity, it did not decrease clinically significant CMV infection (CMV disease or CMV DNAemia requiring preemptive therapy) through week 24 compared with placebo, likely confounded by the fact that it was associated with increased gastrointestinal toxicity which could not be differentiated from acute gastrointestinal graft versus host disease and which resulted in a much higher rate of steroid use in the arm with brincidofovir [76]. At present, it is not being developed as an oral drug for CMV prophylaxis or treatment because of this gastrointestinal toxicity, although an intravenous preparation might possibly alleviate this problem.

Adoptive CMV-specific T-cell therapy may be useful in cases of resistant/refractory CMV infection in organ transplant recipients [77–81]. CMV-specific T-cells undergo in vitro expansion with either viral lysate or CMV synthetic peptides, and then they are infused into the recipient to enhance viral immunity, lessen CMV viral loads, and/or promote resolution of CMV disease. The source of the cells can be "off the shelf" T-cells using HLA-matched third party banked cells, or cells obtained from the transplant recipient (autologous), although the process of generating effector cells can take several weeks. There is more experience in the HSCT field using CMV-specific T cells, with varying degrees of efficacy and no evidence of association with graft versus host disease [82, 83]. Commercialization of HLA-matched third party banked CMV-specific T-cells may allow increased use for resistant/refractory CMV in SOT recipients [84, 85]. As there are only case reports at present, significant efficacy and safety studies will be needed.

Key Points, Research Needs, and Future Trends

The past decade has brought marked improvements in CMV management, in both molecular and cellular diagnostics, novel therapeutics including innovative antiviral and cellular therapies, better prevention, and optimized management of resistant/refractory CMV infection. Nonetheless, many needs and gaps remain, including as outlined here:

- Effective, affordable antiviral therapies for prophylaxis and treatment with limited toxicities, resistance, and drug interactions; the current options are limited by leukopenia, cost, and some development of resistance.
- Vaccines that are able to induce long term immunity and protection, optimally in both previously immune and nonimmune organ transplant recipients; this could

abrogate the need for other methods of prevention, additional diagnostics, etc., and could truly alter the SOT field.

- Molecular diagnostics with better comparability across different testing platforms and perhaps even across different specimen types. Trending test results is crucial, and we are currently quite limited when comparing values from different test centers.
- Cellular mediated immunity assays that are predictive of the risk of infection after transplant, and of risk of recurrent infection after treatment of infection would allow us to focus on those at higher risk for infection, which would enhance care while saving on drugs, diagnostics, and follow-up care.
- Better understanding of the optimal choices of immunosuppression and adjunctive therapies for an individual SOT recipient and how to tailor them to abrogate the risk of CMV infection.
- Optimizing the management of resistant/refractory CMV infection after SOT, including better knowledge of novel antiviral therapies and their use in combination, will be critical in the subset of patients who develop this challenging and morbid infection.

References

1. Balfour HH Jr. Cytomegalovirus: the troll of transplantation. Arch Intern Med. 1979;139(3):279–80.
2. Manuel O, Asberg A, Pang X, Rollag H, Emery VC, Preiksaitis JK, et al. Impact of genetic polymorphisms in cytomegalovirus glycoprotein B on outcomes in solid-organ transplant recipients with cytomegalovirus disease. Clin Infect Dis. 2009;49(8):1160–6.
3. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin Infect Dis. 2010;50(11):1439–47.
4. Kotton CN, Huprikar S, Kumar D. Transplant infectious diseases: a review of the scientific registry of transplant recipients published data. Am J Transplant. 2017;17(6):1439–46.
5. Colvin M, Smith JM, Hadley N, Skeans MA, Uccellini K, Goff R, et al. OPTN/SRTR 2018 annual data report: heart. Am J Transplant. 2020;20(Suppl s1):340–426.
6. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Castro S, et al. OPTN/SRTR 2018 annual data report: kidney. Am J Transplant. 2020;20(Suppl s1):20–130.
7. Kandaswamy R, Stock PG, Gustafson SK, Skeans MA, Urban R, Fox A, et al. OPTN/SRTR 2018 annual data report: pancreas. Am J Transplant. 2020;20(Suppl s1):131–92.
8. Kwong A, Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, et al. OPTN/SRTR 2018 annual data report: liver. Am J Transplant. 2020;20(Suppl s1):193–299.
9. Valapour M, Lehr CJ, Skeans MA, Smith JM, Uccellini K, Goff R, et al. OPTN/SRTR 2018 annual data report: lung. Am J Transplant. 2020;20(Suppl s1):427–508.
10. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102(6):900–31.
11. Burton CE, Sester M, Robinson JL, Eurich DT, Preiksaitis JK, Urschel S. Assigning cytomegalovirus status in children awaiting organ transplant: viral shedding, CMV-specific T cells, and CD27-CD28-CD4+ T cells. J Infect Dis. 2018;218(8):1205–9.
12. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Robinson A, et al. OPTN/SRTR 2016 annual data report: kidney. Am J Transplant. 2018;18(Suppl 1):18–113.
13. Colvin M, Smith JM, Hadley N, Skeans MA, Carrico R, Uccellini K, et al. OPTN/SRTR 2016 annual data report: heart. Am J Transplant. 2018;18(Suppl 1):291–362.

14. Valapour M, Lehr CJ, Skeans MA, Smith JM, Carrico R, Uccellini K, et al. OPTN/SRTR 2016 annual data report: lung. Am J Transplant. 2018;18(Suppl 1):363–433.
15. Basso G, Felipe CR, Cristelli MP, Mansur Siliano J, Viana L, Ferreira Brigido AN, et al. The effect of anti-thymocyte globulin and everolimus on the kinetics of cytomegalovirus viral load in seropositive kidney transplant recipients without prophylaxis. Transpl Infect Dis. 2018;20(4): e12919.
16. Devresse A, Leruez-Ville M, Scemla A, Avettand-Fenoel V, Morin L, Lebreton X, et al. Reduction in late onset cytomegalovirus primary disease after discontinuation of antiviral prophylaxis in kidney transplant recipients treated with de novo everolimus. Transpl Infect Dis. 2018;20(2):e12846.
17. Mallat SG, Tanios BY, Itani HS, Lotfi T, McMullan C, Gabardi S, et al. CMV and BKPyV infections in renal transplant recipients receiving an mTOR inhibitor-based regimen versus a CNI-based regimen: a systematic review and meta-analysis of randomized, controlled trials. Clin J Am Soc Nephrol. 2017;12(8):1321–36.
18. Reusing JO Jr, Feitosa EB, Agena F, Pierrotti LC, Azevedo LSF, Kotton CN, et al. Cytomegalovirus prophylaxis in seropositive renal transplant recipients receiving thymoglobulin induction therapy: outcome and risk factors for late CMV disease. Transpl Infect Dis. 2018;20(5):e12929.
19. Gardiner BJ, Nierenberg NE, Chow JK, Ruthazer R, Kent DM, Snydman DR. Absolute lymphocyte count: a predictor of recurrent cytomegalovirus disease in solid organ transplant recipients. Clin Infect Dis. 2018;67(9):1395–402.
20. Kotton CN. CMV: prevention, diagnosis and therapy. Am J Transplant. 2013;13(Suppl 3):24–40; quiz
21. Atabani SF, Smith C, Atkinson C, Aldridge RW, Rodriguez-Peralvarez M, Rolando N, et al. Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy. Am J Transplant. 2012;12(9):2457–64.
22. Humar A, Lebranchu Y, Vincenti F, Punch J, Abramowicz D, Emily Blumberg E, et al., editors. The IMPACT study: valganciclovir prophylaxis for until 200 days post-transplant in high risk kidney recipients substantially reduces the incidence of CMV disease (abstract). Boston: American Transplant Congress; 2009.
23. Humar A, Michaels M. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. Am J Transplant. 2006;6(2):262–74.
24. Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis. 2017;64(1):87–91.
25. Fisher CE, Alexander J, Bhattacharya R, Rakita RM, Kirby KA, Boeckh M, et al. Sensitivity of blood and tissue diagnostics for gastrointestinal cytomegalovirus disease in solid organ transplant recipients. Transpl Infect Dis. 2016;18(3):372–80.
26. Ritter M, Schmidt T, Dirks J, Hennes P, Juhasz-Boss I, Solomayer EF, et al. Cytomegalovirus-specific T cells are detectable in early childhood and allow assignment of the infection status in children with passive maternal antibodies. Eur J Immunol. 2013;43(4):1099–108.
27. Schmidt T, Ritter M, Dirks J, Gartner BC, Sester U, Sester M. Cytomegalovirus-specific T-cell immunity to assign the infection status in individuals with passive immunity: a proof of principle. J Clin Virol. 2012;54(3):272–5.
28. Pang XL, Fox JD, Fenton JM, Miller GG, Caliendo AM, Preiksaitis JK. Interlaboratory comparison of cytomegalovirus viral load assays. Am J Transplant. 2009;9(2):258–68.
29. Preiksaitis JK, Hayden RT, Tong Y, Pang XL, Fryer JF, Heath AB, et al. Are we there yet? Impact of the first international standard for cytomegalovirus DNA on the harmonization of results reported on plasma samples. Clin Infect Dis. 2016;63(5):583–9.
30. Tong Y, Pang XL, Mabilangan C, Preiksaitis JK. Determination of the biological form of human cytomegalovirus DNA in the plasma of solid-organ transplant recipients. J Infect Dis. 2017; 215(7):1094–101.

31. Lisboa LF, Asberg A, Kumar D, Pang X, Hartmann A, Preiksaitis JK, et al. The clinical utility of whole blood versus plasma cytomegalovirus viral load assays for monitoring therapeutic response. Transplantation. 2011;91(2):231–6.
32. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev. 2010;23(4):689–712.
33. Chemaly RF, Chou S, Einsele H, Griffiths P, Avery R, Razonable RR, et al. Definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients for use in clinical trials. Clin Infect Dis. 2018;68(8):1420–6.
34. Chou S. Approach to drug-resistant cytomegalovirus in transplant recipients. Curr Opin Infect Dis. 2015;28(4):293–9.
35. Jarque M, Melilli E, Crespo E, Manonelles A, Montero N, Torras J, et al. CMV-specific cell-mediated immunity at 3-month prophylaxis withdrawal discriminates D+/R+ kidney transplants at risk of late-onset CMV infection regardless the type of induction therapy. Transplantation. 2018;102(11):e472–e80.
36. Kumar D, Chin-Hong P, Kayler L, Wojciechowski D, Limaye AP, Osama Gaber A, et al. A prospective multicenter observational study of cell-mediated immunity as a predictor for cytomegalovirus infection in kidney transplant recipients. Am J Transplant. 2019;19(9):2505–16.
37. Kumar D, Mian M, Singer L, Humar A. An interventional study using cell-mediated immunity to personalize therapy for cytomegalovirus infection after transplantation. Am J Transplant. 2017;17(9):2468–73.
38. Westall GP, Cristiano Y, Levvey BJ, Whitford H, Paraskeva MA, Paul E, et al. A randomized study of quantiferon-CMV-directed versus fixed duration valganciclovir prophylaxis to reduce late cmv following lung transplantation. Transplantation. 2018.
39. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13512.
40. Navarro D, San-Juan R, Manuel O, Gimenez E, Fernandez-Ruiz M, Hirsch HH, et al. Cytomegalovirus infection management in solid organ transplant recipients across European centers in the time of molecular diagnostics: an ESGICH survey. Transpl Infect Dis. 2017;19(6):e12773.
41. Reischig T, Kacer M, Hruba P, Hermanova H, Hes O, Lysak D, et al. Less renal allograft fibrosis with valganciclovir prophylaxis for cytomegalovirus compared to high-dose valacyclovir: a parallel group, open-label, randomized controlled trial. BMC Infect Dis. 2018;18(1):573.
42. Winston DJ, Saliba F, Blumberg E, Abouljoud M, Garcia-Diaz JB, Goss JA, et al. Efficacy and safety of maribavir dosed at 100 mg orally twice daily for the prevention of cytomegalovirus disease in liver transplant recipients: a randomized, double-blind, multicenter controlled trial. Am J Transplant. 2012;12(11):3021–30.
43. Wiita AP, Roubinian N, Khan Y, Chin-Hong PV, Singer JP, Golden JA, et al. Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis. Transpl Infect Dis. 2012;14(3):248–58.
44. Beam E, Lesnick T, Kremers W, Kennedy CC, Razonable RR. Cytomegalovirus disease is associated with higher all-cause mortality after lung transplantation despite extended antiviral prophylaxis. Clin Transpl. 2016;30(3):270–8.
45. Humar A, Lebranchu Y, Vincenti F, Blumberg EA, Punch JD, Limaye AP, et al. The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in high-risk kidney transplant recipients. Am J Transplant. 2010;10(5):1228–37.
46. Ramanan P, Razonable RR. Cytomegalovirus infections in solid organ transplantation: a review. Infect Chemother. 2013;45(3):260–71.
47. Humar A, Kumar D, Boivin G, Caliendo AM. Cytomegalovirus (CMV) virus load kinetics to predict recurrent disease in solid-organ transplant patients with CMV disease. J Infect Dis. 2002;186(6):829–33.

48. Kliem V, Fricke L, Wollbrink T, Burg M, Radermacher J, Rohde F. Improvement in long-term renal graft survival due to CMV prophylaxis with oral ganciclovir: results of a randomized clinical trial. Am J Transplant. 2008;8(5):975–83.
49. Witzke O, Hauser IA, Bartels M, Wolf G, Wolters H, Nitschke M, et al. Valganciclovir prophylaxis versus preemptive therapy in cytomegalovirus-positive renal allograft recipients: 1-year results of a randomized clinical trial. Transplantation. 2012;93(1):61–8.
50. Vincenti F, Budde K, Merville P, Shihab F, Ram Peddi V, Shah M, et al. A randomized, phase 2 study of ASP0113, a DNA-based vaccine, for the prevention of CMV in CMV-seronegative kidney transplant recipients receiving a kidney from a CMV-seropositive donor. Am J Transplant. 2018;18(12):2945–54.
51. van Gent R, Metselaar HJ, Kwekkeboom J. Immunomodulation by hyperimmunoglobulins after solid organ transplantation: beyond prevention of viral infection. Transplant Rev (Orlando). 2017;31(2):78–86.
52. Carbone J, Gallego A, Fernandez Yanez J, Sousa I, Sarmiento E. Potential immunomodulatory role of specific anticytomegalovirus intravenous immunoglobulin in heart recipients. Transplant Proc. 2016;48(9):3027–9.
53. Fleming JN, Taber DJ, Weimert NA, Nadig S, McGillicuddy JW, Bratton CF, et al. Valganciclovir (VGCV) followed by cytomegalovirus (CMV) hyperimmune globulin compared to VGCV for 200 days in abdominal organ transplant recipients at high risk for CMV infection: a prospective, randomized pilot study. Transpl Infect Dis. 2017;19(6):e12779.
54. Ishida JH, Patel A, Mehta AK, Gatault P, McBride JM, Burgess T, et al. Phase 2 randomized, double-blind, placebo-controlled trial of RG7667, a combination monoclonal antibody, for prevention of cytomegalovirus infection in high-risk kidney transplant recipients. Antimicrob Agents Chemother. 2017;61(2):e01794-16.
55. Asberg A, Humar A, Jardine AG, Rollag H, Pescovitz MD, Mouas H, et al. Long-term outcomes of CMV disease treatment with valganciclovir versus IV ganciclovir in solid organ transplant recipients. Am J Transplant. 2009;9(5):1205–13.
56. Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. Proc Natl Acad Sci USA. 2000;97(14):8039–44.
57. McGavin JK, Goa KL. Ganciclovir: an update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. Drugs. 2001;61(8):1153–83.
58. Asberg A, Jardine AG, Bignamini AA, Rollag H, Pescovitz MD, Gahlemann CC, et al. Effects of the intensity of immunosuppressive therapy on outcome of treatment for CMV disease in organ transplant recipients. Am J Transplant. 2010;10(8):1881–8.
59. Asberg A, Humar A, Rollag H, Jardine AG, Kumar D, Aukrust P, et al. Lessons learned from a randomized study of oral valganciclovir versus parenteral ganciclovir treatment of cytomegalovirus disease in solid organ transplant recipients: the VICTOR trial. Clin Infect Dis. 2016;62(9):1154–60.
60. Natori Y, Humar A, Husain S, Rotstein C, Renner E, Singer L, et al. Recurrence of CMV infection and the effect of prolonged antivirals in organ transplant recipients. Transplantation. 2017;101(6):1449–54.
61. Sullivan T, Brodginski A, Patel G, Huprikar S. The role of secondary cytomegalovirus prophylaxis for kidney and liver transplant recipients. Transplantation. 2015;99(4):855–9.
62. Helantera I, Lautenschlager I, Koskinen P. The risk of cytomegalovirus recurrence after kidney transplantation. Transpl Int. 2011;24(12):1170–8.
63. Gardiner BJ, Chow JK, Price LL, Nierenberg NE, Kent DM, Snydman DR. Role of secondary prophylaxis with valganciclovir in the prevention of recurrent cytomegalovirus disease in solid organ transplant recipients. Clin Infect Dis. 2017;65(12):2000–7.
64. Sun HY, Cacciarelli TV, Wagener MM, Singh N. Preemptive therapy for cytomegalovirus based on real-time measurement of viral load in liver transplant recipients. Transpl Immunol. 2010;23(4):166–9.
65. Martin-Gandul C, Perez-Romero P, Blanco-Lobo P, Benmarzouk-Hidalgo OJ, Sanchez M, Gentil MA, et al. Viral load, CMV-specific T-cell immune response and cytomegalovirus

disease in solid organ transplant recipients at higher risk for cytomegalovirus infection during preemptive therapy. Transpl Int. 2014;27(10):1060–8.
66. Fisher CE, Knudsen JL, Lease ED, Jerome KR, Rakita RM, Boeckh M, et al. Risk factors and outcomes of ganciclovir-resistant cytomegalovirus infection in solid organ transplant recipients. Clin Infect Dis. 2017;65(1):57–63.
67. Avery RK, Arav-Boger R, Marr KA, Kraus E, Shoham S, Lees L, et al. Outcomes in transplant recipients treated with foscarnet for ganciclovir-resistant or refractory cytomegalovirus infection. Transplantation. 2016;100(10):e74–80.
68. Bonatti H, Sifri CD, Larcher C, Schneeberger S, Kotton C, Geltner C. Use of cidofovir for cytomegalovirus disease refractory to ganciclovir in solid organ recipients. Surg Infect. 2017;18(2):128–36.
69. Bhorade SM, Lurain NS, Jordan A, Leischner J, Villanueva J, Durazo R, et al. Emergence of ganciclovir-resistant cytomegalovirus in lung transplant recipients. J Heart Lung Transplant. 2002;21(12):1274–82.
70. Sandkovsky U, Qiu F, Kalil AC, Florescu A, Wilson N, Manning C, et al. Risk factors for the development of cytomegalovirus resistance in solid organ transplantation: a retrospective case-control study. Transplant Proc. 2018;50(10):3763–8.
71. Myhre HA, Haug Dorenberg D, Kristiansen KI, Rollag H, Leivestad T, Åsberg A, et al. Incidence and outcomes of ganciclovir-resistant cytomegalovirus infections in 1244 kidney transplant recipients. Transplantation. 2011;92(2):217–23.
72. Couzi L, Helou S, Bachelet T, Moreau K, Martin S, Morel D, et al. High incidence of anticytomegalovirus drug resistance among D+R− kidney transplant recipients receiving preemptive therapy. Am J Transplant. 2012;12(1):202–9.
73. Papanicolaou GA, Silveira FP, Langston AA, Pereira MR, Avery RK, Uknis M, et al. Maribavir for refractory or resistant cytomegalovirus infections in hematopoietic-cell or solid-organ transplant recipients: a randomized, dose-ranging, double-blind, phase 2 study. Clin Infect Dis. 2018;68(8):1255–64.
74. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med. 2017; 377(25):2433–44.
75. Chou S. Rapid in vitro evolution of human cytomegalovirus UL56 mutations that confer letermovir resistance. Antimicrob Agents Chemother. 2015;59(10):6588–93.
76. Marty FM, Winston DJ, Chemaly RF, Mullane KM, Shore TB, Papanicolaou GA, et al. A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for cytomegalovirus prophylaxis in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2019;25(2):369–81.
77. Brestrich G, Zwinger S, Fischer A, Schmuck M, Rohmhild A, Hammer MH, et al. Adoptive T-cell therapy of a lung transplanted patient with severe CMV disease and resistance to antiviral therapy. Am J Transplant. 2009;9(7):1679–84.
78. Pierucci P, Malouf M, Glanville AR, Beagley L, Smith C, Khanna R. Novel autologous T-cell therapy for drug-resistant cytomegalovirus disease after lung transplantation. J Heart Lung Transplant. 2016;35(5):685–7.
79. Macesic N, Langsford D, Nicholls K, Hughes P, Gottlieb DJ, Clancy L, et al. Adoptive T cell immunotherapy for treatment of ganciclovir-resistant cytomegalovirus disease in a renal transplant recipient. Am J Transplant. 2015;15(3):827–32.
80. Gupta MP, Coombs P, Prockop SE, Hasan AA, Doubrovina E, O'Reilly RJ, et al. Treatment of cytomegalovirus retinitis with cytomegalovirus-specific T-lymphocyte infusion. Ophthalmic Surg Lasers Imaging Retina. 2015;46(1):80–2.
81. Holmes-Liew CL, Holmes M, Beagley L, Hopkins P, Chambers D, Smith C, et al. Adoptive T-cell immunotherapy for ganciclovir-resistant CMV disease after lung transplantation. Clin Transl Immunol. 2015;4(3):e35.

82. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121(26):5113–23.
83. Blyth E, Clancy L, Simms R, Ma CK, Burgess J, Deo S, et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Blood. 2013;121(18):3745–58.
84. Lugthart G, Albon SJ, Ricciardelli I, Kester MG, Meij P, Lankester AC, et al. Simultaneous generation of multivirus-specific and regulatory T cells for adoptive immunotherapy. J Immunother. 2012;35(1):42–53.
85. Gerdemann U, Katari UL, Papadopoulou A, Keirnan JM, Craddock JA, Liu H, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. Mol Ther. 2013;21(11):2113–21.
86. Kotton CN. Updates on antiviral drugs for cytomegalovirus prevention and treatment. Curr Opin Organ Transplant. 2019;24(4):469–75.
87. Asberg A, Humar A, Rollag H, Jardine AG, Mouas H, Pescovitz MD, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant. 2007;7(9):2106–13.
88. Len O, Gavalda J, Aguado JM, Borrell N, Cervera C, Cisneros JM, et al. Valganciclovir as treatment for cytomegalovirus disease in solid organ transplant recipients. Clin Infect Dis. 2008;46(1):20–7.

Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment

25

Jose F. Camargo

Contents

The Virus 573
Epidemiology and Risk Factors 574
Immune Reconstitution 576
Clinical Manifestations 577
Definitions 577
Clinical Manifestations of CMV 579
Diagnosis 582
Quantitative Nucleic Acid Testing 583
Preventive Strategies 585
Preemptive Therapy 585
Prophylaxis 595
Treatment 599
Antivirals 599
Resistance 599
Adoptive CMV-Specific T-Cell Therapy 603
Vaccines 604
Concluding Remarks 605
References 606

The Virus

Human CMV is the prototypic member of the β herpesvirus subfamily, which also includes human herpesviruses 6 and 7. CMV genome is the largest among known human viruses (~230 kb), containing 200 genes encoding proteins [1, 5]. CMV is

J. F. Camargo (✉)
Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, FL, USA
e-mail: jfc31@med.miami.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_25

able to infect several cell types, such as endothelial cells, epithelial cells (including retinal cells), smooth muscle cells, fibroblasts, leukocytes, and dendritic cells. Like other herpesviruses, CMV becomes clinically quiescent after the primary infection is brought under control by the host immune response [4, 10].

Epidemiology and Risk Factors

A recent analysis revealed an overall 50% seroprevalence among adults in the United States [11], but rates in some populations are even higher. Approximately 90% of Mexican-Americans in the United States are seropositive by age 50, as are 88% of HSCT patients in Italy and 96% of individuals in southern Brazil [11–14]. Transplant centers with high volume of international patients can have rates of CMV-seropositive recipients >85% [8].

CMV remains one of the most important complications after HSCT. The overall incidence of CMV reactivation at any level of viremia by day 100 (early CMV reactivation) after allogeneic HSCT in large cohort studies is in the range of 63–69% [8, 9], with reported median time from HSCT to first detectable (>137 IU/mL) CMV viremia of 17 days (IQR, 7–30 days) [8]. In a study that used a cutoff of ≥273 IU/mL, the median time from transplantation to CMV DNAemia was 48 (35–62) days [15]. Of note, in the era of preemptive antiviral therapy, most of the episodes of CMV reactivation correspond to low-grade viremias with less than 40% of patients achieving a plasma viral load of more than 150 IU/mL, and only 12% of patients achieving a viral load of more than 1000 IU/mL [9].

The rate of CMV infection after autologous HSCT is significantly lower in comparison with that in CMV-seropositive patients after allogeneic HSCT [16]. In autologous HSCT, CMV reactivation is reported to occur in up to 41% of CMV-seropositive patients when a prospective monitoring of antigenemia and/or viremia by polymerase chain reaction is adopted. However, more contained frequencies, up to 12%, have been reported when the monitoring criteria were based on a clinically driven diagnostic strategy. The most relevant risk factors appear to be CD34+ selected autografts, total body irradiation, and prior treatment with alemtuzumab, fludarabine, or bortezomib [16–18].

Among allogeneic HSCT recipients, the most important pretransplant risk factor for CMV disease is the serological status of the donor (D) and recipient (R). The risk is highest among seropositive HSCT recipients (i.e., those who were infected prior to transplant) who receive grafts from seronegative donors (i.e., lacking immunity against CMV) and almost null in seronegative recipients from seronegative donors (D− /R+ > D+ /R+ > D+ /R− > D− /R−) [4]. Namely, CMV reactivation occurs in 60–80% of seropositive (R+) individuals, 20–30% among D+/R−, and <5% of cases in D−/R− where infection is typically transfusion-related [1, 6–9]. Thus, strict use of CMV seronegative or leukoreduced blood products is highly recommended in CMV D−/R− recipients. The risk of transfusion-related CMV is higher with filtered

red blood cell products than with leukocyte-reduced platelets (4%) and lowest with use of CMV seronegative products (<2%) [19].

In addition to D/R serostatus, other well-established risk factors for early CMV reactivation include high-dose corticosteroids (in most studies >1 mg/kg/day of prednisone or equivalent is considered high dose); ex vivo or in vivo T-cell depletion (anti-thymocyte globulin (ATG), alemtuzumab, CD34 selection) [3, 20]; acute graft-versus-host disease (GVHD) grades II–IV, especially GVHD requiring systemic steroids; and the use of mismatched or unrelated donors [6]. T-cell depletion by CD34+ selection effectively reduces rates of GVHD, but it is also associated with delayed immune recovery and higher rates of CMV infection compared with those of unmodified HSCT [20]. Acute GVHD and receipt of a graft from a CMV-negative donor by a CMV-positive patient are the most significant clinical risk factors for CMV disease [21].

In 928 HSCT patients who received cyclosporine/methotrexate/ATG GVHD prophylaxis, D− /R+ patients had a substantially lower survival than D+ /R+ patients [22]. Survival rates were also lower for D− /R+ HLA-matched sibling recipients compared with D+ /R+ HLA-matched unrelated donor transplant recipients, demonstrating the significance of donor serostatus.

Among graft sources, umbilical cord blood (UCB) transplantation is associated with delayed immune reconstitution [23] and the highest cumulative incidence of CMV infection, whereas peripheral blood stem cell (PBSC) HSCT is associated with the lowest incidence [24]. Indeed, among CMV-seropositive UCB transplantation recipients, CMV reactivation occurs in up to 100% of patients within 100 days after transplantation [25–27]. HLA-haploidentical (i.e., 5/10 HLA-mismatched related donor) HSCT with conditioning regimens using posttransplant cyclophosphamide (PTCy) are associated with CMV reactivation and disease in 55–74% and 12–15% cases, respectively [28, 29]. By allowing faster recovery of CD4+ T cells, the use of PTCy seems to result in a lower risk of CMV reactivation compared to patients receiving ATG-based conditioning for HLA-haploidentical transplantation [30].

Underlying lymphoma is also a risk factor for CMV infection and progression to clinically significant CMV [8], especially among those with active and stage III/IV lymphoma [31]. CMV reactivation and disease are more common among patients with non-Hodgkin's lymphoma than among those with Hodgkin's disease [31]. Dasatinib use after HSCT to prevent or preemptively treat molecular relapse of Philadelphia chromosome-positive hematological malignancies significantly increased the risk of CMV reactivation in the first year after transplant in CMV-seropositive recipients, independent of acute GVHD [32]. Similarly, CMV tissue-invasive disease can occur after administration of ruxolitinib, a Janus kinase 1/2 inhibitor used for prevention and treatment of steroid-refractory GVHD [33, 34].

In a study of 269 consecutive allogeneic HSCT, the overall incidence of late (occurring beyond day +100) CMV reactivation was 31% [35]. In this study, the

primary determinant of late CMV reactivation was antecedent early reactivation (45% of those who experienced early CMV reactivation experienced late reactivation versus 16% late reactivation rate in those without early reactivation). Other clinical factors associated with the occurrence of late CMV viremia were GVHD, donor-type (mismatched or unrelated donors) and CMV serostatus (CMV-seronegative graft), and type of malignancy (higher risk in those with lymphoid malignancy). Lymphopenia at day +100 and UCB transplantation are additional risk factors for late CMV reactivation.

Even in the absence of antiviral prophylaxis, not all the individuals with the abovementioned risk factors will develop CMV. Indeed more than half of the patients in the placebo arm of the pivotal letermovir prophylaxis trial (NCT02137772) did not develop clinically significant CMV infection by week 24 [36]. This suggests that there are host factors unique to each individual that influence the risk of uncontrolled viremia. It is generally accepted that reconstitution of CMV-specific immunity is the most important factor in controlling the reactivation of the virus and in establishing long-term protection [37–40]; in recent years, assessment of IFNγ production [41–43] or virus-specific CD8+ T-cell functional signatures has been found predictive of risk of CMV following HSCT [44].

Immune Reconstitution

Following infection, CMV remains latent and reactivation controlled primarily by virus-specific CD4+ and CD8+ cytotoxic T lymphocytes (CTL) via coordinated cytokine production and degranulation. The dominant sites of CMV latency or persistence in humans remain unclear, although the risk of transfusion-associated CMV infection from asymptomatic donors implicates mononuclear cells [1]. In seropositive humans, a strikingly high fraction (10–40%) of circulating T lymphocytes target CMV [45], and this proportion of circulating CMV-specific T cells increases with age.

CMV reactivation risk following HSCT is highly dependent on the extent of CMV-specific immune reconstitution. Reconstitution of CD8+ T cells is faster than that of CD4+ T cells after HSCT, which usually occurs around day +100 or later and is indicated by the inversion of the CD4+/CD8+ T-cell ratio. For this reason, the risk of DNA virus reactivation including CMV is highest in the first 100 days post-HSCT. In ATG-treated patients, CD8 recovery might take up to 6 months, and CD4 recovery might not occur after 12 months [46]; complete reconstitution of CD4+ T cells can take up to 2 years after allogenic HSCT [47]. CMV-specific T-cell reconstitution is delayed significantly in recipients of seronegative donors and in marrow recipients [47]. Timing of recovery of CMV-specific CTL responses is also influenced by the type of donor, being severely delayed in recipients of matched unrelated donors (mean 200 ± 117 days) compared with recipients of sibling donors (58 ± 23 days) [43].

In one study that included 148 HSCT recipients [47], among patients with CMV reactivation, the number of CD8+ T cells recovered to and above normal levels within 6 (866×10^6 cells/l) and 12 months (1203×10^6 cells/l), whereas counts in patients with absent CMV reactivation remained within the lower normal range (221 and 300×10^6 cells/l after 6 and 12 months, respectively). Although it seems paradoxical that patients with higher CD8+ T-cell counts were the ones who experienced CMV reactivation, this observation may be attributed to clonal expansion of T cells driven by antigenic exposure to CMV. Higher frequencies of CMV-specific CD4+ and CD8+ T-cell responses are present in subjects experiencing CMV viremia [39]. Absolute lymphocyte count (ALC) and number of virus-specific CD8+ lymphocytes do not discriminate well between HSCT patients with different CMV outcomes [44], whereas functional signatures within the CMV-specific CD8+ T-cell compartment are highly predictive of CMV outcomes, independent of steroid use or donor type. Altogether, these observations indicate that the inability to control CMV reactivation following allogeneic HSCT is due to the impaired function of antigen-specific CD8+ T cells rather than an inability to recover sufficient numbers of CMV-specific T cells.

The polyfunctionality (e.g., the ability to produce 2 or more cytokines) of virus-specific T cells is an important correlate of protective immunity against chronic viral infections. It is estimated that more than half of CD8+ T cells responding to common viruses such as CMV, Epstein–Barr virus, and influenza virus exhibit up to four or five functions [48]. In 118 pediatric HSCT recipients, dual IFN-γ/IL-2-producing $CD8^+$ T cells were present in patients who spontaneously controlled CMV reactivation but were absent from those who required antivirals [40]. Lilleri et al. found that 90% of HSCT adult patients who spontaneously controlled CMV infection had IFN-γ/IL-2-producing CMV-specific T cells [49]. The presence of polyfunctional virus-specific CD8+ T cells is also associated with lower levels of CMV replication and higher frequency of self-resolved episodes. Reduced number of polyfunctional CMV-specific CD8+ T cells measured on day +30 posttransplant can predict risk of clinically significant CMV reactivation in allogeneic HSCT recipients [44]. Robust CMV-specific CTL responses are also characteristic of patients who eradicate viremia in <14 days in response to ganciclovir treatment [50].

Clinical Manifestations

Definitions

The CMV Drug Development Forum published definitions of CMV infection and disease in transplant patients [51]. Although such definitions were developed for the purpose of facilitating consistent reporting of CMV outcomes in clinical trials, they have some applicability in clinical setting.

CMV Syndrome

The definition of probable CMV syndrome requires detection of CMV in blood by viral isolation, rapid culture, antigenemia, or nucleic acid testing (NAT) together with at least two of the following: fever $\geq$38 °C for at least 2 days, new or increased malaise/fatigue, leukopenia or neutropenia, thrombocytopenia, >5% atypical lymphocytes, and elevation of hepatic aminotransferases to 2 times the upper limit of normal.

Pneumonia

Proven CMV pneumonia is defined as the presence of compatible clinical symptoms and/or signs along with documentation of CMV in lung tissue by histopathology, virus isolation, rapid culture, immunohistochemistry, or DNA hybridization. Probable CMV pneumonia is defined as the detection of CMV in BAL fluid (e.g., by PCR or culture) combined with clinical symptoms and/or signs of pneumonia. It should be noted that detection of CMV in BAL is common and results should be interpreted with caution (see "Diagnosis" section). A definite cutoff for CMV DNA load in BAL has not been established.

Gastrointestinal (GI) Disease

Proven GI disease (e.g., esophagitis, colitis) requires symptoms and evidence of macroscopic mucosal lesions plus CMV detection in tissue. In the absence of macroscopic mucosal lesions, GI disease is considered as probable. Proven liver disease requires abnormal liver function tests plus CMV documented in tissue by histopathology, immunohistochemistry, virus isolation, rapid culture, or DNA hybridization techniques and the absence of other documented cause of hepatitis. Probable disease is not a recommended category for CMV hepatitis due to the risk for other confounders such GVHD in allogeneic HSCT recipients, as well as the common occurrence of drug-associated liver dysfunction.

Retinitis

Proven disease requires typical ophthalmological signs judged by an ophthalmologist experienced with the diagnosis of CMV retinitis. Alternatively, the diagnosis might be supported by CMV documented in vitreous fluid by PCR.

Encephalitis

Proven disease requires central nervous system (CNS) symptoms plus detection of CMV in CNS tissue by virus isolation, rapid culture, immunohistochemical analysis, in situ hybridization, or preferably quantitative PCR. In patients with symptoms plus abnormal imaging results or evidence of encephalitis on electroencephalography but in whom CNS tissue is not available, detection of CMV in CSF without visible contamination of blood ("bloody tap") supports the diagnosis and is defined as probable encephalitis.

GU Disease

The detection of CMV in urine by PCR or culture, in the absence of detection of CMV in tissue, is not sufficient for the diagnosis of CMV nephritis or cystitis as asymptomatic viral shedding in urine is common.

Other End-Organ Disease Categories

CMV can also cause disease in other organs (e.g., myocarditis, pancreatitis), and the definitions of these additional disease categories include the presence of compatible symptoms and signs and documentation of CMV in tissue biopsy by virus isolation, rapid culture, immunohistochemistry, or DNA hybridization.

Clinical Manifestations of CMV

Although CMV reactivation can result in tissue-invasive disease, including pneumonia, hepatitis, colitis, retinitis, and encephalitis [1, 2, 6, 37, 38] with high mortality rates, asymptomatic viremia is the most common (>85%) clinical presentation of CMV reactivation in the current preemptive therapy era [8, 9, 15]. Symptomatic patients typically present with CMV syndrome (≈10%) defined as fever, malaise, cytopenias, or transaminitis in patients with concurrent CMV viremia and less commonly (<5%) with tissue-invasive disease [2, 8, 24, 36]. The incidence of CMV disease is higher in allogeneic than autologous HSCT recipients [52].

In the report by Green et al., the cumulative incidence of CMV disease in the first year after transplantation was 11% [9]. Among those with tissue-invasive CMV disease, 62% patients had gastrointestinal tract disease, whereas pneumonia and retinitis were less common, occurring in 35% and 3% patients, respectively. CMV viremia is associated with an increased risk of CMV disease in patients not receiving preemptive therapy [9]; and those with symptomatic CMV reactivation experience higher viremias with a stepwise increase in the median peak viremia for patients with asymptomatic viremia (724 IU/mL; IQR, 181–2049 IU/mL) and CMV syndrome (3075 IU/mL; IQR, 774–35,519 IU/mL) and those with tissue-invasive disease (7393 IU/mL; IQR, 5900–2,185,018 IU/mL) in one study that included 106 HSCT recipients with CMV viremia [8]. Similarly, Ljungman et al. showed that the peak viral load was significantly higher in patients who developed CMV disease than in patients without CMV disease [21].

CMV pneumonia is a potentially fatal disease. Mortality in HSCT recipients with CMV pneumonia can be as high as 60% [37, 38], and outcomes have only modestly improved over the past 25 years [53]. Patients might present with fever, shortness of breath, cough, and hypoxia that can rapidly progress to respiratory failure. Specific risk factors for severe CMV pneumonia include lymphocytopenia, male sex, and severe acute GVHD [54]. Imaging in most patients with CMV pneumonia typically reveals bilateral, diffuse ground-glass opacities. Other common findings are small, consolidated centrilobular (nodule-like) opacities, reticular opacities, and pleural effusions [52]. The gold standard for diagnosis of CMV pneumonia is (1) demonstration of either CMV within cells or atypical

intranuclear inclusions and/or (2) detection of CMV using immunohistochemical staining or in situ hybridization in lung biopsy specimens. CMV can also be detected in BAL specimens using the shell vial culture method. Determination of CMV viral load via PCR in BAL fluid has been used; however this method has not been standardized, and detection of CMV in BAL by PCR needs to be interpreted with caution.

GI disease is the most common type of CMV end-organ disease. A cumulative incidence rate for GI CMV disease of 2% at 2 years after HSCT, with a median time to onset of 88 days and a wide range of 17–1099 days [55]. GI CMV disease is an erosive and/or an ulcerative condition that can occur at any location in the GI tract, from the mouth to rectum. Patients with esophagitis may present with odynophagia and substernal pain secondary to painful ulcerations. Small bowel CMV infection ranges from asymptomatic to upper GI bleeding or obstruction due to severe ulcerations and mucosal inflammation. Patients with CMV colitis may have diarrhea, hematochezia, urgency, tenesmus, and abdominal pain. However, none of these symptoms is pathognomonic. Although CMV colitis can be diagnosed on sigmoidoscopy, total colon examination up to the terminal ileum is preferred [55]. The distinction between GI CMV disease and GVHD is crucial. Histologic distinction between CMV infection and GVHD in the colon relies heavily on identification of viral inclusions and positive CMV-specific immunostains, as the morphologic features (inflammatory changes and ulcerations) of these two entities may be similar [52]. Detection of CMV DNA by PCR in tissue biopsy alone is not sufficient for diagnosis of GI disease in the absence of compatible histological and clinical findings, especially among those with concurrent viremia since blood contamination can occur at the time of biopsy.

CMV CNS disease after allogeneic HSCT usually manifests as ventriculoencephalitis with rapid progression of cognitive dysfunction and occurs as a late-onset disease, with median time to onset ranging from 155 to 285 days after HSCT [56–58]. Incidence of CMV encephalitis has not been established, but it is very rare. As a reference, the incidence of viral encephalitis in a study that included more than 2500 HSCT recipients was only 1.2%, with highest risk among those undergoing T-cell depletion. In that report, HHV-6, HSV, EBV, and JC were more common etiologies of viral encephalitis post-HSCT than CMV [59].

CMV retinitis is also infrequently reported in HSCT recipients, with an estimated prevalence rate of 1–3% [9, 60]. Symptoms include blurring or loss of central vision, scotomata, floaters, or photopsia depending on the anatomic site of retinal destruction and whether retinal detachment has occurred. CMV retinitis is characterized by full-thickness retinal necrosis and edema that is subsequently replaced with scar tissue which can lead to retinal detachment. Bilateral involvement is not uncommon. The diagnosis of retinitis is based on ophthalmologic examination; similar to GI or CNS disease, CMV DNAemia can be absent in cases of CMV retinitis. Diagnosis can be confirmed through PCR analysis of the aqueous humor or vitreous body specimens for the presence of CMV. Treatment includes intravitreal injections of ganciclovir or foscarnet which can be used as an adjunct to systemic therapy. Administration of systemic antivirals to patients with unilateral CMV

retinitis is essential, even among those receiving intravitreal injections, in order to prevent extension of CMV sight-threatening disease into the contralateral eye or other tissues.

In addition to CMV syndrome and end-organ disease, CMV infection has been linked to indirect effects in HSCT recipients. Namely, CMV infection is associated with an increased risk of secondary bacterial and fungal infections following allogeneic HSCT [61, 62] as well as a potentially increased risk of GVHD [63, 64] and increased non-relapse mortality (NRM) [8, 35, 65–67]. CMV disease is an independent risk factor for invasive aspergillosis (adjusted hazard ratio 7.0) [61, 68, 69] and candidemia (adjusted relative risk 16.4) [70]. In addition, >40% of HSCT recipients receiving ganciclovir for CMV reactivation develop neutropenia at a mean of 35 days after starting treatment [8, 71], and ganciclovir use is associated with a 13-fold increased risk of invasive aspergillosis (each week of ganciclovir treatment increases the risk by a factor of 1.4), a much higher risk than that seen with the use of corticosteroids [69]. Nichols et al. reported higher mortality due to bacteremia or invasive fungal infection among CMV D+/R− (18.3%) than D−/R− (9.7%) patients ($P < 0.001$) [62].

Several large cohort studies have shown that CMV reactivation or seropositivity is associated with increased risk of NRM [8, 35, 65–67]. In a recent report by the Center for International Blood and Marrow Transplant Research that included data from 9,469 HSCT recipients who underwent transplantation between 2003 and 2010, CMV reactivation was associated with higher NRM (regardless of the underlying hematologic disease) [67], confirming that CMV remains a serious problem in the current era. At least two contemporary studies have reported an association between a CMV viral load ≥250 IU/mL and increased risk of mortality in the posttransplantation period [9]. In the study by Green et al., CMV DNA levels >500 IU/ml were associated with a 20-fold increase in the risk of death by day 60 [9]. In a recent study, allogeneic HSCT recipients with refractory CMV reactivation (defined as CMV DNAemia lasting for >2 weeks despite administration of a full dose of antiviral drug therapy) within 100 days of transplantation had a higher incidence of CMV disease and non-relapse mortality (11.9% and 17.1%, respectively) than did those without refractory CMV (0.8% and 8.3%, respectively) [72]. At our center, we observed very high probability of NRM among patients who failed to eradicate CMV viremia, whereas early eradication of viremia conferred a survival benefit [8]. Specifically, the probability of NRM was significantly higher among patients who failed to eradicate CMV viremia by treatment day 35 compared with those who cleared CMV viremia within 35 days in response to antiviral therapy (23%, 32%, and 42% vs. 3%, 6%, and 15% at 100, 200, and 365 days post-HSCT; $P = 0.02$, 0.01, and 0.01, respectively). One-year NRM was significantly higher among patients with therapeutic failure compared with those who cleared CMV viremia in response to antiviral therapy (91% vs. 15%; $P < 0.0001$) [8]. Failure to achieve clearance of CMV viremia in response to antiviral therapy is associated with >25-fold increase in 1-year NRM (adjusted HR, 25.9; 95% CI, 7.7–87; $P < 0.0001$) [8]. Most of these deaths were attributed to bacterial and fungal sepsis, confirming the established relationship between CMV and other opportunistic infections.

Diagnosis

The diagnosis of CMV replication and disease in HSCT recipients can be made using different laboratory methods, including histology, pp65 antigenemia, or CMV DNA by quantitative NAT (QNAT) utilizing polymerase chain reaction (PCR) DNA amplification.

Serology. All donors and transplant candidates should be tested for baseline CMV immune status using CMV-IgG serology. CMV-IgM is not routinely recommended due to potential for false positivity. In the rare event there is an indeterminate serology results, the assignment of baseline serologic status should consider the "highest-risk" scenario for CMV prevention purposes. For example, if the HSCT recipient CMV-IgG is borderline or indeterminate, test should be repeated prior to transplant, and if repeat test is not available, it should be considered as positive. If the donor is CMV-IgG indeterminate, it should be considered as positive for CMV seronegative recipients and as negative for CMV-seropositive recipients. Serological testing is not indicated posttransplant as false-negative results can occur in patients receiving immunosuppression, and more importantly, serological testing is unlikely to change management or be useful since it cannot differentiate between previous exposure and active infection.

Histopathology. CMV-infected cells are cytomegalic cells, typically two- to fourfold larger than normal, containing basophilic intranuclear inclusion bodies ("Cowdry bodies") surrounded by a clear halo, giving the appearance of an "owl's eye." Cells show a thickened nuclear membrane and smaller granular intracytoplasmic inclusions. Many of these infected cells can be seen surrounding blood vessels, as the most commonly infected cells are endothelial cells and mesenchymal cells [73]. Although histological appearance is somewhat specific for CMV, it has a low sensitivity. Immunohistochemistry (IHC) or hematoxylin and eosin (H&E) staining can be used to improve sensitivity [74]. The sensitivity has been reported as 82.6% on H&E and 95.7% with IHC [73]. Unfortunately, the most sensitive test for CMV, CMV biopsy PCR, does not seem to have sufficient specificity to be used alone to make a diagnosis of CMV colitis. CMV viral cell culture is also sensitive but labor-intensive and has, therefore, been replaced by nucleic acid detection. Up to 25% of patients with CMV colitis have normal colonic mucosa on endoscopy but histologic evidence of CMV on biopsy. Thus, random multiple biopsies should be performed throughout the colon regardless of the macroscopic appearance on colonoscopy.

Viral culture. Viral culture has a sensitivity of 45% to 78% and a very high specificity (89–100%) [75]. However, results take days to weeks to complete.

Antigenemia. The CMV antigenemia assay consists of the detection of the CMV phosphoprotein 65 (pp65) antigen in neutrophils, which correlates semiquantitatively with viral replication [52]. This assay is relatively easy to perform and does not require expensive equipment, although it is limited by a lack of standardization, including subjective interpretation of results and the need for an adequate neutrophil count (>1000/ml) [52]. CMV PCR is more sensitive than pp65 antigenemia [76, 77], and it has largely replaced other methods for screening and diagnosis of CMV

infection [78]. Primer optimization and improved DNA extraction techniques resulted in high sensitivity (>80%) and specificity (>90%) of CMV PCR in HSCT recipients [77].

Quantitative Nucleic Acid Testing

PCR in blood. The quantification of CMV viral load by quantitative PCR can give important prognostic information, and this technique is now widely available [3], largely replacing the now obsolete detection of antigen (pp65; antigenemia assay). The CMV PCR test has become the standard of care for screening and diagnosis of CMV in HSCT. Molecular quantitative DNA assays in general afford earlier detection of CMV viremia compared to pp65 antigenemia tests [27]. In a study that included more than 700 specimens from HSCT patients, the FDA-approved Cobas AmpliPrep/Cobas TaqMan CMV test had sensitivity and specificity of 85% and 98% when performed in whole blood and 100% and 91% when performed in plasma, respectively [79].

The main disadvantage of CMV PCR testing is the significant inter-assay quantification variability. The variability of viral load tests is greatest near the lower and upper levels of quantitation. Viral load values in most patients are about 10–100-fold higher in whole blood compared with plasma [80]. A number of studies have shown a low correlation between the viral loads determined using different specimen types and molecular platforms [79]. Different primers to amplify CMV DNA by PCR lead to different size amplicons (52–340 base pairs) and, consequently, different viral loads [81]; namely, larger amplicons can lead to under quantitation in some samples and vice versa. However, when the same PCR assay is used, the test performs consistently across laboratories [82].

Despite the development of the World Health Organization (WHO) international standard for CMV DNA (so that quantitative PCR results could be reported in international units (IU) per mL instead of copies per mL), clinical studies in CMV remain difficult to interpret due to testing variability. Caliendo et al. [81] tested 40 genotyped CMV DNA-positive pooled clinical plasma samples in 6 laboratories using 10 quantitative PCR assays calibrated to the WHO international standard. Strikingly, they observed a median variance of 1.50 $\log_{10}$ IU/mL (range, 1.22–2.82 $\log_{10}$ IU/mL), with <60% of the results falling within ± 0.5 $\log_{10}$ IU/mL of the mean viral load of each sample. Because of pre-analytical and other analytical variables, the calibration of assays using the WHO standards alone is not enough to correct the variability across different assays [79, 81]. Transplant clinicians should be aware of this constraint of quantitative assays and always use a single laboratory for viral load testing when assessing viral kinetics and response to therapy so that results can be accurately interpreted.

Another drawback of CMV PCR monitoring is that low viral load values are common and their clinical significance can be difficult to assess. The increased sensitivity of molecular tests results in the prolonged detection of low-level viremia [79] (e.g., 25–200 IU/mL range) which can be difficult to interpret to

the clinician and can result in prolonged therapy and increased toxicity. As outlined above, many patients with low-grade viremia (typically <150 IU/mL) experience spontaneous clearance [8, 44]. On the other hand, sustained viremias >150 IU/mL are associated with significantly reduced probability of spontaneous clearance [8, 83].

It should be noted that due to compartmentalization of CMV in the CNS and GI tract, lack of CMV viremia does not rule out end-organ disease particularly CMV encephalitis, retinitis, or colitis. The opposite is also true; although detection of CMV nucleic acid in any body fluid or tissue specimen reflects CMV infection [51], it is not always indicative of disease.

PCR in BAL. In an elegant study conducted at the Fred Hutchinson Cancer Research Center using archived BAL aliquots from 132 HSCT patients with CMV pneumonia, 99%, 96%, 80%, 29%, and 6% of the patients had CMV detected by PCR, shell vial assay, culture, DFA, and cytology in BAL fluid, respectively. Patients with CMV pneumonia had higher median viral loads (3.9 $\log_{10}$ IU/mL; IQR, 2.6–6.0 $\log_{10}$ IU/mL) than controls: 0 $\log_{10}$ IU/mL, IQR, 0–1.6 $\log_{10}$ IU/mL for patients with non-CMV pneumonia; 0 $\log_{10}$ IU/mL, IQR, 0–1.6 $\log_{10}$ IU/mL for patients with idiopathic pneumonia syndrome; and 1.63 $\log_{10}$ IU/mL, IQR, 0–2.5 $\log_{10}$ IU/mL for patients who were asymptomatic; $P < 0.001$ for all comparisons to patients with CMV pneumonia. Thus, the likelihood for CMV pneumonia increases with increasing DNA viral load in BAL. The authors concluded that CMV DNA levels of >500 IU/mL in BAL can be used to differentiate CMV pneumonia from asymptomatic pulmonary shedding in HSCT recipients. However, considering the high variability in CMV DNA levels between different PCR methods [79, 81], this specific cutoff cannot be easily extrapolated to other centers where different primers and PCR platforms are used to quantify CMV. For example, in a study of CMV pneumonia in immunocompromised patients (including allogeneic HSCT and hematological malignancy) using the Cobas AmpliPrep/Cobas TaqMan CMV test calibrated to the WHO International Standard for CMV DNA, a BAL CMV viral load threshold of 34,800, IU/mL had 91.7% sensitivity and 100% specificity for diagnosis of CMV pneumonia in transplant patients; however, sample size was small, and patient population was heterogeneous [84]. In addition, the viral load cutoff can vary according to how the BAL procedure and processing are performed. Furthermore, CMV DNA levels may vary considerably between patients with varying degrees of severity of CMV pneumonia, which may impact the predictive values of any cutoff. Indeed, CMV shedding in the lower respiratory tract is common, and a low CMV DNA load might well represent asymptomatic infection. Thus, to date, a definite cutoff for CMV DNA load in BAL has not been established [51]. It is important to note that a negative CMV DNA test in the BAL fluid has a negative predictive value close to 100% and therefore excludes the possibility of CMV pneumonia [85].

PCR in tissue. High viral DNA levels detected with PCR in tissue from the relevant organ likely represent CMV disease, especially when blood sampled at the same time does not contain CMV DNA. CMV retinitis is the only condition where the symptoms and/or signs are sufficiently characteristic to allow a diagnosis of proven end-organ disease, even without testing for CMV in a tissue

sample. CMV documented by PCR from tissue biopsies is not sufficient for the diagnosis of CMV GI disease, especially in the absence of macroscopic mucosal lesion or histopathological findings (e.g., viral cytopathic effects). Interpretation of a positive CMV PCR in this setting can be even more difficult in cases of concurrent viremia, where contamination with circulating CMV at the time of tissue sampling could have occurred. Similarly, detection of CMV by PCR in CSF is less relevant in cases of visible contamination of blood ("bloody tap") [51].

Preventive Strategies

Currently, two main strategies are available for prevention of CMV disease following HSCT (Table 1): antiviral prophylaxis and CMV DNA level monitoring followed by preemptive therapy. In the 1990s, even though it had a significant reduction in the rates of CMV infection and disease, ganciclovir (GCV) prophylaxis was discontinued in many centers due to myelotoxicity [86]. Moreover, delayed CMV-immune reconstitution and late-onset CMV disease were observed in GCV preemptive and prophylaxis regimens [37, 87]. Recently, the Food and Drug Administration (FDA) approved letermovir, a non-myelotoxic drug, for CMV prophylaxis in CMV-seropositive HSCT recipients. Studies are now being conducted to establish the optimal duration of letermovir prophylaxis and the occurrence of delayed immune reconstitution or late-onset CMV disease.

It is important to emphasize that not all CMV-seropositive HSCT recipients develop CMV reactivation, even those with risk factors and not receiving prophylaxis. More than half of the patients in the placebo arm of the phase 3 letermovir trial (NCT02137772) did not develop clinically significant CMV infection by week 24 [36]. We have previously reported that 28% of allogeneic HSCT patients who develop detectable CMV DNAemia experience spontaneous clearance [8]. Thus, a significant proportion of CMV-seropositive HSCT recipients might not develop DNAemia or might experience self-resolved episodes. Such individuals are not likely to benefit from prophylaxis or preemptive antiviral therapy, respectively. A recent study showed that deep immune phenotyping of virus-specific CD8+ T-cell functional signatures can facilitate identification of patients at risk for clinically significant CMV infection [44], emerging as a clinical tool to assist in CMV risk stratification and management in HSCT recipients.

Preemptive Therapy

Unlike the case with other DNA viruses such as BK and HHV-6 for which the role of preemptive therapy remains largely unexplored and expectant management of asymptomatic viremia with close monitoring of viral load is almost always adequate, preemptive therapy is generally administered for detection of CMV in blood,

Table 1 Preventive strategies for CMV infection in allogeneic HCT recipients

D−/R− Low risk (<5%)
Leukoreduced blood products
CMV seronegative products
D+/R- Moderate risk (20–30%)
Preemptive therapy (see below) is favored
D+/R+ or D−/R+ High risk (60–80%)
Letermovir prophylaxis
Only indicated in CMV-seropositive
Consider PCR monitoring at least weekly since prophylaxis failure can occur
Requires concurrent administration of acyclovir for HSV/VZV prophylaxis
Preemptive therapy
Consider CMV PCR once or twice weekly until day 100 and then on clinic visits until day 200 and longer monitoring in those with ongoing immunosuppression (e.g., GvHD)
Optimal cutoff for initiation of therapy is unknown but viral loads >150 IU/mL[a] during the first 100 days often fail to resolve without antiviral therapy, particularly among high-risk patients[b].
Transplant centers should derive specific viral load thresholds depending on the CMV PCR assay they use and the population at risk
Valganciclovir is the agent of choice. In cases of severe thrombocytopenia or leukopenia, foscarnet may be preferred
While on therapy, CMV PCR should be monitored weekly. Viral load might not decrease after 2–3 weeks of therapy. If viral load increases (>1 $\log_{10}$) after 2 weeks of adequate therapy, antiviral resistance genetic testing should be ordered
Treat until clearance of viremia with at least one PCR result negative or below level of quantitation
Maintenance therapy does not effectively prevent recurrent viremia
CMV PCR monitoring should continue for at least 4–8 weeks after completion of therapy as recurrent CMV viremia occurs in >50% cases
Immune monitoring
In both prophylactic and preemptive strategies, assessment of CMV immunity may facilitate identification of individuals at risk for clinically significant CMV and guide duration of therapy.

[a]Cobas AmpliPrep/Cobas TaqMan CMV test
[b]T-cell depletion, mismatched donors, haploidentical, umbilical cord blood, GvHD, steroids >1 mg/kg

especially in cases with rising viral load on repeat testing or persistent viremia (>7 days; i.e., no blip).

Preventive preemptive strategies have dramatically decreased the incidence of early CMV disease from 25–30% to <5% according to the latest randomized trials [1, 2, 36]. In the preemptive strategy, antiviral therapy is initiated before the onset of CMV disease when viral load measurements reach a given threshold. One caveat to this approach is that, as noted earlier in this chapter, even low levels of CMV viremia early after allogeneic HSCT have been associated with increased NRM. Another limitation is that the frequency of monitoring (typically once or twice weekly) and the viremia cutoffs for initiation of CMV therapy vary widely across centers [3, 25, 35, 64–67, 88–93]. The recommended monitoring frequency is at least once weekly,

and the duration is at least 100–200 days after HSCT. The prevalence of CMV disease increases with screening intervals >14 days [15]. Even among patients who undergo weekly screening, the incidence of CMV disease can be above 10%. Thus, twice weekly screening, when feasible, might be a better approach during the first 100 days.

Once initiated, preemptive antiviral therapy is continued until virologic clearance (i.e., the virus is no longer detectable by CMV QNAT or has reached levels below a pre-defined viral load threshold). In a recent study in HSCT patients who received preemptive therapy, the median time to treatment-induced clearance was 28 days (IQR, 18–41 days), with a median duration of viremia of 42 days (IQR, 31–54 days) [8]. Delayed initiation of therapy results in prolonged duration of viremia and more protracted antiviral therapy.

Avoidance of marrow suppression is the major rationale for the preferred use of preemptive strategies in allogeneic HSCT in the pre-engraftment period. Disadvantages of the preemptive antiviral strategy include risk of disease prior to treatment initiation in individuals with rapid CMV replication (viral load doubling time can be as short as 2–3 days in high-risk patients [93, 94]), indirect effects of CMV replication, and laboratory costs due to more frequent viral load monitoring [82].

When to start therapy? Perhaps the major drawback of the preemptive approach is the lack of established quantitative cutoffs that are predictive of CMV disease in HSCT recipients due to the lack of standardized quantification assays that perform comparably across laboratories. Many centers in North America have implemented CMV viremia thresholds ranging from 25 to 300 IU/mL for initiation of therapy in high-risk patients [25, 88, 91], whereas some centers in Europe delay initiation of anti-CMV therapy until viremia reaches ≥1500 to 2500 IU/mL [92, 93]. CMV viremia thresholds should be interpreted with caution because CMV DNA levels vary considerably across studies depending on the type of specimen (i.e., higher CMV viral loads in whole blood compared with plasma) and the specific CMV PCR platform [80, 81].

Initiation of preemptive therapy at low CMV levels is associated with shorter episodes of viremia and shorter courses of antiviral therapy [8, 25, 91]. Recently, Boeckh et al. [9] reported an association between CMV viral load ≥250 IU/mL and increased risk of mortality in the early (days 0–60) posttransplantation period, suggesting that preemptive strategies should be aimed at keeping CMV viremia below this threshold. In a large HSCT cohort, a lower treatment threshold (≥25 IU/mL) in patients at greater risk of rapid CMV replication (e.g., T-depleted or steroid-treated recipients) improved preemptive treatment success [88]. The use of the 25 copies/mL viral load threshold for initiation of therapy as part of an intensive strategy significantly reduced the incidence of CMV disease among umbilical cord recipients [25]. In a study that included 213 T cell depleted by CD34+ selection, CMV viremia occurred in >70% of seropositive patients at a median time of 27 days post-HSCT. The risk of persistent viremia in this study increased by 9% for each day of delayed antiviral therapy [20]. Recently, Tan et al. reported significantly shorter median time to resolution of viremia when treatment was initiated at lower viral loads: 15 days for

135–440 IU/mL, 18 days for 441–1000 IU/mL, and 21 days at >1000 IU/mL ($P < 0.001$) [91]; the authors suggested that that preemptive therapy should be started with low-level viremia (135 IU/mL) but also acknowledged that this may unnecessarily treat viremia that might resolve spontaneously. This poses a therapeutic dilemma, because the salutatory effects of early antiviral therapy must be balanced against the risk of drug-related toxicity due to overtreatment, in addition to the financial burden.

Transplant centers are encouraged to derive specific viral load thresholds depending on the CMV QNAT assay they use and the population at risk. In a study of 174 HSCT recipients transplanted at our center, the proportion of patients who experienced spontaneous clearance decreased in a stepwise manner with increasing peak viremia thresholds: 25%, 14%, 4%, 3%, 1%, and 0% for CMV peak viremias of >100, >150, >200, >350, >500, and >1000 IU/mL, respectively [8]. Among patients with a CMV peak viremia <150 IU/mL, 89% achieved spontaneous clearance, compared with only 14% with a peak viremia level ≥150 IU/mL ($P = 0.0001$) [8]. A CMV peak viremia ≥150 IU/mL was strongly associated with a >80% reduced probability of spontaneous clearance independent of other factors including CMV donor serostatus, administration of ATG, and underlying lymphoid malignancy [8]. Among the subgroup of patients who received antiviral therapy, those who started treatment with a preceding CMV value ≥350 IU/mL exhibited more protracted viremia that in many cases required an antiviral course >35 days, beyond which point the risk of therapeutic failure and NRM increased substantially [8]. These data suggest that when using the Cobas AmpliPrep/Cobas TaqMan CMV test, initiation of preemptive therapy should not be delayed until viral load levels >350 IU/mL, and preferably antiviral therapy should be started in those with viremia >150 IU/mL, especially in high-risk allogeneic patients. We did not observe an increased incidence of (val)ganciclovir-related myelotoxicity among patients who received preemptive therapy at lower viral loads (<350 IU/mL) [8]. This suggests that early initiation of antiviral therapy is not only beneficial (i.e., shorter duration of viremia) but also well-tolerated.

In the letermovir trial [36], using thresholds for the preemptive treatment of CMV viremia of >150 copies/mL (equivalent to 137 IU/mL) for high-risk patients and >300 copies/mL for low-risk patients, CMV disease was uncommon (occurring in 1.5% of the patients in the letermovir group and 1.8% of those in the placebo group) and involved the gastrointestinal tract in all cases.

Viral kinetics are more useful than a single viral load [80, 94–96], and therefore preemptive strategies guided by the viral doubling time have been proposed recently [97]. In a cohort that included solid organ ($n = 74$) or HSCT ($n = 119$) recipients, the median doubling time (dt) of CMV DNA plasma levels was 4.3 days (IQR 2.5–7.8) [98], but it may be as short as 2 to 3 days in high-risk allogeneic HSCT recipients such as those receiving ATG or corticosteroids [93, 94]. Some groups have used the CMV dt to initiate preemptive therapy in those with evidence of rapid CMV replication (dt ⩽ 2 days) [93, 97, 99]. This strategy resulted in earlier initiation of therapy and shorter course of antivirals, but larger studies are needed [97].

Who to treat? Among asymptomatic HSCT patients undergoing regular monitoring with CMV PCR, three clinical phenotypes have been recently described [44]: (1) *elite controllers*, CMV-seropositive recipients who despite being at risk do not have evidence of CMV DNAemia on surveillance testing; (2) *spontaneous controllers*, CMV-seropositive recipients who spontaneously resolve low-grade CMV DNAemia (e.g., CMV DNA level <200 IU/ml) without antiviral therapy; and (3) *non-controllers*, CMV-seropositive recipients who experience clinically significant CMV defined as CMV disease [51] or CMV DNAemia leading to preemptive treatment. Non-controllers typically experience high-grade CMV DNAemia (peak >1,000 IU/mL) lasting more than 7 days. In a report that included 149 CMV-seropositive allogeneic HSCT recipients, 29% exhibited features of elite controllers; 20% were spontaneous controllers; and 51% were non-controllers. Since not all individuals at risk develop viremia and among those with CMV viremia only some (non-controllers) require preemptive antiviral therapy, research efforts have recently focused on the use immune monitoring to identify patients at risk of clinically significant CMV.

Interferon gamma (IFN-γ) is the dominant cytokine produced by T cells in response to CMV. QuantiFERON-CMV assay has predictive value for risk of late onset CMV disease in high-risk solid organ transplant (SOT) recipients [100]. Similarly, assessment of CMV cell-mediated immunity determined by production of IFN-γ using an enzyme-linked immunospot (ELISPOT) [41] or the QuantiFERON-CMV assay [42, 101] has been proposed as a method of immune monitoring to predict CMV outcomes following HSCT. A novel strategy that consisted of interrupting anti-CMV therapy upon CMV DNAemia clearance (first undetectable CMV DNA PCR result) and concurrent detection of virus-specific IFN-γ-producing CD8+ T cells at levels of >1 cell/μL was associated with reduced incidence of recurrent CMV DNAemia and shorter duration of therapy [102]. Chemaly and colleagues reported a strong association between low CMV-ELISPOT response and risk of CMV reactivation and progression to clinically significant CMV viremia [103]. However, factors such as the occurrence of GVHD and the use of systemic corticosteroids favored the development of CMV reactivation, regardless of the ELISPOT CMV assay result. In addition, IFN-γ production alone assessed by flow cytometry was not predictive of CMV clinical phenotypes in a recent study [44]. Thus, the clinical utility of IFN-γ-based immune monitoring methods for assessment of risk of CMV following HSCT requires further study.

Deep immunophenotyping of CMV(pp65)-specific CD8+ T cells using IFN-γ, MIP-1β, TNF-α, and IL-2 permits assessment of two donor-derived functional signatures that are highly predictive of CMV outcomes [44]. The first of them, the *non-protective signature* (IL2-IFNγ + TNFα-MIP1 β+), was found at increased levels among patients who experienced early CMV reactivation. Increased skewing toward this signature was highly predictive for CMV reactivation (any DNAemia) and the risk of progression to clinical significant CMV infection [44]. The second signature associated with risk of CMV was the *protective signature* (IL2 + IFNγ + TNFα+MIP1β+); patients whose CD8+ T-cell responses contained a lower relative proportion of cells with this signature had an increased risk of

clinically significant CMV [44]. If prospectively validated in large cohort studies, assessment of these functional signatures – using a readily available technique across transplant centers – could standardize CMV immune monitoring and guide clinical decisions such as initiation and duration of prophylactic and preemptive antiviral therapy [104].

What to start? (Val)ganciclovir is the agent of choice for preemptive therapy (typically 900 mg p.o. every 12 h of valganciclovir or 5 mg/kg i.v. every 12 h of ganciclovir; Table 2). In patients with neutropenia (e.g., <1000/μl) or severe thrombocytopenia (e.g., platelet count <20,000/μl), foscarnet (typically 90 mg/kg i.v. every 12 h) is the preferred agent. Both agents are effective at halting viral replication and preventing CMV disease. In a randomized multicenter trial of foscarnet ($n = 110$) versus ganciclovir ($n = 103$) for preemptive therapy of CMV, the occurrence of CMV disease or death from any cause within 180 days after HSCT was similar in the two treatment groups. Severe neutropenia ($< 0.5 \times 10(9)/L$) occurred in 11% patients on ganciclovir versus 4% patients on foscarnet ($P = 0.04$), and impaired renal function was observed in 5% patients on foscarnet versus 2% patients on ganciclovir ($P = 0.4$) [105].

When to stop? Antiviral preemptive therapy is typically continued for a minimum of 2 weeks and until there is resolution of viremia. With the advent of CMV PCR, one common approach is to discontinue therapy once CMV DNA is no longer detected in blood by PCR or detected but below level of quantitation on two separate specimens drawn a few days or a week apart. Once viremia resolves, many HSCT providers traditionally continue antiviral therapy at reduced dose (e.g., valganciclovir 900 mg daily) for a minimum of 2–4 weeks as maintenance therapy or secondary prophylaxis. The theoretical rationale for maintenance therapy is the high rate of recurrent viremia. In a recent report of 66 allogeneic HSCT recipients, 71% had a second episode of CMV viremia after documented treatment-induced clearance of viremia [106]. The median time to the first recurrence of CMV viremia was 38 days (IQR, 26–72 days).

However, recent data suggests that cessation of antiviral treatment at detection of the first negative CMV DNA load is as safe as doing so upon documentation of two consecutive PCR results and that secondary prophylaxis does not reduce incidence of recurrent viremia. Solano et al. discontinued antiviral therapy at the first negative (undetectable) PCR result (<20 copies/mL or 31 IU/mL), in 38 allogeneic HSCT recipients with preemptively treated CMV viremia, irrespective of the number of days the patient had received antiviral therapy [99]. Interestingly, the cumulative incidence of recurrent viremia was similar to a group of historical controls ($n = 40$) in whom antiviral therapy was discontinued after two consecutive negative PCR results after a minimum of 2 weeks treatment. In an open-labelled study that included SOT and HSCT recipients, Griffiths et al. found no significant difference in the incidence of recurrent viremia (35 vs. 45%) among patients in whom antiviral treatment was interrupted after two consecutive negative PCR results (CMV DNA load <168 IU/mL) and those in whom the threshold used to prompt the inception of antivirals (CMV DNA load <2520 IU/mL) was used to guide discontinuation of therapy, respectively [107]. Similarly, our group recently reported that among

Table 2 Antiviral agents for prophylaxis and treatment of CMV infection

Brand name	Dose	Mechanism of action	FDA approval	Major toxicity	Resistance	Primary indication	Comments
Ganciclovir	Prophylaxis 5 mg/kg IV daily Treatment 5 mg/kg IV every12h	Inhibition of DNA polymerase (pUL54)	1989	Myelosuppression	UL97, UL54	Treatment (and secondary prophylaxis/ maintenance therapy)	Drug of choice to treat CMV infections Myelotoxicity precludes its use for primary prophylaxis Activity dependent on phosphorylation by pUL97 kinase Renal dosing required
Valganciclovir	Prophylaxis 900 mg PO daily Treatment 900 mg PO every 12 h		2001				
Foscarnet	Prophylaxis 90 mg/kg IV daily Treatment 90 mg/kg IV every 12 h	Inhibition of DNA polymerase (pUL54)	1991	Nephrotoxicity, electrolyte imbalances, myelosuppression, penile/vulvar ulceration	UL54	Treatment (and secondary prophylaxis/ maintenance therapy)	Drug of choice for patients who are intolerant or fail to respond to (val) ganciclovir Renal dosing required using calculation of

(continued)

Table 2 (continued)

Brand name	Dose	Mechanism of action	FDA approval	Major toxicity	Resistance	Primary indication	Comments
							creatinine clearance (mL/min/kg)
Cidofovir	5 mg/kg IV weekly or 1 mg/kg IV three times a week	Inhibition of DNA polymerase (pUL54)	1996	Nephrotoxicity, myelosuppression	UL54	Treatment	
Maribavir	400, 800, or 1200 mg PO every 12 h	Competes with ATP for binding to pUL97, thereby inhibiting CMV DNA replication, maturation, encapsidation, and nuclear egress of viral capsids	Drug has been granted fast track status by US FDA	Dysgeusia (>60%)	UL97	Treatment of resistant or refractory CMV. 70% efficacy rate reported in phase 2 trial (**NCT01611974**) Two confirmatory phase 3 studies are underway for resistant or refractory CMV (NCT02931539) and preemptive therapy (NCT02927067)	After successful phase 1 and 2 trials, maribavir, at a potentially suboptimal selected dosage (100 mg BID), failed to show efficacy in the prevention of CMV disease in a phase 3 trial CMV isolates that are UL97 resistant to maribavir remain susceptible to ganciclovir (and vice versa)

Brincidofovir (CMX001)	100 mg PO twice a week	Inhibition of DNA polymerase (pUL54)	No longer under investigation in the United States	Diarrhea (>10%)	UL54	Treatment of resistant or refractory CMV (and mixed dsDNA infections)	Oral lipid conjugate of cidofovir with broad antiviral activity in vitro against dsDNA viruses including herpesviruses, polyomaviruses, adenoviruses, papillomaviruses, and variola virus High oral bioavailability and long half-life, allowing twice weekly oral dosing. No nephrotoxicity or myelosuppression In the phase 3 trial, brincidofovir failed to prevent clinically significant CMV infection through week 24 after HCT

(continued)

Table 2 (continued)

Brand name	Dose	Mechanism of action	FDA approval	Major toxicity	Resistance	Primary indication	Comments
Letermovir	480 mg IV/PO daily beginning between day 0 and 28 (typically before day +10) until day +100 240 mg daily if co-administered with cyclosporine	Inhibits CMV DNA synthesis at a late step by targeting the pUL56 subunit of the terminase enzyme complex	2017	Well-tolerated	UL56	Primary prophylaxis (until day +100) Treatment of resistant or refractory CMV Studies ongoing for use of letermovir for extended primary prophylaxis (until day 200) and for secondary prophylaxis	Most potent in vitro anti-CMV agent to date Superior to placebo in phase 3 trial (NCT02137772) Needs co-administration of acyclovir for HSV/VZV prophylaxis CMV PCR monitoring indicated to screen for breakthrough infections Not recommended for patients with severe hepatic impairment (Child-Pugh class C). Renal dosage adjustments for creatinine clearance (CrCl) of less than 10 mL/min, though serum creatinine should be monitored when administered to patients with a CrCl of less than 50 mL/min.

patients with an initial episode of early CMV reactivation who achieved viral clearance in response to preemptive therapy ($n = 66$) recurrence occurred at a similar rate in patients who received maintenance therapy (72%) and those who did not (79%) [106]. Altogether, these studies indicate that earlier discontinuation of preemptive therapy does not result in increased risk of recurrence viremia. While further studies are needed, assessment of virus-specific cell-mediated immunity might be useful to guide duration of therapy [104]. The efficacy of this approach has been recently demonstrated in SOT recipients [108].

Prophylaxis

Prior to November 2017, preemptive strategy was the most commonly used approach due to the prohibitive toxicity of the until then approved antivirals with CMV activity. With the advent of letermovir, CMV prophylaxis is now feasible for high-risk CMV-seropositive HSCT patients. The main advantage of prophylaxis is prevention of replication in the early posttransplant period potentially reducing the indirect effects of CMV such as predisposition to opportunistic infections and increased NRM. Another theoretical advantage is the elimination of the need for viral load monitoring during prophylaxis, although in the phase 3 letermovir trial patients underwent CMV viral load monitoring while taking the drug, and considering that prophylaxis failure (i.e., breakthrough CMV infection) can occur in up to 18% [36], it is prudent to continue to monitor CMV viral load in patients receiving letermovir prophylaxis.

In a meta-analysis that included 4173 patients enrolled in 19 clinical trials (1981–2017), 14 trials that compared antiviral prophylaxis (acyclovir, valacyclovir, ganciclovir, maribavir, brincidofovir, and letermovir) with placebo demonstrated overall effectiveness in reducing incidence of CMV early infection (odds ratio [OR], 0.49; 95% confidence interval [CI], 0.42–0.58), CMV disease (OR, 0.56; 95% CI, 0.40–0.80), and the use of preemptive therapy (OR, 0.51; 95% CI, 0.42–0.62; 6 trials); however, none demonstrated reduction in all-cause mortality (OR, 0.96; 95% CI, 0.78–1.18) except the phase 3 trial of letermovir (week 24 OR, 0.59; 95% CI, 0.38–0.98) [109].

(Val)ganciclovir. Administration of ganciclovir to allogeneic HSCT recipients from engraftment to day 100 results in low rates of CMV disease (2.7%), but it is associated with increased risk of early invasive fungal infections and late CMV disease [87]. In a recent randomized, double-blind, multicenter trial that included 184 HSCT recipients at high risk for late CMV disease, 6-month valganciclovir prophylaxis was compared with a strategy of CMV PCR-guided preemptive therapy. Although the incidence of a CMV DNAemia level of 1000 copies/mL or greater or a fivefold increase over baseline was reduced in the valganciclovir group (11% vs. 36%; $P < 0.001$), there were no differences between these two strategies in the composite primary end point of death, CMV disease, or other invasive infections by 270 days after HSCT [110]. In a randomized clinical trial of ganciclovir versus acyclovir for prevention of CMV, patients received i.v. ganciclovir 5 mg/kg every

12 h days −7 to −2, followed by i.v. acyclovir 10 mg/kg every 8 h from day −1 until neutrophil engraftment and then randomly assigned to either ganciclovir 5 mg/kg 5 days a week ($n = 45$) or acyclovir 800 mg p.o. five times a day ($n = 46$) until day 100 posttransplant. There were no differences in the cumulative incidence of CMV antigenemia or disease [111]. However, bacterial infections (12.4 per 1000 patient-days) and secondary neutropenia (71%) occurred more frequently in the ganciclovir group.

Five randomized trials suggested that ganciclovir (administered for a median of 101 days) was effective in reducing incidence of CMV infection and CMV disease after allogeneic HSCT but was not associated with a reduction in all-cause mortality, likely secondary to drug discontinuation related to clinically significant myelosuppression [109]. The reported incidence of neutropenia (absolute neutrophil count, ANC <1000 cells/mm^3) in patients receiving valganciclovir prophylaxis is 43% [110]; the incidence of neutropenia with ANC < 500 in the late CMV prevention trial was 7% although in the study protocol growth factors were prescribed for neutropenia <1000 cells/mm^3) [112]. In another randomized trial, neutropenia requiring treatment interruption occurred in 58% of the patients receiving i.v. ganciclovir for prevention of early CMV [113]. In a retrospective study among 72 patients who received preemptive therapy (treatment dose is twice that of prophylaxis dose) with valganciclovir, the occurrence of treatment-associated cytopenia (defined as neutropenia with an ANC <500 cells/mm^3 or requiring administration of growth factors or platelet transfusion while receiving antiviral therapy) was >50%, especially among those patients in whom therapy was started at viral loads >350 IU/mL [8].

(Val)acyclovir. Compared to historical controls, high-dose acyclovir (500 mg/m^2 i.v. every 8 h, or 800 mg p.o. four times a day) from day 7 for 3 months post-transplant in conjunction with weekly CMV pp65 antigenemia monitoring and preemptive treatment was associated with reduced incidence of CMV disease [114]. However, in a recent meta-analysis, acyclovir showed nonsignificant efficacy in preventing CMV infection or disease [109]. In a study that included more than 700 allogeneic HSCT recipients, administration of oral valacyclovir 2 g p.o. four times a day until week 18 after transplantation was more effective than acyclovir 800 mg p. o. four times a day in preventing CMV reactivation and showed a similar safety profile. GI symptoms such as nausea, abdominal pain, or diarrhea were the most common adverse events (reported in approximately 20%) [115]. A retrospective study in which valacyclovir at a dose of 1 g p.o. three times a day was used as primary ($n = 12$) or secondary prophylaxis ($n = 19$) demonstrated significantly lower incidence of CMV reactivation (25 vs. 77%) or recurrence (26 vs. 67%) compared to a control group ($n = 31$) that received acyclovir 400 mg p.o. twice a day for varicella-zoster virus prophylaxis [116]. Milano et al. [25] demonstrated a lower rate of CMV reactivation and disease among seropositive umbilical cord blood transplantation recipients receiving an "intensive" prophylaxis strategy consisting of ganciclovir on days −8 to −2 pretransplantation, high-dose valacyclovir post-transplantation (2 g of valacyclovir p.o. every 8 h or 500 mg/m^2 acyclovir i.v. every 8 h until tolerating oral medications), and twice-weekly serum CMV polymerase

chain reaction testing. Preemptive therapy in this intensive strategy was initiated at viral loads ≥25 copies/mL. CMV disease in the first 100 days was 4.6% in the intensive approach versus 27.6% with the standard strategy, consisting of acyclovir 800 mg p.o. or valacyclovir 500 mg p.o. twice daily for VZV/HSV prophylaxis and initiation of anti-CMV therapy if patients developed ≥500 copies/mL (or ≥100 copies/mL if receiving steroids) on weekly testing. In a follow-up study, the authors showed that a "modified intensive" approach without pretransplant ganciclovir resulted in similar rates of CMV reactivation and CMV disease in umbilical cord transplant recipients compared with those receiving an intensive strategy [117].

Maribavir. A dose-escalation phase 2 trial had demonstrated antiviral activity (reduced CMV infection and need for preemptive therapy) at doses ranging from 100 to 400 mg p.o. twice daily [118]. However, the results of the phase 3 trial demonstrated that maribavir at 100 mg twice daily started after engraftment had no significant effect on incidence of CMV disease, CMV reactivation, or preemptive therapy for CMV after allogeneic HSCT compared with placebo by HSCT week 24 and had no statistically significant effect on mortality [109, 119, 120]. Taste disturbance occurred in up to a third of the patients (18–31%) in the maribavir arm.

Brincidofovir. Similar to maribavir, brincidofovir (CMX001) showed antiviral activity in the phase 2 trial but was not superior to placebo in the phase 3 trial. The phase 2 dose-ranging trial demonstrated significantly lower CMV events with brincidofovir at a dose of 100 mg p.o. twice weekly when started after engraftment through week 13 post-HSCT [121]. Yet, the same dose of brincidofovir did not improve CMV-related outcomes in the phase 3 (SUPPRESS) trial that evaluated treatment against placebo beginning a median of 15 days post-HSCT [122]. Furthermore, brincidofovir was associated with increased rates of diarrhea (61%), acute GVHD with gastrointestinal involvement (57%), other gastrointestinal adverse events, and a nonsignificant increased risk of death when compared with placebo [121, 122]. Whether the development of an intravenous formulation of brincidofovir that does not result in gastrointestinal accumulation but retains the drug's spectrum, antiviral activity, and lack of nephrotoxicity or myelotoxicity could provide a more effective and safer prevention of CMV remains to be studied [122].

Letermovir. In the phase 3 trial that included 495 adult seropositive HSCT patients with undetectable CMV DNA at randomization, fewer patients in the letermovir group (letermovir started at a median of 9 days posttransplantation) than in the placebo group had clinically significant CMV infection (defined as CMV disease or CMV viremia leading to preemptive treatment) or were imputed as having a primary end point event by week 24 after transplantation (122 of 325 patients [37.5%] vs. 103 of 170 [60.6%], $P < 0.001$). Clinically significant CMV occurred in 18 versus 42% of patients in the letermovir and placebo groups, respectively. Administration of letermovir through week 14 (approximately 100 days) after HSCT was also associated with reduced all-cause mortality at week 24. However, this survival benefit was not statistically significant at week 48. The rates of myelotoxic and nephrotoxic events were similar in the letermovir

group and the placebo group. Gastrointestinal disorders are common with administration of letermovir (52–76%); peripheral edema, cough, headache, and fatigue occur at a rate >10% in letermovir-treated patients. In the phase 3 trial, atrial fibrillation or flutter occurred in 4.6% and 1.0% of letermovir and placebo-treated patients, respectively. Drug-drug interactions are another important consideration as letermovir is a weak-to-moderate inhibitor of cytochrome P-450 3A (CYP3A) and a weak-to-moderate inducer of CYP2C9 and CYP2C19 enzymes [36]. Letermovir increases levels of cyclosporine, and it is administered at a dose 240 mg per day in patients taking cyclosporine. For patients with tacrolimus or sirolimus levels >8 ng/mL, empirical reductions of 50% and monitoring of immunosuppressant drug levels are advisable [109]. The dose of statins should also be reduced if co-administered with letermovir [109]. Letermovir reduces the levels of voriconazole, and an alternative agent for anti-mold prophylaxis such as posaconazole should be considered.

Lin et al. reported 15 patients who received letermovir as secondary prophylaxis for a median duration of 127 days (18–270) with no reactivation (TCT 2019, abstract #128). However, other small case series have shown a breakthrough CMV infection rate of 30% in 6 HSCT patients receiving letermovir for this indication (TCT 2019, abstracts #406 and #416). Thus, there are currently insufficient data to recommend the use of letermovir for secondary prophylaxis. However, in a recent French study that included 80 HSCT recipients with at least one prior episode of CMV (infection or disease), letermovir was found to be effective for secondary prophylaxis with <6% of the patients developing breakthrough infections [123].

It should be noted that concurrent administration of acyclovir or equivalent for HSV/VZV prophylaxis is indicated in patients receiving letermovir prophylaxis as this agent only has anti-CMV activity. Also, CMV PCR monitoring is highly recommended in patients receiving letermovir prophylaxis as breakthrough infections can occur [124, 125]. Recently, breakthrough CMV pneumonia was reported in a haploidentical cord blood transplant recipient receiving letermovir prophylaxis. Resistance in this case was conferred by a UL56 C325F mutation [124].

Other considerations. Simultaneous reactivation of DNA viruses is a common phenomenon following allogeneic HSCT [126]. In the experience of the author, breakthrough CMV DNAemia can occur in patients receiving cidofovir for other indications such as BK hemorrhagic cystitis or adenovirus infections. Thus, in patients with mixed viral DNA infection who have not cleared CMV DNAemia prior to initiation of cidofovir, CMV preemptive or maintenance therapy might need to be continued while on cidofovir. Brincidofovir, an oral lipid conjugate of cidofovir, has more potent activity than cidofovir against CMV and has been shown to be useful for the treatment of mixed DNA viral infection [127].

In a large cohort study, Marty et al. showed that sirolimus-containing GVHD prophylaxis regimens are associated with a significant reduction in the incidence of CMV reactivation [128]. This concept was confirmed in recent studies where risk for CMV DNAemia increased with increasing tacrolimus concentrations and decreased with increasing sirolimus concentrations among HSCT recipients receiving GVHD prophylaxis [129, 130].

A Cochrane systematic review of polyvalent immunoglobulins or hyperimmune CMV-IVIG prophylaxis that included 6 trials including CMV infections in 986 hematological malignancy and HSCT participants showed no difference in the occurrence of CMV infection or all-cause mortality, compared to control subjects [131].

Treatment

Antivirals

Four antiviral drugs are approved by the US Food and Drug Administration for treatment of CMV (Table 2): ganciclovir (1989), foscarnet (1991), cidofovir (1996), and the prodrug valganciclovir (2001). Intravenous ganciclovir is the recommended initial treatment for severe or life-threatening CMV disease. Because of the risk of nephrotoxicity, foscarnet and cidofovir are considered second and third line drugs, respectively, for HSCT recipients unable to tolerate valganciclovir or ganciclovir. Although letermovir can be considered in selected cases for salvage therapy in patients with highly resistant UL97/UL54 mutation CMV [132, 133], currently there is insufficient data to support its use for secondary prophylaxis or treatment.

CMV DNA levels should be measured weekly while on treatment. More frequent assessments are not recommended as they do not provide useful information and might make the clinical picture confusing due to variability of the PCR testing. Importantly, the CMV levels often continue to rise during the first week or two of therapy [52], and this should not be interpreted as failure to therapy nor lead to concerns about resistance. Also, in order to be able to accurately assess response to therapy, it is important to send baseline CMV DNA levels on the day of initiation of therapy, especially if the PCR results triggering initiation of therapy were obtained >2–3 days prior to first dose of antiviral.

A minimum of 2–3 weeks of antiviral therapy should be administered to patients with CMV reactivation. Duration of therapy should be guided by resolution of signs/symptoms in cases of CMV disease and by resolution of viremia in cases of asymptomatic reactivation.

Resistance

CMV resistance has been increasingly recognized and may be caused by mutations targeting the UL54 DNA polymerase and the UL97 kinase. Antiviral drug resistance should be suspected when CMV viremia (DNAemia or antigenemia) fails to improve (i.e., >1 $\log_{10}$ increase in CMV DNA levels in blood or serum) after 2 weeks of appropriately dosed and delivered antiviral therapy. Antiviral drug resistance should also be suspected when CMV end-organ disease occurs during prolonged antiviral therapy (>6 weeks of antiviral drug exposure, including 2 weeks of full-dose

therapy) in the presence of risk factors [5]. In adult drug-naive patients, it is unusual for resistance to develop during the first 6 weeks of therapy.

Refractory CMV infection is defined as CMV viremia that increases (i.e., >1 $\log_{10}$ increase in CMV DNA levels in blood or serum between peak viral load within the first week and the peak viral load at ≥ 2 weeks as measured in the same laboratory with the same assay) after at least 2 weeks of appropriately dosed antiviral therapy [134]; this is similar to resistance, except that known genetic mutations conferring resistance are not detected (clinical resistance). A modest quantitative increase in viral loads during the first 2 weeks of anti-CMV therapy may occur; this is not an indication of drug resistance and may not necessitate a change of therapy [5].

Risk factors for CMV resistance in HSCT recipients [5, 134] include:

i) Host factors: prolonged antiviral CMV drug exposure (>3 months); previous antiviral CMV drug exposure; recurrent CMV infection; inadequate antiviral CMV drug absorption and bioavailability; inadequate antiviral CMV oral prodrug conversion; variation in antiviral CMV drug clearance; subtherapeutic antiviral CMV drug level; poor patient compliance with antiviral drug regimen; T-cell depletion; haploidentical, allogeneic, or cord blood HSCT; delayed immune reconstitution; CMV-seropositive recipient and CMV-seronegative donor; treatment with ATG; active GVHD; young age;
congenital immunodeficiency syndromes
ii) Viral factors: CMV viral load rise while receiving treatment (after >2 weeks of adequate dosing); failure of CMV viral load to fall despite appropriate treatment; rise in CMV viral load after initial decline while receiving appropriate treatment; intermittent low-level CMV viremia; and high CMV viral loads

CMV resistance has been reported in the range 0% to 10% [135–139]. At our center CMV resistance was observed in 3% of allogeneic HSCT recipients [8]. Among high-risk patients, such as haploidentical transplants, the incidence of CMV resistance has been reported to be as high as 14.5% [140].

Genotypic testing is commonly done on the same plasma or whole-blood specimens used for viral load assays. DNA sequencing is currently the standard for detection of drug resistance, and the analysis is more reliable if the CMV viral load is at least 1000 IU/mL [5]. The accuracy of detection of variant CMV subpopulations is reduced with insufficient CMV DNA present in the sample (e.g., <500 IU/mL). False negatives can occur if mutant strains comprising 20% to 30% of the viral population are not reported [5]. Detection of emerging drug-resistant mutant CMV subpopulations can be improved by deep sequencing, but this technology is not widely available [141, 142]. In some cases where drug-resistant viral genomes and disease have become localized to a particular body site without significant viremia, a tissue-specific sample (i.e., vitreal fluid, cerebrospinal fluid, or biopsy) may be more informative.

In order to understand antiviral resistance mechanisms, it is important to understand key proteins encoded in the 236 kb CMV genome [5]:

- *UL54* (pol) encodes the highly conserved CMV DNA polymerase pUL54.
- pUL97, a protein kinase, is the product of the *UL97* and phosphorylates some CMV viral and cellular proteins and controls the phosphorylation of ganciclovir in CMV-infected cells.
- pUL56, which is encoded by *UL56*, is one of two proteins forming the CMV terminase complex responsible for the process of replication, cleavage, and packaging of new virions.

(Val)ganciclovir, (brin)cidofovir, and foscarnet all target and inhibit CMV DNA polymerase pUL54 preventing viral DNA synthesis. In order to become active, ganciclovir must undergo activation to ganciclovir-triphosphate upon phosphorylation by pUL97 and cellular kinases in the CMV-infected cell. Maribavir competes with ATP for binding to pUL97, and it does not require intracellular phosphorylation and is independent of pUL54. Since maribavir inhibits UL97, it impairs phosphorylation of ganciclovir, and these two drugs should not be co-administered [143]. Letermovir has a unique mechanism of action as it is the only antiviral that targets the pUL56 subunit of the CMV terminase complex.

CMV resistance to (val)ganciclovir can result from modification of pUL97, whereas foscarnet and (brin)cidofovir activity is independent of pUL97 and is therefore unaffected by *UL97* mutations. *UL54* mutations induce various patterns of cross-resistance among all these drugs. Although ganciclovir and valganciclovir are affected by both UL97 and UL54 mutations, in the clinical setting, *UL97* mutations are much more likely to emerge first. CMV isolates that are resistant to maribavir may remain susceptible to ganciclovir (and vice versa) [5]. Due to its mechanism of action, letermovir can overcome any *UL97/UL54* mutations, but antiviral activity can be affected by *UL56* mutations.

A comprehensive review of the specific CMV codon mutations conferring different degrees of antiviral resistance is beyond the scope of this chapter, and excellent reviews are available on this topic [5, 144–149]. For ganciclovir, the most common *UL97* mutations confer a three- to tenfold increase in the EC_{50} (drug concentration required to reduce viral growth in cell culture by 50%), but combinations of UL97 and UL54 mutations result in higher levels of resistance [5]. Seven viral UL97 kinase mutations (amino acid substitutions M460 V/I, H520Q, C592G, A594V, L595S, and C603W) are established as the genetic basis of ganciclovir resistance in most cases [5, 148]. Namely, M460 V/I, H520Q, A594V, L595S, L595F, and C603W confer 5–15-fold increase in the EC_{50}, whereas C592G confers two- to fivefold increase. Viral *UL54* DNA polymerase gene mutations can confer resistance to any or all of the traditional CMV polymerase inhibitors ganciclovir, foscarnet, and cidofovir. Unlike UL97, there is no compact list of canonical resistance mutations [148] conferring resistance to maribavir. Maribavir-resistant mutations map to the *UL97* and the *UL27* genes [149]. Amino acid substitutions conferring resistance to letermovir selected in vitro map primarily to the pUL56 and rarely to the pUL89 and pUL51 subunits of the CMV terminase complex [149]. *UL56* mutations in the codon range 231–369 can confer impressive levels of resistance to letermovir [148, 150]. Breakthrough CMV viremia during letermovir

treatment can occur in the presence of the *UL56* V236M mutation [36]. The *UL56* mutation C325Y, which confers high-level resistance to letermovir, has been described in both SOT and HSCT recipients [36, 124, 132, 151, 152]. Critical letermovir binding residues are not critical to the biological function of the terminase complex [148]. Considering that letermovir was approved in 2017 and it has been mostly used for primary prophylaxis, the fact that UL56 have already been identified in letermovir-treated patients [36, 124, 132, 151] suggests low genetic barrier to resistance [124, 132, 148, 150–153].

When refractory or resistant CMV is suspected during (val)ganciclovir therapy, genotypic analysis for drug resistance should be obtained immediately, and empiric switch of antiviral therapy to foscarnet should be considered while awaiting genotypic analysis results, especially in symptomatic patients or those considered high risk for CMV disease (e.g., T-cell depletion, unrelated donors, GVHD on high-dose steroids) and those with the risk factors for CMV resistance outlined above. Foscarnet therapy should be continued in patients with CMV end-organ disease (regardless of the *UL97* mutation) and those with documented high-grade *UL97*-resistant mutations (e.g., M460V, A594V, H520Q, L595S, L595F, C603W). In the absence of CMV end-organ disease, dose escalation of ganciclovir (i.e., 7.5–10 mg/kg every 12 h) can be considered to overcome *UL97* mutations conferring low-grade resistance such as C592G [5, 144, 148]. Hematologic toxicity occurs in most patients receiving high-dose ganciclovir for more than 4 weeks. If there are additional *UL54* mutations increasing the overall level of resistance, combination therapy (e.g., ganciclovir plus foscarnet) should be strongly considered and tailored to the specific resistance pattern [5, 148]. For ganciclovir-foscarnet-cidofovir-resistant strains, investigational agents alone or in combination with standard therapies, as well as adoptive T-cell therapy [154–161], can be considered.

The full potential of novel therapies such as maribavir, brincidofovir, and letermovir for the treatment of CMV in HSCT remains to be established. In a recent randomized trial that included 47 allogeneic HSCT recipients, maribavir (doses from 400–1200 mg twice a day) was effective for treatment of refractory or resistant CMV infection, with 70% of patients achieving undetectable CMV DNA within 6 weeks of treatment [162]. Dysgeusia occurred in two thirds of the maribavir-treated patients but only led to discontinuation in one patient [162]. Brincidofovir can be used for ganciclovir-resistant CMV infection, unless resistance is acquired through a *UL54* mutation [5, 127, 163]. Letermovir might have a role in the treatment of *UL97* or *UL54* mutants [132, 164]. However, current evidence for the use of brincidofovir or letermovir in the treatment of refractory or resistant CMV infection is only anecdotal.

As it is the case with any serious infection in immunocompromised hosts, tapering of immunosuppression, in particular steroids, is a critical step in the management of resistant/refractory CMV infection. Adjunctive therapies such as CMV-Ig (or IVIG) and adaptive infusions of CMV-specific T cells may improve antiviral host defenses [154–161, 165–167]. There is in vitro and case report level of evidence for other agents such as leflunomide [168–174], artesunate [175–178], and mTOR inhibitors [128, 179]. However, the clinical efficacy of such therapies remains questionable. In patients who require ongoing immunosuppression for

GVHD prophylaxis or treatment, switching immunosuppressive therapy to an mTOR inhibitor may be reasonable [128, 179].

Adoptive CMV-Specific T-Cell Therapy

Administration of CMV-specific T cells obtained from the patient (autologous) [160, 161], the stem cell donor [155, 156, 158], or a third-party donor [154, 155, 159] has been shown to be safe and effective in the treatment and prevention of CMV infection in transplant recipients. The infusion of in vitro expanded autologous CMV(IE-1/pp65)-specific T cells was successfully used for the treatment of a lung transplant recipient with respiratory failure due to a ganciclovir-foscarnet resistant strain of CMV [160]. Cells exhibited a late effector/memory (CCR7- CD45 RA-) T-cell phenotype. In a recent open-label phase 1 clinical trial of 22 SOT patients with recurrent or ganciclovir-resistant CMV infection, the use of 1–6 infusions of in vitro-expanded autologous CMV-specific T cells led to improvement in symptoms, including complete resolution or reduction in DNAemia and CMV-associated end-organ disease and/or the cessation or reduced use of antiviral drugs, in 11 (84%) of the 13 treated patients [161]. Adoptive T-cell therapy was associated with no therapy-related serious adverse events. In vitro-expanded cells were predominantly $CD3^{+}CD8^{+}$ T cells with polyfunctional capacity (i.e., capable of expressing IFN-γ, TNF, and CD107a).

Administration of CMV-specific T-cell lines generated by short-term culture from the blood of transplant donors to 16 allogeneic HSCT recipients with early CMV infection resulted in no need for antiviral therapy in half of the cases and low rates (13%) of recurrent CMV [180]. In a follow-up study, none of the seven patients who were treated prophylactically with T-cell therapy required CMV antiviral therapy within the next 6 months [181]. In another study, among seven allogeneic HSCT recipients with persistent CMV viremia after 4 weeks of antiviral therapy and/or lacking CMV-specific T-cell proliferation who underwent adoptive transfer of donor-derived CMV-specific T cells, complete response was observed in five (71%) and partial response (transient reduction in viremia) in two [166]. In a phase 2 trial, a single dose of adoptively transferred donor-derived CMV-specific CD8+ T lymphocytes administered after day 28 posttransplant to 50 allogeneic HSCT recipients did not reduce the overall rate of CMV reactivation but did reduce the need for preemptive therapy and shortened the duration of antiviral therapy [167], without increasing the rates of GVHD. There were no serious, immediate infusion-related toxicities. Adoptive transfer of virus-specific T cells (VSTs; specific for EBV, CMV, AdV, HHV-6, and BKV), generated from allogeneic stem cell transplant donors, administered to 11 HSCT recipients, was shown to be safe in all subjects and produced an overall 94% virological and clinical response rate that was sustained long term [158]. In another study of 17 patients treated with transplant donor ($n = 16$) or third-party ($n = 1$) CMV(pp65)-specific CD8+ T cells, 15 (88%) cleared viremia, including 3 of 5 with overt disease. In responding patients, the infused CMV(pp65)-specific CD8+ T cells could be detected for a period of 120 days to up to 2 years after infusion [155].

Despite this compelling evidence, the use of cellular therapy in the clinical setting has been limited by logistical difficulties. Namely, the use of autologous or donor-derived CMV-specific T cells is problematic due to the cost and complexity of individualized product manufacture, the time needed for custom manufacturing (typically several weeks), which may preclude the immediate availability of T cells for urgent medical need, and the requirement for seropositive donors [154] (although recent data demonstrate that CMV-specific T cells that recognize atypical epitopes of CMV pp65 can be derived from naïve T cells taken from CMV-seronegative people and might be protective in vivo) [182]. In recent years, there have been efforts to generate HLA-matched VST lines from healthy seropositive donors, which can be available for immediate use as an "off-the-shelf" product. In a multicenter study of banked third-party VSTs (specific for EBV, CMV, and AdV) that included 23 allogeneic HSCT recipients with severe or refractory CMV infection, complete or partial responses at 6 weeks post-infusion were documented in 74% of the cases, with no immediate infusion-related adverse events or de novo (grades 2–4) GVHD [157]. In a phase 2 trial, a total of 23 allogeneic HSCT recipients received 1–3 infusions of off-the-shelf third-party polyclonal VSTs (T-cell lines specific for 12 viral antigens from EBV, CMV(IE-1/pp65), AdV, HHV-6, and BKV), including a total of 17 patients who received VSTs for persistent CMV, 8 of them with antiviral-resistant CMV; the response rate (complete and partial) was 94% [154]. Importantly, the in vivo persistence of VSTs from the infused line for up to 12 weeks was documented in 69% of the patients. Infusions were well-tolerated without serious immediate toxicities and no cases of de novo grades 2–4 GVHD or cytokine release syndrome. Of note, the authors could identify a suitable VST line for 54 (96%) of the 56 patients screened for study participation [154] suggesting that off-the-shelf products could be easily available to patients in urgent need for therapy. A recent study that included 28 allogeneic HSCT patients with persistent or recurrent CMV treated with infusions of partially HLA-matched, third-party, ex vivo-expanded VSTs showed that at 12 months, the cumulative incidence of overall response was 93% [159]. Acute GVHD occurred in only two patients, despite a median HLA match between VSTs and the recipient of two of six antigens. This data support the long-term safety and durability of off-the-shelf therapy.

Altogether these data support the applicability of CMV-specific T-cell therapy for the management of CMV infection in allogeneic HSCT recipients.

Vaccines

An effective CMV vaccine would have potential benefits not only for women of childbearing age (to prevent congenital CMV infection) but also for SOT and HSCT patients. In HSCT recipients, protection from CMV viremia is critically dependent upon the reconstitution and expansion of CMV-specific T-cell subsets, such as pp65 tegument (UL83) and the immediate early 1 (IE1, UL123) T cells [183]. Thus, there is significant interest in the development of immunization strategies that enhance CMV-specific immunity and prevent CMV viremia by triggering robust, functional,

and durable CMV-specific T-cell responses. A comprehensive review of the literature on prophylactic and therapeutic CMV vaccine development is out of the scope of this chapter, and excellent reviews are available on this topic [184–187]. To the date, only few CMV vaccines have been evaluated in randomized clinical trials in HSCT population [188, 189].

In a phase 2 trial of a CMV therapeutic DNA vaccine (TransVax) [188] that contains plasmids encoding CMV glycoprotein B and phosphoprotein pp65 formulated with poloxamer CRL1005 and benzalkonium chloride, 19 (48%) of 40 vaccine recipients required CMV-specific antiviral therapy, compared with 21 (62%) of 34 controls ($p = 0.145$). Although the rates of clinically significant viremia did not differ from those noted for placebo, vaccine significantly reduced the occurrence and recurrence of CMV viremia and improved the time-to-event for viremia episodes compared with placebo. The vaccine was well-tolerated. A phase 3 clinical trial is ongoing (NCT01877655).

CMVPepVax is a chimeric peptide composed of a cytotoxic CD8 T-cell epitope from CMV pp65 and a tetanus T-helper epitope. It is formulated with the adjuvant PF03512676, a Toll-like receptor 9 agonist, which augments cellular immunity. CMVPepVax, administered subcutaneously on days 28 and 56, was shown to be safe in allogeneic HSCT recipients, with no secondary graft failures or cases of GVHD in vaccinated subjects [189]. The vaccine was immunogenic providing proof of concept that HSCT recipients are able to mount vaccine-driven CMV-specific T-cell response within 6 weeks post-HSCT, when they are at the highest risk for CMV reactivation. However, CMVPepVax includes an HLA A∗0201 restricted CD8 T-cell peptide epitope of the pp65 protein, and consequently, its use is restricted to patients who are HLA A∗0201 positive. A phase 2 clinical trial is ongoing (NCT02396134).

CMV-MVA Triplex is a newer CMV vaccine developed by constructing a recombinant attenuated poxvirus-modified vaccinia Ankara encoding 3 immunodominant CMV antigens: UL83 (pp65), UL123 (IE1-exon4), and UL122 (IE2-exon5). In healthy subjects, Triplex vaccinations induced significant expansion of pp65-specific IFNγ+ CD8+ T cells, indicating that vaccine was able to expand a functional subset of CMV-specific T cells, even in the absence of CMV viremia. Expansion of durable CMV-specific T cells was observed in both CMV-seropositive and CMV-seronegative participants. This high immunogenicity of Triplex is in contrast to previously reported responses in CMV-seropositive healthy adults that were either low or detectable only after in vitro stimulation for both CMVPepVax peptide vaccine [190] and the TransVax CMV DNA vaccine [191]. Phase 2 trial with CMV-MVA Triplex is ongoing (NCT02506933).

Concluding Remarks

In conclusion, despite enormous advancements on molecular diagnostics and therapeutics over the last two decades, CMV remains a serious complication of HSCT associated with significant morbidity and mortality. However, a number of emerging

developments in the fields of CMV prophylaxis, treatment, and precision medicine have transformed the panorama by promising to improve outcomes of HSCT patients and facilitate clinical decision-making of HSCT providers, including:

1. The recent advent of effective and well-tolerated agents, such as letermovir, for prevention of CMV reactivation in seropositive HSCT recipients
2. The promising efficacy of investigational antiviral agents, such as maribavir, for the treatment of refractory or resistant CMV infection in transplant recipients
3. The revolutionary use of immune monitoring, based on deep immune phenotyping of virus-specific CD8+ T cells, emerging as a bench-to-bedside clinical tool to assist in CMV risk stratification and management of HSCT recipients
4. The remarkable development of highly effective off-the-shelf virus-specific T-cell therapies for treatment of CMV infection
5. CMV remains a serious complication of HSCT associated with significant morbidity and mortality
6. Effective and well-tolerated agents, such as letermovir, for prevention of CMV reactivation in seropositive HSCT recipients are now available
7. Investigational antiviral agents, such as maribavir, might have a role for the treatment of refractory or resistant CMV infection in transplant recipients
8. Immune monitoring, based on deep immune phenotyping of virus-specific CD8+ T cells, has emerged as a bench-to-bedside clinical tool to assist in CMV risk stratification and management of HSCT recipients
9. Highly effective off-the-shelf virus-specific T-cell therapies for treatment of CMV infection seem promising for the treatment of refractory or resistant CMV infection

References

1. Boeckh M, Geballe AP. Cytomegalovirus: pathogen, paradigm, and puzzle. J Clin Invest. 2011;121(5):1673–80.
2. de la Camara R. CMV in hematopoietic stem cell transplantation. Mediterr J Hematol Infect Dis. 2016;8(1):e2016031.
3. Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood. 2009;113(23):5711–9.
4. Camargo JF, Komanduri KV. Emerging concepts in cytomegalovirus infection following hematopoietic stem cell transplantation. Hematol Oncol Stem Cell Ther. 2017;10:233–8.
5. El Chaer F, Shah DP, Chemaly RF. How I treat resistant cytomegalovirus infection in hematopoietic cell transplantation recipients. Blood. 2016;128(23):2624–36.
6. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. Hematol Oncol Clin North Am. 2011;25(1):151–69.
7. Solano C, Gimenez E, Albert E, et al. Pre-engraftment cytomegalovirus DNAemia in allogeneic hematopoietic stem cell transplant recipients: incidence, risk factors, and clinical outcomes. Bone Marrow Transplant. 2019;54(1):90–8.
8. Camargo JF, Kimble E, Rosa R, et al. Impact of cytomegalovirus viral load on probability of spontaneous clearance and response to preemptive therapy in allogeneic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2018;24(4):806–14.

9. Green ML, Leisenring W, Xie H, et al. Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. Lancet Haematol. 2016;3(3):e119–27.
10. Minton K. Viral immunity: how CMV bypasses immune memory. Nat Rev Immunol. 2010;10(5):288.
11. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin Infect Dis. 2010;50(11):1439–47.
12. Hyde TB, Schmid DS, Cannon MJ. Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. Rev Med Virol. 2010;20(5):311–26.
13. Ljungman P, Brandan R. Factors influencing cytomegalovirus seropositivity in stem cell transplant patients and donors. Haematologica. 2007;92(8):1139–42.
14. Souza MA, Passos AM, Treitinger A, Spada C. Seroprevalence of cytomegalovirus antibodies in blood donors in southern, Brazil. Rev Soc Bras Med Trop. 2010;43(4):359–61.
15. Lodding IP, da Cunha Bang C, Sorensen SS, et al. Cytomegalovirus (CMV) disease despite weekly preemptive CMV strategy for recipients of solid organ and hematopoietic stem cell transplantation. Open Forum Infect Dis. 2018;5(5):ofy080.
16. Styczynski J. Who is the patient at risk of CMV recurrence: a review of the current scientific evidence with a focus on hematopoietic cell transplantation. Infect Dis Ther. 2018;7(1):1–16.
17. Marchesi F, Pimpinelli F, Ensoli F, Mengarelli A. Cytomegalovirus infection in hematologic malignancy settings other than the allogeneic transplant. Hematol Oncol. 2018;36(2):381–91.
18. Ljungman P, de la Camara R, Cordonnier C, et al. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. Bone Marrow Transplant. 2008;42(4):227–40.
19. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood. 2003; 101(10):4195–200.
20. Huang YT, Neofytos D, Foldi J, et al. Cytomegalovirus infection after CD34(+)-selected hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22(8):1480–6.
21. Ljungman P, Perez-Bercoff L, Jonsson J, et al. Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. Haematologica. 2006;91(1):78–83.
22. Kalra A, Williamson T, Daly A, et al. Impact of donor and recipient cytomegalovirus serostatus on outcomes of antithymocyte globulin-conditioned hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22(9):1654–63.
23. Komanduri KV, St John LS, de Lima M, et al. Delayed immune reconstitution after cord blood transplantation is characterized by impaired thymopoiesis and late memory T-cell skewing. Blood. 2007;110(13):4543–51.
24. Melendez-Munoz R, Marchalik R, Jerussi T, et al. Cytomegalovirus infection incidence and risk factors across diverse hematopoietic cell transplantation platforms using a standardized monitoring and treatment approach: a comprehensive evaluation from a single institution. Biol Blood Marrow Transplant. 2019;25(3):577–86.
25. Milano F, Pergam SA, Xie H, et al. Intensive strategy to prevent CMV disease in seropositive umbilical cord blood transplant recipients. Blood. 2011;118(20):5689–96.
26. Takami A, Mochizuki K, Asakura H, Yamazaki H, Okumura H, Nakao S. High incidence of cytomegalovirus reactivation in adult recipients of an unrelated cord blood transplant. Haematologica. 2005;90(9):1290–2.
27. Dahi PB, Perales MA, Devlin SM, et al. Incidence, nature and mortality of cytomegalovirus infection after double-unit cord blood transplant. Leuk Lymphoma. 2015;56(6):1799–805.
28. Law AD, Salas MQ, Lam W, et al. Reduced-intensity conditioning and dual T Lymphocyte suppression with antithymocyte globulin and post-transplant cyclophosphamide as graft-versus-host disease prophylaxis in haploidentical hematopoietic stem cell transplants for hematological malignancies. Biol Blood Marrow Transplant. 2018;24(11):2259–64.

29. Slade M, Goldsmith S, Romee R, et al. Epidemiology of infections following haploidentical peripheral blood hematopoietic cell transplantation. Transpl Infect Dis. 2017;19:e12629: 1–10.
30. Tischer J, Engel N, Fritsch S, et al. Virus infection in HLA-haploidentical hematopoietic stem cell transplantation: incidence in the context of immune recovery in two different transplantation settings. Ann Hematol. 2015;94(10):1677–88.
31. Torres HA, Kontoyiannis DP, Aguilera EA, et al. Cytomegalovirus infection in patients with lymphoma: an important cause of morbidity and mortality. Clin Lymphoma Myeloma. 2006;6(5):393–8.
32. Prestes DP, Arbona E, Nevett-Fernandez A, et al. Dasatinib use and risk of cytomegalovirus reactivation after allogeneic hematopoietic-cell transplantation. Clin Infect Dis. 2017;65(3):510–3.
33. von Hofsten J, Johnsson Forsberg M, Zetterberg M. Cytomegalovirus retinitis in a patient who received ruxolitinib. N Engl J Med. 2016;374(3):296–7.
34. Kroger N, Shahnaz Syed Abd Kadir S, Zabelina T, et al. Peritransplantation ruxolitinib prevents acute graft-versus-host disease in patients with myelofibrosis undergoing allogenic stem cell transplantation. Biol Blood Marrow Transplant. 2018;24(10):2152–6.
35. Ozdemir E, Saliba RM, Champlin RE, et al. Risk factors associated with late cytomegalovirus reactivation after allogeneic stem cell transplantation for hematological malignancies. Bone Marrow Transplant. 2007;40(2):125–36.
36. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med. 2017;377(25):2433–44.
37. Boeckh M, Leisenring W, Riddell SR, et al. Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. Blood. 2003;101(2):407–14.
38. Reusser P, Riddell SR, Meyers JD, Greenberg PD. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. Blood. 1991;78(5):1373–80.
39. Ozdemir E, St John LS, Gillespie G, et al. Cytomegalovirus reactivation following allogeneic stem cell transplantation is associated with the presence of dysfunctional antigen-specific CD8 + T cells. Blood. 2002;100(10):3690–7.
40. Krol L, Stuchly J, Hubacek P, et al. Signature profiles of CMV-specific T-cells in patients with CMV reactivation after hematopoietic SCT. Bone Marrow Transplant. 2011;46(8):1089–98.
41. Nesher L, Shah DP, Ariza-Heredia EJ, et al. Utility of the enzyme-linked immunospot interferon-gamma-release assay to predict the risk of cytomegalovirus infection in hematopoietic cell transplant recipients. J Infect Dis. 2016;213(11):1701–7.
42. Tey SK, Kennedy GA, Cromer D, et al. Clinical assessment of anti-viral CD8+ T cell immune monitoring using QuantiFERON-CMV(R) assay to identify high risk allogeneic hematopoietic stem cell transplant patients with CMV infection complications. PLoS One. 2013;8(10):e74744.
43. Fleming T, Dunne J, Crowley B. Ex vivo monitoring of human cytomegalovirus-specific CD8 (+) T-cell responses using the QuantiFERON-CMV assay in allogeneic hematopoietic stem cell transplant recipients attending an Irish hospital. J Med Virol. 2010;82(3):433–40.
44. Camargo JF, Wieder ED, Kimble E, et al. Deep functional immunophenotyping predicts risk of cytomegalovirus reactivation after hematopoietic cell transplantation. Blood. 2019;133(8):867–77.
45. Sylwester AW, Mitchell BL, Edgar JB, et al. Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. J Exp Med. 2005;202(5):673–85.
46. Suarez JF, Rosa R, Lorio MA, et al. Pretransplant CD4 count influences immune reconstitution and risk of infectious complications in human immunodeficiency virus-infected kidney allograft recipients. Am J Transplant. 2016;16(8):2463–72.
47. Heining C, Spyridonidis A, Bernhardt E, et al. Lymphocyte reconstitution following allogeneic hematopoietic stem cell transplantation: a retrospective study including 148 patients. Bone Marrow Transplant. 2007;39(10):613–22.

48. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood. 2006;107(12):4781–9.
49. Lilleri D, Fornara C, Chiesa A, Caldera D, Alessandrino EP, Gerna G. Human cytomegalovirus-specific CD4+ and CD8+ T-cell reconstitution in adult allogeneic hematopoietic stem cell transplant recipients and immune control of viral infection. Haematologica. 2008;93(2):248–56.
50. Pelak O, Stuchly J, Krol L, et al. Appearance of cytomegalovirus-specific T-cells predicts fast resolution of viremia post hematopoietic stem cell transplantation. Cytometry B Clin Cytom. 2017;92(5):380–8.
51. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis. 2017;64(1):87–91.
52. Ariza-Heredia EJ, Nesher L, Chemaly RF. Cytomegalovirus diseases after hematopoietic stem cell transplantation: a mini-review. Cancer Lett. 2014;342(1):1–8.
53. Erard V, Guthrie KA, Seo S, et al. Reduced mortality of cytomegalovirus pneumonia after hematopoietic cell transplantation due to antiviral therapy and changes in transplantation practices. Clin Infect Dis. 2015;61(1):31–9.
54. Torres HA, Aguilera E, Safdar A, et al. Fatal cytomegalovirus pneumonia in patients with haematological malignancies: an autopsy-based case-control study. Clin Microbiol Infect. 2008;14(12):1160–6.
55. van Burik JA, Lawatsch EJ, DeFor TE, Weisdorf DJ. Cytomegalovirus enteritis among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2001;7(12):674–9.
56. Wolf DG, Lurain NS, Zuckerman T, et al. Emergence of late cytomegalovirus central nervous system disease in hematopoietic stem cell transplant recipients. Blood. 2003;101(2):463–5.
57. Jeong TD, Sung H, Choi SH, et al. Cytomegalovirus ventriculoencephalitis with compartmentalization of antiviral-resistant cytomegalovirus in a T cell-depleted haploidentical peripheral blood stem cell transplant recipient. Diagn Microbiol Infect Dis. 2012;74(3):307–10.
58. Reddy SM, Winston DJ, Territo MC, Schiller GJ. CMV central nervous system disease in stem-cell transplant recipients: an increasing complication of drug-resistant CMV infection and protracted immunodeficiency. Bone Marrow Transplant. 2010;45(6):979–84.
59. Schmidt-Hieber M, Schwender J, Heinz WJ, et al. Viral encephalitis after allogeneic stem cell transplantation: a rare complication with distinct characteristics of different causative agents. Haematologica. 2011;96(1):142–9.
60. Song WK, Min YH, Kim YR, Lee SC. Cytomegalovirus retinitis after hematopoietic stem cell transplantation with alemtuzumab. Ophthalmology. 2008;115(10):1766–70.
61. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood. 2002;100(13):4358–66.
62. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. J Infect Dis. 2002;185(3):273–82.
63. Cantoni N, Hirsch HH, Khanna N, et al. Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. Biol Blood Marrow Transplant. 2010;16(9):1309–14.
64. Broers AE, van Der Holt R, van Esser JW, et al. Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. Blood. 2000;95(7):2240–5.
65. Mariotti J, Maura F, Spina F, et al. Impact of cytomegalovirus replication and cytomegalovirus serostatus on the outcome of patients with B cell lymphoma after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20(6):885–90.
66. Green ML, Leisenring WM, Xie H, et al. CMV reactivation after allogeneic HCT and relapse risk: evidence for early protection in acute myeloid leukemia. Blood. 2013;122(7):1316–24.

67. Teira P, Battiwalla M, Ramanathan M, et al. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. Blood. 2016;127(20):2427–38.
68. Garcia-Vidal C, Upton A, Kirby KA, Marr KA. Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: biological risk factors for infection according to time after transplantation. Clin Infect Dis. 2008;47(8):1041–50.
69. Thursky K, Byrnes G, Grigg A, Szer J, Slavin M. Risk factors for post-engraftment invasive aspergillosis in allogeneic stem cell transplantation. Bone Marrow Transplant. 2004;34(2):115–21.
70. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis. 2000;181(1):309–16.
71. Venton G, Crocchiolo R, Furst S, et al. Risk factors of ganciclovir-related neutropenia after allogeneic stem cell transplantation: a retrospective monocentre study on 547 patients. Clin Microbiol Infect. 2014;20(2):160–6.
72. Liu J, Kong J, Chang YJ, et al. Patients with refractory cytomegalovirus (CMV) infection following allogeneic haematopoietic stem cell transplantation are at high risk for CMV disease and non-relapse mortality. Clin Microbiol Infect. 2015;21(12):1121 e9–15.
73. Baniak N, Kanthan R. Cytomegalovirus colitis: an uncommon mimicker of common colitides. Arch Pathol Lab Med. 2016;140(8):854–8.
74. Goodman AL, Murray CD, Watkins J, Griffiths PD, Webster DP. CMV in the gut: a critical review of CMV detection in the immunocompetent host with colitis. Eur J Clin Microbiol Infect Dis. 2015;34(1):13–8.
75. Garrido E, Carrera E, Manzano R, Lopez-Sanroman A. Clinical significance of cytomegalovirus infection in patients with inflammatory bowel disease. World J Gastroenterol. 2013;19(1):17–25.
76. Cardenoso L, Pinsky BA, Lautenschlager I, et al. CMV antigenemia and quantitative viral load assessments in hematopoietic stem cell transplant recipients. J Clin Virol. 2013;56(2):108–12.
77. Boeckh M, Huang M, Ferrenberg J, et al. Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. J Clin Microbiol. 2004;42(3):1142–8.
78. Limaye AP, Huang ML, Leisenring W, Stensland L, Corey L, Boeckh M. Cytomegalovirus (CMV) DNA load in plasma for the diagnosis of CMV disease before engraftment in hematopoietic stem-cell transplant recipients. J Infect Dis. 2001;183(3):377–82.
79. Babady NE, Cheng C, Cumberbatch E, Stiles J, Papanicolaou G, Tang YW. Monitoring of cytomegalovirus viral loads by two molecular assays in whole-blood and plasma samples from hematopoietic stem cell transplant recipients. J Clin Microbiol. 2015;53(4):1252–7.
80. Kraft CS, Armstrong WS, Caliendo AM. Interpreting quantitative cytomegalovirus DNA testing: understanding the laboratory perspective. Clin Infect Dis. 2012;54(12):1793–7.
81. Preiksaitis JK, Hayden RT, Tong Y, et al. Are we there yet? Impact of the first international standard for cytomegalovirus DNA on the harmonization of results reported on plasma samples. Clin Infect Dis. 2016;63(5):583–9.
82. Hirsch HH, Lautenschlager I, Pinsky BA, et al. An international multicenter performance analysis of cytomegalovirus load tests. Clin Infect Dis. 2013;56(3):367–73.
83. Waggoner J, Ho DY, Libiran P, Pinsky BA. Clinical significance of low cytomegalovirus DNA levels in human plasma. J Clin Microbiol. 2012;50(7):2378–83.
84. Beam E, Germer JJ, Lahr B, et al. Cytomegalovirus (CMV) DNA quantification in bronchoalveolar lavage fluid of immunocompromised patients with CMV pneumonia. Clinical Transplantation. 2018;32:e13149: 1–8.
85. Boeckh M, Stevens-Ayers T, Travi G, et al. Cytomegalovirus (CMV) DNA quantitation in bronchoalveolar lavage fluid from hematopoietic stem cell transplant recipients with CMV pneumonia. J Infect Dis. 2017;215(10):1514–22.
86. Goodrich JM, Bowden RA, Fisher L, Keller C, Schoch G, Meyers JD. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. Ann Intern Med. 1993;118(3):173–8.

87. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. 1996; 88(10):4063–71.
88. Green ML, Leisenring W, Stachel D, et al. Efficacy of a viral load-based, risk-adapted, preemptive treatment strategy for prevention of cytomegalovirus disease after hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2012;18(11):1687–99.
89. Gimeno C, Solano C, Latorre JC, et al. Quantification of DNA in plasma by an automated real-time PCR assay (cytomegalovirus PCR kit) for surveillance of active cytomegalovirus infection and guidance of preemptive therapy for allogeneic hematopoietic stem cell transplant recipients. J Clin Microbiol. 2008;46(10):3311–8.
90. Peres RM, Costa CR, Andrade PD, et al. Surveillance of active human cytomegalovirus infection in hematopoietic stem cell transplantation (HLA sibling identical donor): search for optimal cutoff value by real-time PCR. BMC Infect Dis. 2010;10:147.
91. Tan SK, Waggoner JJ, Pinsky BA. Cytomegalovirus load at treatment initiation is predictive of time to resolution of viremia and duration of therapy in hematopoietic cell transplant recipients. J Clin Virol. 2015;69:179–83.
92. Solano C, Munoz-Cobo B, Gimenez E, et al. Pre-emptive antiviral therapy for active CMV infection in adult allo-SCT patients guided by plasma CMV DNAemia quantitation using a real-time PCR assay: clinical experience at a single center. Bone Marrow Transplant. 2013;48(7):1010–2.
93. Gimenez E, Munoz-Cobo B, Solano C, Amat P, Navarro D. Early kinetics of plasma cytomegalovirus DNA load in allogeneic stem cell transplant recipients in the era of highly sensitive real-time PCR assays: does it have any clinical value? J Clin Microbiol. 2014;52(2):654–6.
94. Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD. The dynamics of human cytomegalovirus replication in vivo. J Exp Med. 1999;190(2):177–82.
95. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet. 2000;355(9220):2032–6.
96. Munoz-Cobo B, Solano C, Costa E, et al. Dynamics of cytomegalovirus (CMV) plasma DNAemia in initial and recurrent episodes of active CMV infection in the allogeneic stem cell transplantation setting: implications for designing preemptive antiviral therapy strategies. Biol Blood Marrow Transplant. 2011;17(11):1602–11.
97. Solano C, Gimenez E, Pinana JL, et al. Preemptive antiviral therapy for CMV infection in allogeneic stem cell transplant recipients guided by the viral doubling time in the blood. Bone Marrow Transplant. 2016;51(5):718–21.
98. Lodding IP, Sengelov H, da Cunha-Bang C, et al. Clinical application of variation in replication kinetics during episodes of post-transplant cytomegalovirus infections. EBioMedicine. 2015;2(7):699–705.
99. Solano C, Gimenez E, Pinana JL, et al. When should preemptive antiviral therapy for active CMV infection be withdrawn from allogeneic stem cell transplant recipients? Bone Marrow Transplant. 2017;52(10):1448–51.
100. Manuel O, Husain S, Kumar D, et al. Assessment of cytomegalovirus-specific cell-mediated immunity for the prediction of cytomegalovirus disease in high-risk solid-organ transplant recipients: a multicenter cohort study. Clin Infect Dis. 2013;56(6):817–24.
101. Clari MA, Munoz-Cobo B, Solano C, et al. Performance of the QuantiFERON-cytomegalovirus (CMV) assay for detection and estimation of the magnitude and functionality of the CMV-specific gamma interferon-producing CD8(+) T-cell response in allogeneic stem cell transplant recipients. Clin Vaccine Immunol. 2012;19(5):791–6.
102. Navarro D, Amat P, de la Camara R, et al. Efficacy and safety of a preemptive antiviral therapy strategy based on combined virological and immunological monitoring for active cytomegalovirus infection in allogeneic stem cell transplant recipients. Open Forum Infect Dis. 2016;3(2):ofw107.

103. El Haddad L, Ariza-Heredia E, Shah DP, et al. The ability of a cytomegalovirus ELISPOT assay to predict outcome of low-level CMV reactivation in hematopoietic cell transplant recipients. J Infect Dis. 2019;219(6):898–907.
104. Auletta JJ. T'ing off on posttransplant CMV. Blood. 2019;133(8):777–9.
105. Reusser P, Einsele H, Lee J, et al. Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. Blood. 2002;99(4):1159–64.
106. Camargo JF, Anderson AD, Rosa R, Kimble E, Komanduri KV, Morris MI. Use of maintenance therapy and incidence of recurrent Cytomegalovirus DNAemia among allogeneic hematopoietic cell transplant recipients. Transpl Infect Dis. 2019;21(2):e13054.
107. Griffiths PD, Rothwell E, Raza M, et al. Randomized controlled trials to define viral load thresholds for cytomegalovirus pre-emptive therapy. PLoS One. 2016;11(9):e0163722.
108. Kumar D, Mian M, Singer L, Humar A. An interventional study using cell-mediated immunity to personalize therapy for cytomegalovirus infection after transplantation. Am J Transplant. 2017;17(9):2468–73.
109. Chen K, Cheng MP, Hammond SP, Einsele H, Marty FM. Antiviral prophylaxis for cytomegalovirus infection in allogeneic hematopoietic cell transplantation. Blood Adv. 2018;2(16):2159–75.
110. Boeckh M, Nichols WG, Chemaly RF, et al. Valganciclovir for the prevention of complications of late cytomegalovirus infection after allogeneic hematopoietic cell transplantation: a randomized trial. Ann Intern Med. 2015;162(1):1–10.
111. Burns LJ, Miller W, Kandaswamy C, et al. Randomized clinical trial of ganciclovir vs acyclovir for prevention of cytomegalovirus antigenemia after allogeneic transplantation. Bone Marrow Transplant. 2002;30(12):945–51.
112. Kimball LE, Stevens-Ayers T, Green ML, et al. A multicenter, longitudinal, interventional, double blind randomized clinical trial in hematopoietic cell transplant recipients residing in remote areas: lessons learned from the late cytomegalovirus prevention trial. Contemp Clin Trials Commun. 2016;4:84–9.
113. Winston DJ, Ho WG, Bartoni K, et al. Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients. Results of a placebo-controlled, double-blind trial. Ann Intern Med. 1993;118(3):179–84.
114. Nakamura R, Cortez K, Solomon S, et al. High-dose acyclovir and pre-emptive ganciclovir to prevent cytomegalovirus disease in myeloablative and non-myeloablative allogeneic stem cell transplantation. Bone Marrow Transplant. 2002;30(4):235–42.
115. Ljungman P, de La Camara R, Milpied N, et al. Randomized study of valacyclovir as prophylaxis against cytomegalovirus reactivation in recipients of allogeneic bone marrow transplants. Blood. 2002;99(8):3050–6.
116. Vusirikala M, Wolff SN, Stein RS, et al. Valacyclovir for the prevention of cytomegalovirus infection after allogeneic stem cell transplantation: a single institution retrospective cohort analysis. Bone Marrow Transplant. 2001;28(3):265–70.
117. Hill JA, Pergam SA, Cox E, et al. A modified intensive strategy to prevent cytomegalovirus disease in seropositive umbilical cord blood transplantation recipients. Biol Blood Marrow Transplant. 2018;24(10):2094–100.
118. Winston DJ, Young JA, Pullarkat V, et al. Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem cell transplant recipients: a multicenter, randomized, double-blind, placebo-controlled, dose-ranging study. Blood. 2008;111(11):5403–10.
119. Marty FM, Ljungman P, Papanicolaou GA, et al. Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplants: a phase 3, double-blind, placebo-controlled, randomised trial. Lancet Infect Dis. 2011;11(4):284–92.
120. Marty FM, Boeckh M. Maribavir and human cytomegalovirus-what happened in the clinical trials and why might the drug have failed? Curr Opin Virol. 2011;1(6):555–62.
121. Marty FM, Winston DJ, Rowley SD, et al. CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation. N Engl J Med. 2013;369(13):1227–36.

122. Marty FM, Winston DJ, Chemaly RF, et al. A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for cytomegalovirus prophylaxis in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2019;25(2):369–81.
123. Robin C, Thiebaut A, Alain S, de Fontbrune FS, Berceanu A, D'Aveni M, Ceballos P, Redjoul R, Nguyen-Quoc S, Bénard N, Pahlavan-Grumel G, Cordonnier C. Letermovir for secondary prophylaxis of CMV infection and disease after allogeneic hematopoietic cell transplantation: results from the French compassionate program. Biol Blood Marrow Transplant. 2020. pii: S1083-8791(20)30060-4. https://doi.org/10.1016/j.bbmt.2020.01.027.
124. Knoll BM, Seiter K, Phillips A, Soave R. Breakthrough cytomegalovirus pneumonia in hematopoietic stem cell transplant recipient on letermovir prophylaxis. Bone Marrow Transplant. 2019;54(6):911–2.
125. Lischka P, Michel D, Zimmermann H. Characterization of cytomegalovirus breakthrough events in a phase 2 prophylaxis trial of letermovir (AIC246, MK 8228). J Infect Dis. 2016;213(1):23–30.
126. Hill JA, Mayer BT, Xie H, et al. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. Blood. 2017;129(16):2316–25.
127. Camargo JF, Morris MI, Abbo LM, et al. The use of brincidofovir for the treatment of mixed dsDNA viral infection. J Clin Virol. 2016;83:1–4.
128. Marty FM, Bryar J, Browne SK, et al. Sirolimus-based graft-versus-host disease prophylaxis protects against cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation: a cohort analysis. Blood. 2007;110(2):490–500.
129. Guglieri-Lopez B, Perez-Pitarch A, Garcia-Cadenas I, et al. Effect of sirolimus exposure on the need for preemptive antiviral therapy for cytomeglovirus infection after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2019;25(5):1022–30.
130. Pinana JL, Perez-Pitarch A, Guglieri-Lopez B, et al. Sirolimus exposure and the occurrence of cytomegalovirus DNAemia after allogeneic hematopoietic stem cell transplantation. Am J Transplant. 2018;18(12):2885–94.
131. Raanani P, Gafter-Gvili A, Paul M, Ben-Bassat I, Leibovici L, Shpilberg O. Immunoglobulin prophylaxis in hematological malignancies and hematopoietic stem cell transplantation. Cochrane Database Syst Rev. 2008;(4):CD006501.
132. Turner N, Strand A, Grewal DS, et al. Use of letermovir as salvage therapy for drug-resistant cytomegalovirus retinitis. Antimicrob Agents Chemother. 2019 Feb 26;63(3); e02337–18: 1–6
133. Kaul DR, Stoelben S, Cober E, et al. First report of successful treatment of multidrug-resistant cytomegalovirus disease with the novel anti-CMV compound AIC246. Am J Transplant. 2011;11(5):1079–84.
134. Chemaly RF, Chou S, Einsele H, et al. Definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients for use in clinical trials. Clin Infect Dis. 2019;68(8):1420–6.
135. Chou SW. Cytomegalovirus drug resistance and clinical implications. Transpl Infect Dis. 2001;3(Suppl 2):20–4.
136. Gilbert C, Roy J, Belanger R, et al. Lack of emergence of cytomegalovirus UL97 mutations conferring ganciclovir (GCV) resistance following preemptive GCV therapy in allogeneic stem cell transplant recipients. Antimicrob Agents Chemother. 2001;45(12):3669–71.
137. Hantz S, Garnier-Geoffroy F, Mazeron MC, et al. Drug-resistant cytomegalovirus in transplant recipients: a French cohort study. J Antimicrob Chemother. 2010;65(12):2628–40.
138. Allice T, Busca A, Locatelli F, Falda M, Pittaluga F, Ghisetti V. Valganciclovir as pre-emptive therapy for cytomegalovirus infection post-allogenic stem cell transplantation: implications for the emergence of drug-resistant cytomegalovirus. J Antimicrob Chemother. 2009;63(3):600–8.
139. Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies. Biol Blood Marrow Transplant. 2003;9(9):543–58.
140. Shmueli E, Or R, Shapira MY, et al. High rate of cytomegalovirus drug resistance among patients receiving preemptive antiviral treatment after haploidentical stem cell transplantation. J Infect Dis. 2014;209(4):557–61.

141. Chou S, Ercolani RJ, Sahoo MK, Lefterova MI, Strasfeld LM, Pinsky BA. Improved detection of emerging drug-resistant mutant cytomegalovirus subpopulations by deep sequencing. Antimicrob Agents Chemother. 2014;58(8):4697–702.
142. Sahoo MK, Lefterova MI, Yamamoto F, et al. Detection of cytomegalovirus drug resistance mutations by next-generation sequencing. J Clin Microbiol. 2013;51(11):3700–10.
143. Chou S, Marousek GI. Maribavir antagonizes the antiviral action of ganciclovir on human cytomegalovirus. Antimicrob Agents Chemother. 2006;50(10):3470–2.
144. Kotton CN, Kumar D, Caliendo AM, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102(6):900–31.
145. Hakki M, Chou S. The biology of cytomegalovirus drug resistance. Curr Opin Infect Dis. 2011;24(6):605–11.
146. Chou S. Comparison of cytomegalovirus terminase gene mutations selected after exposure to three distinct inhibitor compounds. Antimicrob Agents Chemother. 2017 Oct 24;61(11); e01325–17: 1–11.
147. Chou S. A third component of the human cytomegalovirus terminase complex is involved in letermovir resistance. Antivir Res. 2017;148:1–4.
148. Chou S. Approach to drug-resistant cytomegalovirus in transplant recipients. Curr Opin Infect Dis. 2015;28(4):293–9.
149. Piret J, Boivin G. Clinical development of letermovir and maribavir: overview of human cytomegalovirus drug resistance. Antivir Res. 2019;163:91–105.
150. Goldner T, Hempel C, Ruebsamen-Schaeff H, Zimmermann H, Lischka P. Geno- and phenotypic characterization of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. Antimicrob Agents Chemother. 2014;58(1):610–3.
151. Cherrier L, Nasar A, Goodlet KJ, Nailor MD, Tokman S, Chou S. Emergence of letermovir resistance in a lung transplant recipient with ganciclovir-resistant cytomegalovirus infection. Am J Transplant. 2018;18(12):3060–4.
152. Frietsch JJ, Michel D, Stamminger T, et al. In vivo emergence of UL56 C325Y cytomegalovirus resistance to letermovir in a patient with acute myeloid leukemia after hematopoietic cell transplantation. Mediterr J Hematol Infect Dis. 2019;11(1):e2019001.
153. Chou S, Satterwhite LE, Ercolani RJ. New locus of drug resistance in the human cytomegalovirus UL56 gene revealed by in vitro exposure to letermovir and ganciclovir. Antimicrob Agents Chemother. 2018 Aug 27;62(9); e00922–18: 1–6
154. Tzannou I, Papadopoulou A, Naik S, et al. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, Epstein-Barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2017;35(31):3547–57.
155. Koehne G, Hasan A, Doubrovina E, et al. Immunotherapy with donor T cells sensitized with overlapping pentadecapeptides for treatment of persistent cytomegalovirus infection or viremia. Biol Blood Marrow Transplant. 2015;21(9):1663–78.
156. Gerdemann U, Katari UL, Papadopoulou A, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. Mol Ther. 2013;21(11):2113–21.
157. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121(26):5113–23.
158. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014;6(242):242ra83.
159. Withers B, Blyth E, Clancy LE, et al. Long-term control of recurrent or refractory viral infections after allogeneic HSCT with third-party virus-specific T cells. Blood Adv. 2017;1(24):2193–205.
160. Brestrich G, Zwinger S, Fischer A, et al. Adoptive T-cell therapy of a lung transplanted patient with severe CMV disease and resistance to antiviral therapy. Am J Transplant. 2009;9(7):1679–84.

161. Smith C, Beagley L, Rehan S, et al. Autologous adoptive T-cell therapy for recurrent or drug-resistant cytomegalovirus complications in solid organ transplant recipients: a single-arm open-label phase I clinical trial. Clin Infect Dis. 2019;68(4):632–40.
162. Papanicolaou GA, Silveira FP, Langston AA, et al. Maribavir for refractory or resistant cytomegalovirus infections in hematopoietic-cell or solid-organ transplant recipients: a randomized, dose-ranging, double-blind, phase 2 study. Clin Infect Dis. 2019;68(8):1255–64.
163. El-Haddad D, El Chaer F, Vanichanan J, et al. Brincidofovir (CMX-001) for refractory and resistant CMV and HSV infections in immunocompromised cancer patients: a single-center experience. Antivir Res. 2016;134:58–62.
164. Chong PP, Teiber D, Prokesch BC, et al. Letermovir successfully used for secondary prophylaxis in a heart transplant recipient with ganciclovir-resistant cytomegalovirus syndrome (UL97 mutation). Transpl Infect Dis. 2018;20(5):e12965.
165. Macesic N, Langsford D, Nicholls K, et al. Adoptive T cell immunotherapy for treatment of ganciclovir-resistant cytomegalovirus disease in a renal transplant recipient. Am J Transplant. 2015;15(3):827–32.
166. Einsele H, Roosnek E, Rufer N, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood. 2002;99(11):3916–22.
167. Blyth E, Clancy L, Simms R, et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. Blood. 2013;121(18):3745–58.
168. Waldman WJ, Knight DA, Blinder L, et al. Inhibition of cytomegalovirus in vitro and in vivo by the experimental immunosuppressive agent leflunomide. Intervirology. 1999;42(5–6):412–8.
169. Waldman WJ, Knight DA, Lurain NS, et al. Novel mechanism of inhibition of cytomegalovirus by the experimental immunosuppressive agent leflunomide. Transplantation. 1999;68(6):814–25.
170. Avery RK, Bolwell BJ, Yen-Lieberman B, et al. Use of leflunomide in an allogeneic bone marrow transplant recipient with refractory cytomegalovirus infection. Bone Marrow Transplant. 2004;34(12):1071–5.
171. Avery RK, Mossad SB, Poggio E, et al. Utility of leflunomide in the treatment of complex cytomegalovirus syndromes. Transplantation. 2010;90(4):419–26.
172. Ehlert K, Groll AH, Kuehn J, Vormoor J. Treatment of refractory CMV-infection following hematopoietic stem cell transplantation with the combination of foscarnet and leflunomide. Klin Padiatr. 2006;218(3):180–4.
173. Battiwalla M, Paplham P, Almyroudis NG, et al. Leflunomide failure to control recurrent cytomegalovirus infection in the setting of renal failure after allogeneic stem cell transplantation. Transpl Infect Dis. 2007;9(1):28–32.
174. El Chaer F, Mori N, Shah D, et al. Adjuvant and salvage therapy with leflunomide for recalcitrant cytomegalovirus infections in hematopoietic cell transplantation recipients: a case series. Antivir Res. 2016;135:91–6.
175. Efferth T, Romero MR, Wolf DG, Stamminger T, Marin JJ, Marschall M. The antiviral activities of artemisinin and artesunate. Clin Infect Dis. 2008;47(6):804–11.
176. Germi R, Mariette C, Alain S, et al. Success and failure of artesunate treatment in five transplant recipients with disease caused by drug-resistant cytomegalovirus. Antivir Res. 2014;101:57–61.
177. Shapira MY, Resnick IB, Chou S, et al. Artesunate as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation. Clin Infect Dis. 2008;46(9):1455–7.
178. Drouot E, Piret J, Boivin G. Artesunate demonstrates in vitro synergism with several antiviral agents against human cytomegalovirus. Antivir Ther. 2016;21(6):535–9.
179. Sabe N, Gonzalez-Costello J, Rama I, et al. Successful outcome of ganciclovir-resistant cytomegalovirus infection in organ transplant recipients after conversion to mTOR inhibitors. Transpl Int. 2012;25(7):e78–82.

180. Peggs KS, Verfuerth S, Pizzey A, et al. Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. Lancet. 2003;362(9393):1375–7.
181. Peggs KS, Thomson K, Samuel E, et al. Directly selected cytomegalovirus-reactive donor T cells confer rapid and safe systemic reconstitution of virus-specific immunity following stem cell transplantation. Clin Infect Dis. 2011;52(1):49–57.
182. Hanley PJ, Melenhorst JJ, Nikiforow S, et al. CMV-specific T cells generated from naive T cells recognize atypical epitopes and may be protective in vivo. Sci Transl Med. 2015;7(285):285ra63.
183. La Rosa C, Longmate J, Martinez J, et al. MVA vaccine encoding CMV antigens safely induces durable expansion of CMV-specific T cells in healthy adults. Blood. 2017;129(1):114–25.
184. Diamond DJ, La Rosa C, Chiuppesi F, et al. A fifty-year odyssey: prospects for a cytomegalovirus vaccine in transplant and congenital infection. Expert Rev Vaccines. 2018;17(10):889–911.
185. Anderholm KM, Bierle CJ, Schleiss MR. Cytomegalovirus vaccines: current status and future prospects. Drugs. 2016;76(17):1625–45.
186. Plotkin S. The history of vaccination against cytomegalovirus. Med Microbiol Immunol. 2015;204(3):247–54.
187. Kharfan-Dabaja MA, Nishihori T. Vaccine therapy for cytomegalovirus in the setting of allogeneic hematopoietic cell transplantation. Expert Rev Vaccines. 2015;14(3):341–50.
188. Kharfan-Dabaja MA, Boeckh M, Wilck MB, et al. A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2012;12(4):290–9.
189. Nakamura R, La Rosa C, Longmate J, et al. Viraemia, immunogenicity, and survival outcomes of cytomegalovirus chimeric epitope vaccine supplemented with PF03512676 (CMVPepVax) in allogeneic haemopoietic stem-cell transplantation: randomised phase 1b trial. Lancet Haematol. 2016;3(2):e87–98.
190. La Rosa C, Longmate J, Lacey SF, et al. Clinical evaluation of safety and immunogenicity of PADRE-cytomegalovirus (CMV) and tetanus-CMV fusion peptide vaccines with or without PF03512676 adjuvant. J Infect Dis. 2012;205(8):1294–304.
191. Wloch MK, Smith LR, Boutsaboualoy S, et al. Safety and immunogenicity of a bivalent cytomegalovirus DNA vaccine in healthy adult subjects. J Infect Dis. 2008;197(12):1634–42.

Herpes Simplex and Varicella Zoster in Transplant Candidates and Recipients

26

Richard A. Zuckerman

Contents

Introduction 618
Disease Epidemiology 618
Herpes Simplex 618
Varicella 621
Clinical Impact in Transplant 623
HSV 623
VZV 625
Disease Diagnostics and Clinical Evaluation 626
Pretransplant 626
Posttransplant 627
Management 631
Clinical Disease 631
Prevention 635
Primary Infection 635
Pretransplant Vaccination (Table 5) 637
Posttransplant Vaccination (Table 5) 638
Posttransplant Postexposure prophylaxis for VZV 639
Prevention of Reactivation 640
Antiviral Medication 641
VZV Vaccination (Table 9) 643
Special Considerations 646
Infection Control 646
Pregnancy 647
IV Acyclovir Shortage 647
Conclusion 648
Key Points 648
References 648

R. A. Zuckerman (✉)
Infectious Disease and International Health, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA
e-mail: richard.a.zuckerman@hitchcock.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_26

Introduction

HSV-1, -2, and VZV are the only three α-herpesviruses to infect humans. They are characterized by rapid replication, the ability to infect multiple tissues, and to establish latency in neurons. Humans have been infected with herpes viruses since we evolved from our primate ancestors. The herpes simplex viruses (HSV-1 and HSV-2) evolved from a common primate virus, but now only infect humans. The evolutionary origin of varicella zoster virus is not as clear, but likely also co-evolved with primates and humans in a similar fashion many millions of years ago [1]. It is difficult to out-smart millions of years of evolution, so despite significant advances in medicine we have still not been able to eradicate these pathogens from our population. Herpes viruses are challenging in part due to the fact that they integrate into the DNA of human cells and use our cellular mechanisms to reproduce and to evade eradication. Despite this, we have lived with these viruses with a minimum of morbidity to the population; however, as we perturb normal human systems, as in the setting of transplantation, we agitate this delicate balance. Thus, we see increased incidence of serious and potentially life-threatening manifestations from herpes viruses in the setting of transplant and immune compromise. In fact, even in the era of modern antiviral prophylaxis, herpesviruses (HSV/VZV) remain the most common viral infections in solid organ recipients, accounting for 51% of viral infections diagnosed in the first year after transplant [2].

Disease Epidemiology

Herpes Simplex

While genetically very similar, HSV-1 and HSV-2 have historically had different epidemiology, risks, and clinical manifestations. HSV-1 is the classic oropharyngeal virus and is acquired early in life, while HSV-2 has been associated mostly with genital infections and is usually acquired after sexual debut. However, the epidemiology of HSV is changing. The prevalence of both HSV 1 and 2 is decreasing in the US (Fig. 1) [3]. Additionally, an increasing number of persons are infected with HSV-1 genitally. The WHO estimates that "140 million people aged 15–49 years are infected with genital HSV-1 infection, primarily in the Americas, Europe, and Western Pacific. Fewer people in high-income countries are becoming infected with HSV-1 as children, likely due to better hygiene and living conditions, and are instead at risk of contracting it genitally through oral sex after they become sexually active." [4] In fact, they estimate that half a billion people 15–49 are infected with HSV-1 or -2 genitally. Few data exist on the seroprevalence of HSV in transplant recipients, but it is felt to be similar to that in the general population, varying by age and other usual risk factors.

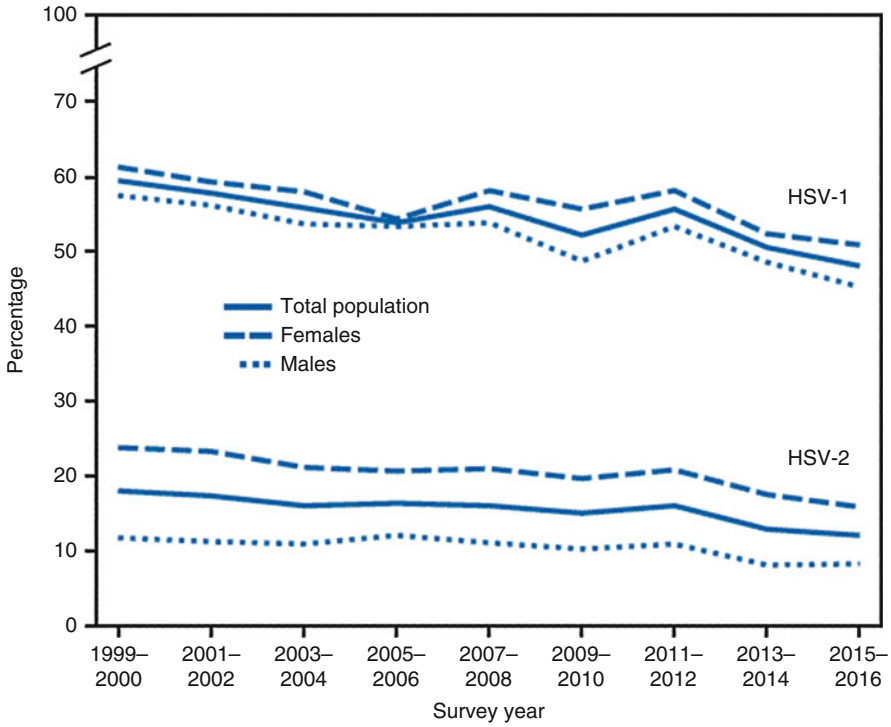


Fig. 1 Age-adjusted trends in the prevalence of Herpes Simplex Virus Type 1 (HSV-1) and Herpes Simplex Virus Type 2 (HSV-2) among adolescents and adults aged 14–49 years – United States, 1999–2000 Through 2015–2016 [3]

These statistics have important implications for transplant patients and their clinicians. While lower rates of seropositivity in a population may predict lower rates of clinically evident HSV reactivation after transplant, they could potentially create a larger pool of seronegative transplant recipients at risk for acute primary infection, which may be more severe and potentially life-threatening in immunocompromised patients. Transplant volumes continue to increase, and more transplant recipients will have risk for HSV acquisition, so clinicians will need to adapt to this changing epidemiology. Acquisition of HSV from a transplant donor is very rare, so the majority of primary HSV will be via acquisition from sexual or other close contacts.

The incidence of clinically apparent HSV disease in seropositive transplant patients not receiving prophylaxis ranges from 35 to >80% [5, 6]. In the era of modern antiviral prophylaxis, the incidence of HSV reactivation is low. Figure 2 shows the probability of HSV reactivation after solid organ transplantation (SOT) depending on the antiviral prophylaxis regimen [7]. Patients on universal prophylaxis for CMV with antivirals that have activity against HSV have very low rates of HSV reactivation. A similar phenomenon is also evident in hematopoietic stem

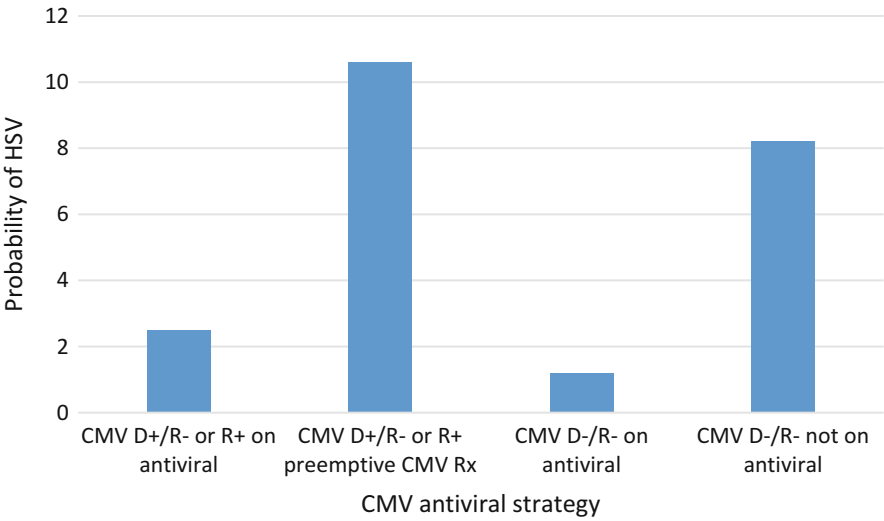


Fig. 2 Probability of HSV infection after organ transplantation according to the CMV serostatus and antiviral preventive strategy. (Probability of HSV infection at one year depending on antiviral therapy. Regardless of CMV prevention strategy, shows the effectiveness of antiviral prophylaxis on HSV reactivation (p <0.001 comparison of antiviral versus not). HSV, herpes simplex virus; CMV, cytomegalovirus, D, donor; R, recipient; + seropositive; − seronegative [7])

cell transplant (HSCT) recipients, where routine extended antiviral prophylaxis for VZV also prevents symptomatic HSV reactivation. Moving forward, an increasing number of transplant recipients may be on anti-CMV medications that do not have activity against HSV (i.e., letermovir and maribavir), and some organ recipients may be on a preemptive preventive strategy, so HSV reactivation epidemiology will need to be monitored.

Most HSV reactivation in SOT and HSCT occurs in the immediate post-transplant period when immunosuppression is most profound. In SOT, without antiviral prophylaxis, 8–10% of patients will reactivate HSV in the first year, whereas with antiviral prophylaxis, that rate drops to <2.5% (Fig. 2) [7]. Historically, agents associated with compromised cell-mediated immunity have been associated with increased risk of HSV reactivation after transplantation. In HSCT, risk increases similarly with compromised cell-mediated immunity in the setting of anti-GvHD therapy, but in fact, HSV reactivation is most common earlier during the profound neutropenia associated with conditioning therapy, particularly in association with mucositis [8].

Acquisition of HSV in a seronegative recipient can occur at any time after transplant. Despite this, the clinical severity of primary disease may be related to the relative immune status of the at-risk individual, which is greatest soon after transplant and also during treatment of rejection or graft versus host disease. Few studies have assessed the incidence of HSV reactivation with various immune suppression regimens, but a recent review in SOT did not suggest any particular agents to be associated with higher risk [7].

Clinical Pearls

- HSV seroincidence is declining, but genital HSV-1 is increasing.
- There is expectation of a growing pool of seronegative transplant recipients at risk of acute primary HSV infection.
- As antiviral prophylaxis guidelines change for CMV with more narrow spectrum antiviral agents, HSV prevention will need to be revisited.

Varicella

Varicella is a highly contagious virus, so is extraordinarily common throughout the world. The incidence and prevalence of clinical manifestations and complications of varicella is correlated with the strength of local and regional vaccination campaigns. For example, in the United States, where >90% of children have been vaccinated since the vaccine was introduced in 1995, morbidity, and deaths from varicella have declined by >90% [9]. In fact, it is estimated that more than 3.5 million cases of varicella, 9,000 hospitalizations, and 100 deaths are prevented yearly by varicella vaccination in the United States.

VZV seroprevalence in the USA varies by age, with approximately 98% of persons seropositive by age 19–40 [10]. Children are eligible for VZV vaccination at 1 year old, so in the current era, the majority transplant patients are likely to have some immunity to VZV. Approximately 3% of adult SOT recipients are seronegative for VZV and therefore susceptible to primary infection [11]. In seropositive recipients, without extended prophylaxis, about 2.5% of organ transplant patients will have an episode of HZ reactivation in the first year after transplant; however, the incidence of HZ remains relatively constant for years after transplant, such that about 5% of patients will have a HZ reactivation within 3 years and about 9% by 6 years [12]. The low rate in organ transplant patients is likely in part related to the fact that they receive less intense immunosuppression and historically have been on universal CMV prophylaxis during the period of most intense immune suppression. This is in contrast to HSCT, with more intense immunosuppression, where over 20% of patients will reactivate varicella [13]. With shorter course prophylaxis in HSCT, reactivation rates may approach 40–50% after stopping prophylaxis [14]. A recent review of 1000 consecutive autologous HSCT patients showed that even with a year of prophylaxis, 21% of patients had reactivation disease within 5 years, 82% after stopping antiviral prophylaxis (Fig. 3) [13]. Additionally, 16% of those who did reactivate had a second episode. Nevertheless, within a year of stopping prophylaxis, cumulative HSCT VZV reactivation incidence stabilizes, whereas in SOT, cases continue to present at a steady rate for many years. In solid organ recipients, lung recipients appear to be at highest risk (55.1 cases per 1000 person-years of follow-up) for zoster reactivation as compared with heart (40.0), kidney (24.4), and liver (18.3), which is significantly higher than the incidence of 3.6 per 1000 person years in the general population [12].

Persons who receive the varicella vaccination as their primary exposure to varicella still can get reactivation disease, but the risk of reactivation is ~79%

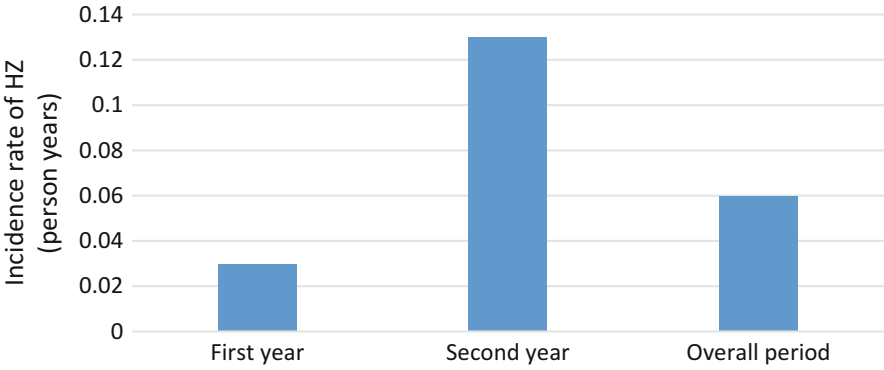


Fig. 3 Incidence of HZ after autologous HSCT. Shows the high rate of HZ reactivation in 194 patients who received approximately 1 year of antiviral prophylaxis. Overall 21% of patients had HZ reactivation in the 5 years of follow-up [13].

lower and the clinical manifestations are usually less severe when reactivation occurs [15]. Interestingly, half of zoster reactivation in vaccines was with wild-type virus suggesting the possibility of breakthrough infection. Eventually, in locations with high vaccination rates, the population will age to a point where the majority of transplant recipients will have been vaccinated as their sole exposure to varicella. This will likely reduce the population incidence and severity of clinical manifestations. More importantly, with the availability of the new adjuvanted varicella vaccination to boost cell-mediated immunity and prevent reactivation (Shingrix®, GlaxoSmithKline), varicella reactivation could eventually become rare in transplant recipients through appropriately directed vaccination strategies. More research will be necessary to evaluate these options in organ and marrow recipients and donors. Regardless, without more aggressive vaccination efforts throughout the world, we will surely continue to see persons developing wild-type disease and its manifestations.

The primary risk factor for VZV reactivation after transplant is loss of cell-mediated immunity. This is more profound early after transplant (after conditioning or induction therapy), and in the setting of treatment of rejection or graft versus host disease. Transplant patients, and in particular those undergoing HSCT, may have significant enough immune compromise to make them susceptible to acute acquisition even if they are seropositive for VZV, though this is rare. Importantly, even though risk of reactivation is higher with decreased CMI, the risk of primary VZV acquisition is associated with lack of antibody to varicella. This is why serostatus prior to transplant is important and also why varicella-specific antibody works to prevent VZV acquisition. As with HSV, acute primary varicella is more likely to be severe if acquired during periods of more significant immune compromise. Acquisition of vaccine strain from a recently immunized individual is extraordinarily rare, with only 11 confirmed cases to date, and all index cases had a rash [16].

Clinical Pearls

- Most patients going into transplant will be seropositive for VZV.
- VZV immunization has decreased the incidence of serious and nonserious clinical disease significantly.
- Even with prophylaxis, many transplant recipients will still develop VZV reactivation disease.
- Transmission of vaccine strain is very rare.

Clinical Impact in Transplant

HSV

Reactivation HSV: The majority of HSV disease in transplant recipients is reactivation disease, which appears as painful vesicular or ulcerative lesions, the majority of which is oropharyngeal. Asymptomatic HSV reactivation is very common, with HSV being found in the oropharynx in >70% of renal transplant and leukemia patients [17]. Genital herpes reactivation is less common, in part likely reflecting lower seroprevalence of HSV-2 [18]. HSV reactivation in transplant recipients can be more severe and prolonged than in immunocompetent patients. Most symptomatic HSV disease occurs early after transplantation and in the setting of intensified immunosuppressive therapy, such as treatment of organ rejection or graft versus host disease [19, 20]. HSV seropositive recipients are at risk of clinical reactivation post-transplant in the absence of antiviral prophylaxis even if they have not had prior clinical HSV disease. Clinically evident HSV recurrences are characterized by lesions that are similar to primary infection but may also present in an atypical fashion (ulcers, plaques, and hemorrhagic lesions) (Table 1) [21, 22].

The majority of HSV reactivation in HSCT recipients is oropharyngeal and/or gingivostomatitis. There is an association between mucositis and HSV reactivation in HSCT recipients, such that all HSCT recipients usually receive antivirals to prevent HSV-associated mucositis. HSV-associated mucositis has a more severe and prolonged course than non-HSV-associated mucositis [8, 23]. In patients receiving cytotoxic chemotherapy or conditioning chemotherapy, HSV should be considered in the setting of complex mucositis that does not improve as expected or is associated with vesicles. Patients often have vesicles or painful ulcers, but classic HSV lesions may not be present. Often patients develop bleeding ulcers and scabbing on the lips. Even with appropriate prophylaxis, up to 25% of marrow recipients may still shed HSV [24]. Oropharyngeal HSV reactivation may descend into the lower respiratory tract in critically ill and intubated patients and rarely can cause pneumonia [25]. In addition to oropharyngeal reactivation, HSV may occur in genital, peri-anal, and other mucocutaneous locations. Rarely, post-transplant patients may have a more disseminated or fulminant clinical course of HSV reactivation, including esophagitis, pneumonitis, enteritis, hepatitis, and disseminated disease [26].

Table 1 Clinical Issues and Risk factors for VZV and HSV infections in transplant patients

	Clinical issues	Risk factors
VZV		
Primary	Disseminated pruritic rash, >98% symptomatic Starts on face and spreads to trunk, spares hands and feet Lesions in various stages More severe in immunocompromised Rarely causes end-organ damage (liver, CNS, lung, and visceral)	Seronegative Inhalation or direct contact Transmission by donor very rare (SOT only)
Reactivated	Localized Shingles: ≤2 unilateral dermatomes Disseminated: >2 dermatomes or 2 noncontiguous May be atypical or severe/disseminated in transplant patients Cranial nerve/ophthalmic involvement, vasculopathy as a complication PHN or superinfection as complications	Decreased VZV-CMI Older age More immunosuppression No shingles vaccine (possible)
HSV		
Primary	Vesicular and/or ulcerative lesions may extend locally in the orolabial, genital, or perianal regions More severe and prolonged in transplant Dissemination rare (skin, liver, and CNS)	Seronegative Contact with symptomatic or asymptomatic Transmission from donor rare (SOT only)
Reactivated	Asymptomatic reactivation common Lesions similar to primary infection HSV-associated mucositis in HSCT more severe Disseminated disease rare	More immunosuppression Neutropenia, mucositis (HSCT) HSV seronegative donor in allo-HSCT

Modified from Zuckerman and Limaye [35]. See text for references
HSV, herpes simplex virus; VZV, varicella zoster virus; SOT, solid organ transplant; PHN, post-herpetic neuralgia; CMI, cell mediated immunity; CNS, central nervous system

Primary HSV: Since asymptomatic viral shedding is frequent, HSV is most often acquired from persons without clinical lesions. HSV seronegative transplant recipients may acquire primary HSV from intimate contacts, with a usual incubation period of 3–7 days. HSV usually appears as classic vesicular and/or ulcerative lesions and may extend locally in the orolabial, genital, or perianal regions. Rarely, primary HSV infection can be associated with more severe and disseminated disease, including skin, liver, CNS, and other organs. Primary infection from an organ allograft is rare, but if it occurs is fulminant and has a poor outcome [27]. Transmission of HSV from stem cell products has not been described.

HSV should be considered in the differential diagnosis of clinically appropriate syndromes regardless of serostatus prior to transplant. In all transplant recipients (organ and marrow), visceral or disseminated disease can occur, including disseminated cutaneous disease, esophagitis, hepatitis, CNS disease, and pneumonitis. Fever, leukopenia, and hepatitis are common presenting signs of disseminated disease.

Clinical Pearls
- HSV can present with atypical lesions and manifestations in transplant patents.
- HSV-associated mucositis can be more severe and prolonged.
- Transplant patients may acquire HSV after transplant.

VZV

Reactivation VZV: VZV remains latent lifelong in trigeminal and dorsal root ganglia, and is generally symptomatic when reactivation occurs. VZV reactivation usually presents as cutaneous herpes zoster (HZ) or "shingles" (Table 1). HZ typically presents as a painful vesicular rash that involves ≤2 adjacent unilateral dermatomes. Recurrence is rare, but does occur, typically in a different dermatome from the prior episode. The rash can be similar to HSV (vesicles, scabs) but can often be differentiated based on location as VZV rash does not cross the midline and VZV usually does not recur in the same location like HSV. Because there is a spectrum of immune control in transplant recipients, patients may have atypical lesions (plaques, nodules) and varying rates of progression of lesions, including a more prolonged onset and slower rate of crusting and scabbing. It is not uncommon for patients to develop new lesions for many days to a week after onset. Recurrent HZ (>2 episodes), particularly in the same dermatome, should prompt evaluation for another cause, most commonly HSV. Viral testing is recommended for recurrent lesions in this setting.

Secondary complications are common with HZ. The incidence of post-herpetic neuralgia (PHN, chronic neuropathic pain at the site of the skin lesions) is higher in transplant patients (20–40%) than in the general population (18%) [28, 29]. With open skin lesions, patients are at risk for bacterial superinfection.

Transplant recipients have a greater chance of multidermatomal and disseminated disease than immunocompetent persons [30]. Disseminated VZV is defined by a distribution of greater than 2 dermatomes, or involvement of 2 noncontiguous dermatomes. Rarely, patients may develop invasive complications (CNS, visceral) without skin manifestations (zoster sine herpete). Immunocompromised children may present with hemorrhagic lesions [31]. VZV reactivation may also present with ophthalmologic findings, with or without rash. Herpes zoster ophthalmicus (HZO) and progressive outer retinal necrosis (PORN) are sight-threatening emergencies that require prompt ophthalmologic evaluation and treatment (see ▶ Chap. 64, "Ophthalmic Infections in Transplant"). The nasociliary nerve innervates the tip of the nose and the globe, so a vesicular rash on the nose (Hutchinson sign) should prompt consideration of ophthalmic involvement.

In addition to direct nerve involvement of VZV causing inflammatory neuritis, patients with VZV reactivation are at risk for VZV vasculopathy, which may lead to neurovascular complications such as ischemic or hemorrhagic stroke and granulomatous small and large vessel disease. VZV vasculopathy is more common in immune-compromised individuals, including HIV and renal transplant, and can present weeks to months after HZ, or even without prior clinically evident HZ

disease [32]. The course is often complicated and chronic, with poor response to therapy and is associated with significant morbidity.

Primary VZV: Varicella zoster virus is highly infectious to seronegative persons and is primarily transmitted through respiratory droplets but can also be spread through direct contact with active skin lesions. Rarely, virus may be aerosolized from patients with active skin lesions and transmitted to mucosal surfaces. Only one organ donor transmitted infection has been reported; there have been no reports of stem cell transmission [33]. The incubation period for varicella is 10–21 days after exposure to the rash, but may be shorted in immunocompromised patients. Patients are infectious from 1–2 days prior to lesion formation until all lesions are crusted.

Clinically, primary varicella presents as a disseminated pruritic rash that often starts on the face and spreads down the trunk, with relative sparing of the hands and soles of the feet; mucosal involvement can occur. Lesions begin as papules then progress to vesicles, followed by crusting and scabbing. New lesions continue to appear for several days such that patients have lesions of various stages (papules, vesicles, and crusted lesions) at the same time. Clinical manifestations may depend on the immune status of the patient and can be atypical in transplant patients [34]. Highly immunosuppressed patients may have more severe primary infection with rapid progression and multiorgan involvement, including pneumonia or CNS disease.

Clinical Pearls

- Transplant recipients may have HZ lesions develop for many days with reactivation.
- Patients with primary VZV are infectious prior to lesions developing and until lesions are crusted.
- Recurrent HZ, particularly multiple episodes in the same dermatome, should prompt evaluation for HSV infection.
- VZV vasculopathy can be a devastating neurologic complication of reactivation.

Disease Diagnostics and Clinical Evaluation

Pretransplant

All pretransplant recipients should have IgG serologic testing for HSV and VZV. Serum IgG level and not IgM level is recommended given the potential for isolated false-positive IgM levels which have no clinical value outside of the diagnosis of acute herpesvirus infection in the appropriate clinical setting [36]. The majority of patients even without a history of clinical VZV infection will be seropositive; nevertheless, negative serology prior to transplant is an important part of identifying prevention (vaccine, immunoprophylaxis) efforts prior to and after transplant. If IVIG administration is anticipated, patients should have serologic testing prior to the IVIG as this can give falsely positive results.

Type-specific testing for HSV (HSV-1, HSV-2) is recommended for risk stratification of potential for oral and/or genital reactivation or acquisition after transplant. Many patients may not be aware of their serostatus and thus may need counseling regarding the meaning of a positive test. The CDC has good information for practitioners regarding the significance of a positive test (https://www.cdc.gov/std/herpes/screening.htm). Given the potentially sensitive nature of a positive serologic test for HSV-2, practitioners should counsel patients prior to and after testing.

Patients with a history of pretransplant, clinical HSV reactivation should be counseled on the possibility of an increase in frequency and/or severity after prophylaxis is stopped. Seronegative recipients should be made aware of their serostatus to provide counseling regarding the potential for HSV acquisition after transplant.

Donor serologic testing for HSV and VZV is not routinely done as it has not been an important part of posttransplant care. In organ transplant, donor serology does not predict the rare case of allograft transmission of HSV and donor Immunity is not transferred to the recipient. In allogeneic stem cell transplant, having a seropositive donor may decrease the incidence of HSV reactivation after transplant, but knowledge of donor serology has not been used to alter care after transplant [37]. In allo-HSCT, it is not known if donor VZV serostatus has any bearing on post-transplant VZV reactivation or acquisition. However, given that most donors will be seropositive, donor serostatus is not routinely checked.

Posttransplant

HSV

Most patients present with typical orolabial and genital lesions and can be diagnosed clinically. However, HSV in an immunocompromised host may present atypically so laboratory confirmation may be necessary. To sample for HSV, the lesion should be unroofed, scraped, and sent for Direct Fluorescent Antibody (DFA) testing, viral culture, or polymerase chain reaction (PCR). Earlier sampling of HSV lesions improves the diagnostic yield. Direct Fluorescent Antibody (DFA) testing of mucocutaneous lesions, bronchoalveolar lavage (BAL), and other clinical samples can provide rapid results; however, the sample needs to include enough cells for staining. Compared with tissue culture, the sensitivity is between 60% and 75% and specificity is 85–99% [38]. PCR for HSV DNA is fourfold more sensitive than tissue culture for diagnosing mucocutaneous HSV and has largely replaced culture and DFA for HSV diagnosis in most body sites (Table 2) [39]. HSV PCR testing availability has increased dramatically and is available at most medical centers though may be performed at an outside reference laboratory. Turnaround time may vary depending on availability, so alternative on-site laboratory tests (DFA, culture, Tzanck, and histopathology) may be useful. Cerebrospinal fluid PCR is the gold standard for diagnosing HSV meningitis or meningo-encephalitis with a sensitivity of 98% and specificity of 100% [40]. FDA-

approved rapid multiplex nucleic acid amplification testing (NAAT) on CSF is becoming the standard of care at many medical centers, making CNS infections more easily identified quickly. It should be noted that HSV PCR testing may be negative in the CSF very early in the disease, so in patients with a compatible clinical syndrome and other diagnostic testing (EEG showing temporal lobe findings, MRI with temporal lobe encephalitis), a repeat LP may be necessary [41]. The significance of HSV DNA detected in other secretions (e.g., BAL) should be evaluated in the context of the patients' clinical disease as HSV shedding may occur outside of clinically significant reactivation. Histopathologic evaluation for confirmation is recommended and can be done on BAL-acquired cells. HSV DNA can also be detected in the blood of patients without clinically evident disease [42]. However, the clinical significance of finding HSV DNA in the blood outside of patients with clinical syndromes consistent with disseminated disease has not been well established [43].

HSV culture should be done when other testing is not readily available or when drug resistant HSV disease is suspected. Genotypic testing for HSV resistance testing is available, but not often used for clinical care because the database for clinical resistance is not complete. Tissue or cell-block histopathology can be helpful with immunocytochemistry for HSV and is recommended to confirm a diagnosis where PCR or other tests may represent contamination (e.g., BAL contaminated from oropharynx). Serologic testing is rarely useful for diagnosing acute infection as increased IgM levels in HSV may occur with reactivation and not a new acquisition [44]. Novel diagnostic tests are being evaluated for clinical use, including Next-Generation Sequencing (NGS) and functional immunologic assays (T-cells), but these are not in wide clinical use.

Diagnosis of HSV keratitis remains primarily a clinical diagnosis based on characteristic features of the corneal lesion on slit lamp microscopy. Patients with ocular disease should be referred to an ophthalmologist for appropriate diagnosis and treatment.

VZV

As with HSV, the majority of primary and reactivation VZV can be diagnosed based on clinical presentation [45]. However, as discussed previously, VZV can present with atypical lesions due to altered immune status and can also present in deep sites (CNS, visceral) necessitating the use of diagnostic tests. VZV diagnostics are similar as for HSV, with a few minor differences (Table 2). When available, PCR is the most sensitive and specific rapid diagnostic test to perform on scrapings, vesicle fluid, serum, spinal fluid, and other tissues. However, PCR may not always be available in real-time on all sample types at most medical centers. As with HSV, PCR of CSF is the gold standard for CNS disease. PCR testing may be positive only early in the setting of VZV vasculopathy, but in immunocompetent patients, CSF VZV IgG has been proposed to be more sensitive [46]. This has not been evaluated in transplant patients. PCR of saliva has been found to be more sensitive than serum PCR in patients with HZ skin lesions, but is not commonly used clinically [47]. In the rare circumstance where there is concern for vaccine strain associated

Table 2 Laboratory Methods for Diagnosis of HSV and VZV

Test	Advantage	Disadvantage
Direct Fluorescent Antibody (DFA)	Rapidly available Virus-specific Good specificity, 85–99%	Lower sensitivity (40–90%) than PCR Limited sample types (needs cells to stain; e.g., not CSF) Time/labor intensive
PCR	Most sensitive (98%) Done on most sample types	Not available at all centers High cost Positive result other than in CSF requires interpretation
Culture (standard tissue based)[a]	Identify type-specific HSV Able to isolate virus for drug susceptibility testing	Turn-around time Labor intensive Sensitivity affected by storage and transport (30–90%)
Culture (shell vial)[a]	Faster turnaround than tissue culture (24–48 h) Similar sensitivity to tissue culture	Labor intensive Does not grow virus for phenotypic sensitivity testing
Tzanck Smear	Rapid Direct visualization	Fresh lesion needed Requires experience Nonspecific to HSV or VZV
Histopathology with immunohistochemistry	Can prove tissue-invasive disease Specific to HSV or VZV	Samples more difficult to acquire Long turn-around time
Serology (IgG, IgM)	Useful to guide pre-transplant risk stratification and prevention Can be used to document response to primary VZV vaccine series[a] CSF VZV IgG can be used for CNS vasculopathy	Insensitive marker for acute infection False-positive IgM
Drug resistance phenotypic assay (plaque reduction assay)[b]	Clinically reliable Can test multiple medications	Need positive culture (see above re: sensitivity) Turn around time
Drug resistance genotypic assay	Rapid	Available for research use, commercial assays not readily available Resistance mutations not completely characterized
Virus-specific immune responses (e.g., CD4/CD8 cell-mediated immunity)[b]	Can show functional immune responses Potential to help guide prevention	Expensive, complex Not validated

(continued)

Table 2 (continued)

Test	Advantage	Disadvantage
Next-generation sequencing (NGS)[c]	Sensitive and specific Can detect specific pathogens and resistance genes Many sample types	Investigational Not widely available Expensive Not validated

Modified from Lee and Zuckerman [53]. See text for references

[a]Culture and phenotypic resistance testing are not commonly used in clinical practice for VZV (see text); however culture may assist in confirming HSV vs. VZV infection if other tests are unavailable. Culture (standard tissue based), Culture (Shell vial), Drug resistance phenotypic assay (plaque reduction assay)

[b]Practitioners should be aware of sensitivity of their laboratory assay for vaccine-induced antibody as some assays have reduced sensitivity. Can be used to document response to primary VZV vaccine series

[c]Purely investigational/hypothetical. Virus-specific immune responses (e.g.....), Next-generation sequencing (NGS)

disease, CDC has PCR testing available for the OKA (vaccine) strain. DFA, though cumbersome, may be more readily available in some centers for rapid identification of VZV skin and mucosal lesions. Viral culture is rarely used clinically for the diagnosis of VZV infections given its low sensitivity (48%) [48]. For HSV, culture is often done to evaluate for antiviral resistance, but given the low sensitivity of culture for VZV, susceptibility testing, if done, has been via PCR for thymidine kinase and polymerase mutations which may confer resistance, but have been poorly described in clinically significant resistance [49]. Given the presumed low likelihood of clinically significant antiviral resistance in VZV infections, routine testing is not recommended. Nevertheless, for patients with a high clinical suspicion, PCR testing may be available on an experimental basis. Nonresponse to acyclovir may be a function of the higher IC50 of VZV for acyclovir than in HSV, so failure is usually due to inadequate dosing or duration of antivirals [50].

Commercially available serologic testing for VZV detects both natural infection and vaccine-induced antibody. However, care should be taken with interpretation of negative results as some commercial assays have been reported to have reduced sensitivity for detection of vaccine-induced immunity [51]. Nevertheless, test characteristics have been improving so clinicians should be aware of which assay is used in their institution [52].

Clinical Pearls

- Serology is only useful to document pretransplant status and response to VZV primary vaccine series, though some assays have reduced sensitivity for vaccine-induced immunity.
- PCR is the most sensitive and specific diagnostic test in most samples.
- HSV resistance testing takes time, so treatment may need to be changed prior to results.

Management

Clinical Disease

HSV

HSV treatment in the immunocompromised host depends on the serostatus of the patient, degree of immune compromise, and clinical manifestations. Acute primary HSV in an immunocompromised patient may spread rapidly so early initiation of therapy is recommended, including starting with IV therapy if there is any clinical concern for progression or dissemination. In seropositive patients, the majority of mucocutaneous disease can be treated with oral therapy (acyclovir, valacyclovir, or famciclovir) (Table 3). It is important to remember that by the time clinical HSV symptoms arise, there has been significant viral production in deep nerve and epithelial cells. As terminators of viral production, nucleoside analogues (acyclovir and derivatives) are effective at diminishing new virions from being produced, but do not stop the ongoing damage from the many virions already in the tissues by the time symptoms are present. Therefore, early administration of antivirals has a greater chance of altering disease trajectory. In immunocompromised patients, viral production is less likely to be controlled by the innate and adaptive immune system and lesions have a tendency to persist for longer and take longer to crust or resolve; therefore, it is reasonable to initiate therapy when the diagnosis is made later than the usually recommended 72 h window for herpes infections. Once crusting of all lesions occurs, initiation of antiviral therapy is unlikely to alter the outcome. Therapy should be continued until complete healing of the lesions and a minimum of 5–7 days [53].

In the immunocompromised patient, intravenous acyclovir should be used for more extensive mucocutaneous disease and when there is disseminated, visceral, or CNS involvement. It goes without saying that early initiation of therapy is associated with improved outcomes in patients with more severe disease. Therapy in severe disease should be continued for a minimum of 14 days. If identified and treated early, HSV-infected organs (not known at the time of transplant) have been used successfully for transplantation and retransplantation [54]. Liver transplantation can be performed in cases of fulminant HSV hepatitis without recurrence in the new organ; however, recurrence has been reported in a patient due to acyclovir-resistant HSV [55].

Children clear acyclovir more rapidly than adults and need higher doses. Dosing guidelines are extrapolated from studies in other pediatric populations and appear in Table 3. Epidemiologically, children are more likely to be seronegative for HSV, so at risk for primary infection after transplant.

Ocular HSV manifestations are treated with various topical and/or systemic therapies and should be done in coordination with an ophthalmologist. Transplant clinicians should be aware that there are risks of epithelial toxicity with various topical agents, so oral therapies may be used as either an adjunct or as primary therapy of ocular HSV infections such as keratitis and endotheliitis [56]. Treatments may need to be continued for an extended period of time depending on the extent of

Table 3 Recommendations for HSV treatment in transplant patients [53]

	Agents	Comments
Limited mucocutaneous disease		
Adult	Acyclovir 400 mg three times a day Valacyclovir 1000 mg twice a day Famciclovir 500 mg twice a day Acyclovir 5 mg/kg IV every 8 h (if unable to take PO or more extensive disease)	Therapy should be started based on clinical diagnosis, pending laboratory confirmation Therapy should be continued until complete healing of all lesions or at least 5–7 days, some give longer courses for more extensive disease
Pediatric	Acyclovir 10 mg/kg IV every 8 h Acyclovir 80 mg/kg divided qid (not to exceed 800 mg/dose)	
Severe, visceral/ disseminated/ CNS disease		
Adult	Acyclovir IV 10 mg/kg every 8 h	Intravenous therapy should be continued until resolution of disease, or 14 days, and then oral medication may be given. For CNS infection may consider 21 days of IV therapy. Continue for 21 days for disseminated or CNS infection
Pediatric	Acyclovir IV 45–60 mg/kg/day in three divided doses	
Acyclovir-resistant HSV		
	Foscarnet 80–120 mg/kg/day IV in two or three divided doses Cidofovir IV 5 mg/kg IV q wk. given with provenecid Topical cidofovir or foscarnet Topical trifluridine	Resistance should be laboratory-confirmed, although empiric therapy can be started Reduce immunosuppression, if possible
Chronic Suppression		
	Valacyclovir 500 mg po BID (preferred over 1 g daily) Famciclovir 250 mg po BID Acyclovir 400 mg po BID	Patients with recurrences (≥2) after cessation of antiviral prophylaxis Can be continued long-term

Modified from Lee and Zuckerman [53]. See text for references
Dosages are for GFR≥50, adjustment is necessary for renal insufficiency
HSV, herpes simplex virus; PO, orally; IV, intravenously

infection, viral integration into the corneal layers, and the risk of relapse following cessation of treatment.

Chronic Suppression

Acyclovir and valacyclovir are well tolerated for chronic suppression of HSV, and associated with minimal risk and toxicity at the doses required for suppression. Therefore, patients entering transplant on chronic suppression and those with bothersome recurrences (≥2) after cessation of antiviral prophylaxis may be candidates for suppressive therapy. Some patients may prefer to remain on this therapy for years or indefinitely, but it is reasonable to consider attempting to stop suppressive therapy in some individuals when immune suppressive therapy is tapered, particularly in those patients not requiring suppressive therapy prior to transplant.

Suppressive therapy can be safely continued for many years and is associated with less frequent acyclovir-resistant HSV than episodic therapy in immunocompromised patients [57]. It is important to give appropriately recommended doses of antiviral therapy to avoid increased risk of clinical breakthrough or resistance. Clinicians must consider the toxicities, costs, risks of transmission, adherence, and psychosocial issues when discussing chronic suppression with patients.

Acyclovir Resistance

Acyclovir resistance can occur in up to 10.9% of immunocompromised hosts and is more common in HSCT than in SOT patients [24]. Patients typically present with lesions that do not respond to high-doses of antiviral therapy. Often, they have had prior acyclovir exposure and/or recurrent disease. HSV resistance to nucleoside analogues is usually due to diminished or absent thymidine kinase (TK) activity (e.g., UL23 gene) or altered DNA polymerase. Drugs that utilize TK (acyclovir, famciclovir, valacyclovir, ganciclovir) are all affected by TK mutations. Evaluation should include laboratory confirmation of HSV disease, including a viral culture as testing for acyclovir resistance generally relies on phenotypic assays. This is most commonly a plaque reduction assay which gives an IC50, the concentration of a drug that is required for 50% inhibition in vitro. Given that testing relies on growth of the virus, results may be delayed for weeks or even months. Genotypic testing may be performed more rapidly than phenotypic testing but is not routinely available for clinical use and requires cautious interpretation. NGS may be a future option for rapid detection of HSV resistance [58].

Foscarnet is the usual first-line therapy medication for acyclovir-resistant HSV infections, but may be limited by toxicity. Renal function and electrolytes need to be followed closely and some patients may develop genital ulcerations that may be mistaken for herpes lesions [59]. Cidofovir is an option, but due to its long half-life, toxicity is difficult to attenuate once it manifests. Probenecid is used with cidofovir to protect against renal toxicity. Cidofovir and foscarnet can also be compounded for topical use, if necessary. Brincidofovir and helicase-primase inhibitors (e.g., amenamevir and pritelivir) are currently in later stages of development and may be available in the near future. Recurrent acyclovir-resistant HSV disease may require repeated courses of therapy. However, subsequent recurrences may be susceptible to acyclovir therapy [60].

Clinical Pearls

- Nucleoside analogues (acyclovir, etc.) are effective in preventing new virions from being produced, but do not prevent damage from the significant amount of virus present by the time patients have symptoms.
- For limited mucocutaneous lesions, oral therapy can be used.
- Suppressive therapy is associated with less frequent acyclovir-resistant HSV than episodic therapy in immunocompromised patients.

VZV

Primary Varicella

Aggressive pretransplant screening/vaccination and posttransplant, postexposure prophylaxis should lead to very few patients developing primary varicella disease. In those at risk, primary varicella can be highly morbid in the posttransplant setting so should be treated rapidly with IV therapy, preferably within 24 h of onset of symptoms or rash (Table 4). Additional supportive measures may include IV hydration (to avoid nephrotoxicity), lesion care to prevent super-infection and pain control. Some patients with severe disease (e.g., pneumonitis, CNS disease) may require ICU-level care. IVIG and VZV-specific immune globulin have been used in some cases of severe disease, but the efficacy of this treatment has not been confirmed [61]. After crusting of all lesions or when patients have improved clinically and no new lesions are forming, most patients can be safely transitioned to oral therapy. It is important to remember that the doses of acyclovir (both IV and oral) required to treat varicella are higher than for HSV due to the higher IC50 of varicella.

Reactivation Disease

Patients with disseminated, CNS or other, visceral varicella should be treated initially with IV acyclovir and supportive care similarly to those with acute primary

Table 4 Recommendations for VZV treatment in transplant patients

	Agents	Comments
Localized HZ, uncomplicated		
Adult	Acyclovir 800 mg 5× a day Valacyclovir 1000 mg 3× a day Famciclovir 500 mg 3× a day	Therapy should be started based on clinical diagnosis Therapy should be continued until complete crusting of all lesions or at least 7 days, longer courses may be necessary for lesions slow to resolve
Pediatric	Valacyclovir 20 mg/kg tid (2–18 years old) Acyclovir 800 mg po 5× a day (≥12 years old) Acyclovir 10 mg/kg IV every 8 h (<2 years old or unable to take PO)	
Acute primary or Severe (visceral/ disseminated/ CNS, including vasculopathy and HZO)		
Adult	Acyclovir IV 10 mg/kg every 8 h	Intravenous therapy should be continued until significant improvement of disease, and then oral medication may be given. For CNS infection may consider 14–21 days of IV therapy, followed by oral therapy.
Pediatric	Acyclovir IV 45–60 mg/kg/day in three divided doses	

Modified from Pergam and Limaye [62]. See text for references

Follow renal function carefully on therapy. Dosages are for GFR≥50, adjustment is necessary for renal insufficiency

VZV, varicella zoster virus; HZ, herpes zoster; HZO, herpes zoster opthalmicus; PO, orally; IV, intravenously

disease [62]. Also, patients with ocular or facial nerve involvement are at risk for persistent sequalae, so should receive initial IV therapy, with transition to oral therapy after initial stabilization. Those with suspected VZV vasculopathy should receive IV therapy, the duration of which is not well established. Most experts would treat until symptoms resolve, and for a minimum of 2–3 weeks with longer courses sometimes needed. The use of steroids has been advocated by some in the setting of complex vasculopathy that does not respond initially to antiviral therapy or has the potential for significant permanent neurologic sequelae, with no controlled trials evaluating their efficacy [63].

The majority of cutaneous dermatomal HZ is treated with oral antiviral agents, acyclovir, valacyclovir, or famciclovir. As with HSV, antiviral therapy should be given for at least 7 days or until lesions have crusted over, which may be delayed in immunocompromised hosts. IV therapy is recommended for young children and/or those who cannot tolerate oral therapy.

Controlled trials of adjunctive therapies (e.g., steroids, gabapentin, and amitriptyline) given during the HZ episode to prevent PHN have not been conducted in transplant recipients. Due to their uncertain effectiveness and potential toxicities, these agents are not recommended routinely.

Clinical Pearls

- Posttransplant patients who develop primary varicella should be treated with intravenous acyclovir.
- Localized nonsevere dermatomal HZ can be treated with oral therapy in most adults.
- Patients with severe HZ disease should receive IV acyclovir as initial therapy.
- Patients with HZ involvement of the eye(s) should be assessed by ophthalmology.

Prevention

Primary Infection

Acute primary infection with HSV or VZV can be morbid in the transplant recipient. Therefore, pretransplant assessment for prior infection is important. For HSV, patients should be asked about prior orolabial, genital, perianal, or other recurrent mucocutaneous reactivation. For varicella, many patients may recall having had chicken pox or exposure to someone with chicken pox as a child. Alternatively, some recipients may have had a prior episode of shingles. HSV primary infection can be asymptomatic, so it is important to remember that many patients are seropositive even without recollection of having had herpes lesions. While asymptomatic VZV infection is uncommon, adult recollection of prior disease may be inaccurate, with 71–93% of adults with negative or uncertain history being seropositive [64]. Even with a reported history of infection or immunization, all transplant recipients should have IgG serologic testing for HSV and VZV. Serology is usually adequate to

evaluate for evidence of prior infection; however, some patients with hematologic malignancy may have immunoglobulin deficiencies and negative serology.

HSV

There is no FDA-approved vaccine to prevent HSV infections, though there are some candidates in the pipeline. Current HSV prevention techniques are focused on behavioral and antiviral methods. Seronegative transplant recipients should be counseled regarding the risks of HSV-1 and HSV-2 acquisition. It is important to avoid contact with persons with active lesions, as these patients are highly infectious. However, persons may acquire HSV from asymptomatic individuals, so providers are encouraged to provide standard counseling to recipients regarding HSV acquisition. The virus can be acquired from persons who have never had lesions. Condoms are effective, but do not completely protect against HSV transmission. In serodiscordant couples, daily antiviral therapy taken by the seropositive partner can prevent HSV-2 transmission to the seronegative partner [65]. This may be considered as an option, but has not been evaluated in the SOT population. There are no controlled studies looking at the efficacy of postexposure prophylaxis to prevent HSV acquisition, so it is not routinely recommended.

VZV

The incidence of primary VZV in transplant recipients remains low. However, VZV is highly transmissible via the airborne route; therefore, prevention of primary infection in seronegative patients relies most heavily on vaccination rather than behavioral interventions. Thankfully, there are effective vaccines to prevent primary infection with VZV (Varivax, ProQuad). Because the only current preparations are live vaccines, timing of immunization needs to be considered carefully in the transplant population (Table 5). Each transplant patient should be evaluated individually, with the risks and benefits weighed with regard to the potential for adverse outcome from the vaccine, the likelihood of response to immunization, and risk of acquisition of VZV in the community. It should be noted that there have only been rare cases of Oka strain disseminated disease after immunization, even in the most profoundly immunocompromised patients [66]. Additionally, because acyclovir is active against the Oka strain, it can be used for treatment of complications of the vaccine in the rare event this should happen [67]. Unfortunately, antiviral resistance in the Oka strain has now been reported [68]. Seronegative patients who receive the primary immunization series should have serology checked to confirm adequate response to vaccine, which may be poorer in immunocompromised patients [69]. However, care should be taken in interpretation of a negative test given the reduced sensitivity of some assays [51].

Seronegative patients who are not able to be immunized should take particular care to avoid situations where they may be exposed to persons with primary varicella. Additionally, they should avoid close contact with persons with shingles as there has been transmission from patients with the rash of HZ. A very important part of pretransplant counseling is ensuring contacts of transplant recipients are up to date on their immunizations. Preventing primary VZV disease in close contacts can

be an effective way to keep transplant patients safe, so eligible contacts (>1 year old, no history of disease, no contraindication) should be vaccinated with the live-vaccine series. Some practitioners express concern with immunizing close contacts of immunosuppressed patients with a live vaccine out of concern the potential to transmit the vaccine strain to the compromised host. Transmission of the vaccine strain is extraordinarily rare, and it is significantly higher risk for contacts to remain unvaccinated and potentially bring the highly-transmissible wild-type varicella into the home should this occur [70]. Vaccinees should keep the site covered, particularly if a localized rash develops and transplant patients should be isolated from contacts who develop a significant rash. Close contacts who are eligible should be considered for the adjuvanted zoster vaccine as well (Shingrix®, GlaxoSmithKline).

Pretransplant Vaccination (Table 5)

SOT

Pretransplant immunization in the SOT population is more straightforward than in the HSCT population. Seronegative patients without significant immunocompromise can be safely immunized prior to transplant. Response rates are lower in end-stage organ disease, so the full two-dose series should be given [71]. The vaccination series should be completed at least 4 weeks prior to transplant. The majority of patients ineligible or unable to be immunized will be unlikely to be able to get the primary series after transplant unless a non-live vaccine is deemed effective (see below).

Table 5 VZV Immunization in seronegative SOT and HSCT

		Pretransplant	Posttransplant
SOT		Varivax, preferred pretransplant	Varivax may be safe in select posttransplant patients on stable, low-dose immunosuppression. Ongoing study of RZV in VZV seronegative patients (Clinical trials.gov NCT03685682)
HSCT	Autologous	Varivax, two shot series (though one is better than none). Prior to stem-cell harvest, if possible	Varivax, two shot series, when eligible for live-virus vaccination[a]
	Allogeneic	No recipient vaccine	Varivax, 2 shot series, when eligible for live-virus vaccination[a]

Varivax: two-shot series recommended, 4 weeks apart for >12 years old, 3 months apart for 1–12 years old. Proquad may be considered in eligible children. RZV (recombinant zoster vaccine, Shingrix®). Hold acyclovir during live-vaccine administration

[a]2-1-5 Rule = Not until 2 years post HSCT and >1 year off all immunosuppressive therapy and at least 5 months since last dose of IVIG/VZIG or most recent plasma transfusion. Check varicella serology at least 1–2 months after second dose of Varivax to ensure seroconversion. Make sure close contacts are immunized.

HSCT

Immunization of the allogeneic HSCT recipient prior to transplant will likely not provide reliable immunity after transplant so is not routinely recommended. There has been no study of immunization of the allogeneic DONOR against varicella, but this could be a viable option to improve immunity or responses to vaccination after transplant given data with other vaccines [72]. Further research is necessary before making this recommendation. Seronegative autologous transplant patients should receive the primary immunization series if there is enough time prior to transplant or chemotherapy to wait the approximately 4 weeks necessary to avoid immunosuppression after each immunization in the two-shot series. If possible, the entire two-shot series should be given prior to transplant; but if not feasible, a single shot is preferred to none. Some autologous patients may be on maintenance immunosuppression, or their primary disease/prior chemotherapy may be so immunocompromising that the live-virus vaccine should be avoided. If there is no good time to safely administer the vaccine, it is reasonable to wait until after transplant. As stated, the risk of vaccine must be taken into account on an individual basis. In general, immunizations in autologous patients should be attempted prior to stem cell harvest to optimize the likelihood of improved immunity after transplant; however, there are no studies of this method with primary VZV. Many patients are on acyclovir prophylaxis prior to transplant. This medication should be held with live-varicella immunization as it decreases the efficacy of the immunization because the Oka strain is susceptible. Additionally, patients should not receive the primary VZV immunization if they have had IVIG within the past 5 months; however, in a seronegative patient who may be eligible for the primary vaccine series, the lower likelihood of response should be evaluated with regard to the risk of potential primary VZV infection in the future.

Posttransplant Vaccination (Table 5)

SOT

The live varicella vaccine has been given safely to very select post-SOT patients, mainly pediatric patients on lower immunosuppression [71]. Current guidelines recommend vaccination for only select kidney and liver transplant recipients who are seronegative posttransplant and receiving long-term, low-level immunosuppression [62]. Given the impressive humoral (anti-gE) as well as cell-mediated immune responses to Shingrix® (aka, adjuvanted vaccine, or recombinant zoster vaccine (RZV), GlaxoSmithKline), there is a current study looking at this vaccine in seronegative post-transplant patients (Clinical trials.gov NCT03685682).

HSCT

Seronegative HSCT recipients are usually eligible to receive live virus vaccines after transplant assuming they are 2 years out from transplant, immunosuppressive medications have been discontinued for at least a year and they have not received

IVIG for at least 5 months [73]. Even though immune reconstitution occurs earlier after autologous transplantation, there has been no delineation between allogeneic and autologous transplant with regard to timing of administration of live vaccines after transplant.

Posttransplant Postexposure prophylaxis for VZV

Seronegative transplant recipients are at risk for developing severe primary infection after exposure and should receive postexposure prophylaxis after significant exposure (Table 6) [74]. While cell-mediated immunity is important to prevent reactivation of VZV, antibody is a better correlate of protection against primary infection. Therefore, the mainstay for postexposure prophylaxis in high-risk individuals is the administration of immunoprophylaxis in the form of VZV-specific antibodies or with nonspecific immunoglobulin preparations [31]. VARIZIG™ (Saol Therapeutics) is the only VZV-specific immune globulin preparation available currently and recommended within 10 days of exposure [74].

Antiviral agents have been used successfully either alone or in combination with immunoprophylaxis [75]. Therefore, antivirals are recommended if immunoprophylaxis is not able to be given within 10 days of exposure or as an adjunct to immunoprophylaxis in high-risk patients. Current guidelines suggest the use of valacyclovir due to improved oral bioavailability, but acyclovir is likely effective as well [62]. There have been no controlled trials of antiviral medication, so timing of administration is based on case series and expert opinion. Historically, antivirals were recommended starting day 7 after exposure and for 7 days of treatment [76]. However, due to concerns about early and prolonged viral replication, some experts recommend starting earlier and continuing for longer [77]. Additionally, immunoprophylaxis has been suggested to delay viral production so a longer course is often given in the setting of immunoprophylaxis [77].

Patients who are seropositive for varicella are presumed to be protected against new infection. However, some patients with profound immunosuppression early

Table 6 Postexposure prophylaxis for VZV seronegative transplant recipients

VZV-infected exposure	*Primary infection*: primarily airborne, also direct contact with lesions *HZ*: direct contact with active lesions or airborne material from rash
Definition of contact	*Outpatient*: household contact, nontransient face-to-face contact *Inpatient*: exposure in the same room, face-to-face contact with an infectious staff member or patient, visit by a contagious person
Prophylaxis:	*Immunoprophylaxis*: VARIZIG within at least 10 days of exposure; IVIG if VARIZIG not available. *Antiviral*: If unable to give immunoprophylaxis within 10 days. Also, may be used as an adjunct to immunoprophylaxis, particularly if IVIG is used. Valacyclovir either for a 7-day course of therapy beginning 7–10 days after VZV exposure or, alternatively, from day 3 to 28 following exposure.

VZV seropositive HSCT patients on high-dose steroids or T-cell depleted grafts should be considered for immunoprophylaxis if they have a known VZV exposure. See text for references

after transplant or in the setting of treatment of rejection or graft versus host disease may be at risk of VZV acquisition even if seropositive, though this is rare. These patients are commonly on antiviral prophylaxis that may protect against VZV replication; however, it is recommended to follow them closely if they are exposed. HSCT patients are at higher risk than SOT, so postexposure immunoprophylaxis should be considered in patients on high-dose steroids and those with T-cell depleted grafts if they have a high-risk exposure [73].

Clinical Pearls

- HSV prevention is through mostly behavioral changes, but antiviral prophylaxis in the seropositive partner may also be considered.
- The only current option for immunization for VZV seronegative patients as a live vaccine and HSCT and SOT patients have different recommendations around transplant.
- VZV seronegative patients unable to safely receive Varivax should receive postexposure prophylaxis for high-risk exposures.
- Some highly immunocompromised seropositive HSCT patients should receive postexposure prophylaxis for high-risk exposures

Prevention of Reactivation

As seen in the epidemiologic curves, reactivation of HSV and/or VZV occurs most frequently within defined time frames of immune compromise. Therefore, most seropositive transplant recipients should receive some form of preventive antiviral therapy during the highest-risk periods. A discussion of HSV and VZV prevention would not be complete without mention of CMV prevention because (a) CMV can be highly morbid in the transplant population, (b) most transplant recipients get some form of CMV prevention modality, and (c) many of the anti-CMV medications have activity against HSV and VZV. Use of a CMV universal prophylaxis regimen with activity against HSV and VZV usually obviates the need for specific prophylaxis against these viruses. However, as preemptive regimens evolve and new drugs are developed specific for CMV and without activity against HSV and VZV (Table 7), clinicians will need to modify prevention algorithms.

In SOT, VZV reactivation occurs at a steady rate for years after transplant, so preventive antivirals are not usually guided by VZV risk given that there are no data to support the many years of antiviral treatment that would be required to prevent HZ after SOT [53, 62]. For HSCT, most significant HSV reactivation typically occurs early during neutropenia, with varicella risk generally more significant and prolonged than HSV after transplant, so antiviral prophylaxis duration usually centers around HZ risk. Moving forward, the use RZV as a preventive modality will hopefully reduce the risk of HZ such that extended antiviral prophylaxis for VZV may not be necessary in many transplant recipients, but there are currently not

Table 7 Currently available therapies for CMV and adequacy against HSV/VZV

CMV therapy	Adequate for HSV/ VZV?
Ganciclovir	Yes
Valganciclovir	Yes
Foscarnet	Yes
Cidofovir	Yes
Letermovir	No
Brincidofovir	Yes [78]
Maribavir	No

enough data to support attenuation of antiviral prophylaxis regimens in recipients of RZV after transplant.

Antiviral Medication

SOT

As stated, many SOT recipients receive antiviral medication to prevent CMV replication, the majority of which also covers HSV. Additional HSV and VZV-specific prophylaxis may be necessary in patients not receiving agents active against these viruses. Table 8 outlines recommended antiviral prophylaxis regimens and duration depending on the serologic status of the recipient. Typically, one month if HSV/VZV prophylaxis is given to recipients not on CMV prevention with activity against these viruses, though some centers give longer courses (e.g., 3 months) [53]. New epidemiologic data may help guide future recommendations [2]. Some centers will give about a month of antiviral therapy to HSV seronegative recipients not on HSV/VZV-active CMV therapy even if VZV seronegative out of concern for the rare potential transmission of HSV from the organ. In addition to the immediate post-transplant period, patients should receive HSV/VZV-active antiviral medication during immunosuppression intensification for organ rejection, especially with cytolytic therapies such as antithymocyte globulin and alemtuzimab. Antiviral dosing is garnered from studies of similarly immunosuppressed patients and has been successful in practical use. Increased doses of acyclovir or valacyclovir should be used for patients with a history of frequent and/or severe recurrences, and some clinicians opt to give chronic suppression to patients who have an episode of severe HSV disease after transplant.

Pediatric patients are more likely to be HSV and VZV seronegative. Aside from immunization against VZV in seronegative children, antiviral prophylaxis in pediatric patients is usually driven by serostatus. Dosing for antivirals is based on studies in immunosuppressed children and IV formulations may need to be used in children not able to reliably take p.o. medications. Though cidofovir has in vitro activity against HSV and could be used weekly in children, breakthrough HSV stomatitis has been reported with this regimen [79].

Table 8 Antiviral prophylaxis for HSV and VZV after transplant

	Serology	Antiviral prophylaxis	Comments
SOT	HSV+ VZV+/−	CMV prophylaxis[a] or Acyclovir 400–800 mg twice a day Valacyclovir 500 mg twice a day Pediatric dosing: <40 kg: Acyclovir (60–90 mg/kg PO in three divided doses) Valacyclovir (20 mg/kg PO twice a day, 3mo to 11 years) For IV, 5 mg/kg every 8 h	Administer for at least 1 month During treatment of rejection episodes (for at least 1 month) See also VZV pre/post vaccine prevention
	HSV− VZV+	CMV prophylaxis[a] In patients not on CMV prophylaxis there is no research, but some centers give VZV prophylaxis for 1 month after transplant	RZV after transplant, if eligible
	HSV− VZV−	In patients not on CMV prophylaxis, some centers give a short course of antiviral prophylaxis around transplant due to very low HSV risk from organ	See also VZV seronegative prevention
HSCT	VZV+ HSV+/−	Acyclovir 800 mg twice a day Valacyclovir 500 mg twice a day Unable to take p.o.: IV acyclovir 5 mg/kg IV every 12 h or 250 mg/m^2 IV every 12 h	Prophylaxis for at least one year, also during treatment of GvHD Consider RZV after transplant
	VZV− HSV+	Acyclovir 400 mg twice a day Valacyclovir 500 mg twice a day Unable to take p.o.:IV acyclovir 5 mg/kg IV every 12 h or 250 mg/m^2 IV every 12 h	From start of conditioning to engraftment or resolution of mucositis See also VZV seronegative prevention
	VZV− HSV−	No HSV/VZV-specific prophylaxis	See also VZV seronegative prevention

HSV, herpes simplex virus; VZV, varicella zoster virus; CMV, cytomegalovirus; SOT, solid organ transplant; HSCT, hematopoietic stem cell transplant; PO, orally; IV, intravenously, RZV, recombinant zoster vaccine

[a]CMV prophylaxis with recommended doses of ganciclovir, valganciclovir, valacyclovir or acyclovir are adequate for HSV prevention. If agent without HSV/VZV activity used for CMV prophylaxis (Table 7), additional HSV-specific prophylaxis is necessary. Dosages are for GFR>50, adjustment is necessary for renal insufficiency. Stop HSV/VZV prophylaxis if patient on treatment for CMV with activity against HSV/VZV (e.g., ganciclovir, valganciclovir, foscarnet, and cidofovir)

HSCT

Antiviral therapy significantly decreases HSV and VZV reactivation in HSCT patients [57]. The majority of HSCT patients are either getting preemptive prevention for CMV or letermovir, so will need some antiviral medication with activity against HSV and VZV. Some patients are on prophylaxis with acyclovir or valacyclovir prior transplant as a part of treatment protocols, this can just be continued into transplant. HSV risk is greatest early after transplant, so antiviral

therapy should be started with conditioning and continued at least through engraftment or when mucositis resolves. In VZV seropositive patients, antiviral therapy should be continued for an extended duration. At least one year has been considered to be the norm due to the high rate of HZ without antiviral therapy or with stopping antiviral therapy earlier than a year after transplant [13]. Some centers continue treatment for at least a year, or 6 months after completion of anti-T cell therapy, whichever is longer. One study used CD4 count as a marker for discontinuation, but patients had an unacceptably high rate of reactivation after treatment cessation [14]. In the future, immunologic biomarkers (e.g., VZV-specific immunity) may be used to determine when to stop antiviral therapy, but no such studies have been done to date. Finally, some patents may receive therapy for CMV reactivation that is active against HSV and VZV; acyclovir or valacyclovir can be stopped during these periods.

Clinical Pearls

- Most transplant patients should receive some antiviral prophylaxis with activity against HSV and VZV.
- Clinicians should be aware that some CMV prophylaxis does not have anti-HSV/VZV activity.
- HSV/VZV prophylaxis should be resumed during intensification of immunosuppression.

VZV Vaccination (Table 9)

Until 2017, the only commercially available vaccine to prevent HZ reactivation was the live-attenuated Oka vaccine (Zostavax®, Merck and Co.), which had incomplete response rates and was contraindicated in most transplant patients [80]. Since its approval, the new adjuvanted subunit varicella vaccination (Shingrix®, GlaxoSmithKline Biologicals) has been a complete game-changer with regard to prevention of VZV reactivation and postherpetic neuralgia. In immune competent patients, it is >90% effective in preventing HZ episodes and postherpetic neuralgia [81]. Studies are ongoing in transplant patients, but preliminary reports suggest good safety and efficacy such that current recommendations favor the use of the RZV over the live attenuated version [82]. Guidelines and recommendations can often lag behind treatment practice, but practically speaking, the live-attenuated vaccine is no longer used in transplant patients. Additionally, previous receipt of Zostavax® does not provide reliable long-term HZ prevention; therefore, patients (SOT and HSCT) eligible by CDC or other guidelines should receive RZV regardless of prior Zostavax® immunization.

Due to the adjuvant, RZV is associated with frequent injection site and systemic reactions such as fatigue, myalgias, headache, and fever. Because it is not a live vaccine, there is no concern with progressive clinical VZV disease or transmission. Patients should be counseled to expect the common side effects and treat them with supportive care.

Table 9 VZV immunization in seropositive SOT and HSCT patients

		Pretransplant	Posttransplant
SOT		RZV (where eligible, >50 years old). Some centers give RZV to all VZV seropositive adults ≥18-years old prior to transplant, further research ongoing.	RZV likely safe in low-risk, postrenal transplant patients. Safety and efficacy of RZV not confirmed in controlled trials in other organ recipients, some centers give to high-risk (NCT 03993717, NCT03493776, peds NCT 04006808),
HSCT	Autologous	Consider RZV if eligible based on ACIP guidelines and significant duration before transplant, otherwise wait until after transplant. If administered, consider prior to stem cell harvest.	RZV for all ≥18yo; 50–70 days after transplant and 1–2 months later
	Allogeneic	No recipient vaccine ? donor vaccination	No data, suggested to give to patients >50 years old, >2 years from transplant, off immunosuppression, no GvHD

RZV, recombinant zoster vaccine (Shingrix); SOT, solid organ transplant; HSCT, hematopoietic stem cell transplant. See text for references

SOT

Pretransplant Vaccination

Where possible, SOT patients who are eligible should receive the RZV prior to transplant [62]. RZV is recommended for the pretransplant population >50 years of age; however, studies are ongoing in younger patient populations. The vaccine should be given at least a few weeks prior to transplant to give an opportunity for immune response and to get past the phase of vaccine side effects prior to transplant. If vaccine is given closer to transplant, there is no contraindication to getting the transplant since this is not a live vaccine.

Posttransplant Vaccination

Despite the excitement at the prospect of having a non-live vaccine to give to transplant patients, there are theoretical risks that a potently adjuvanted vaccine could increase the risk of acute or chronic allograft rejection. Therefore, some centers are not routinely giving this immunization to all posttransplant patients until more research is done. To date, the only published trial in SOT is a randomized double-blind placebo-controlled study of the safety and immunogenicity of RZV in renal transplant patients that were highly selected based on factors that put them at low risk for rejection [83]. This study showed excellent immunogenicity and no increased risk of rejection or adverse events. Efficacy to prevent clinical HZ disease was not assessed. Additional ongoing research will hopefully answer questions regarding safety and efficacy in higher-risk patients in the coming years. At the

current time, it is reasonable to consider giving the RZV to renal and other transplant patients at low risk for rejection as defined by published literature, though some centers are using RZV in patients at higher risk for HZ (lung, heart). It should be noted that some patients are on anti-CMV therapy with activity against VZV for extended courses after transplant (e.g., lung recipients), so are generally protected against HZ reactivation during antiviral treatment. In these patients, it may be preferable to wait until closer to cessation of antiviral therapy to consider immunization as patients will have more time since transplant and likely be more clinically and immunologically stable. RZV can be given to patients taking antivirals because there is no known attenuation of vaccine response by antiviral medication to RZV. Unfortunately, there is significant variability between transplant centers in immunization practices after transplant; more research is needed to assist with standard practice guidelines.

HSCT

Pretransplant Vaccination

To date, there has been no evaluation of vaccination in the pretransplant setting as a preventive therapy for HZ after HSCT. However, some patients receiving chemotherapy and biological medications for their primary disease may be candidates for immunization prior to the decision to pursue transplantation. This is an area ripe for research. In the allogeneic recipient, recovery of cell-mediated immunity to varicella is highly variable though poorly characterized in the modern era. Patients undergoing a T-cell depleted allo-HSCT may not recover cell-mediated varicella responses unless they have clinical reactivation of VZV [84]. Non-T-cell depleted patients may reconstitute cell-mediated immunity, but recent data are sparse and GvHD prophylaxis likely attenuates this [85]. Currently, donor VZV serostatus is not routinely tested in allogenic donors and it is not known if boosting cell-mediated VZV-specific immunity with RZV in a donor will assist in reconstituting recipient immunity after transplant.

Autologous transplant patients were the subjects of a trial of a gamma-irradiated killed varicella immunization (that is not currently available), the first in a four shot series of immunizations was given prior to transplant [86]. HZ prevention efficacy of this series was similar to the efficacy of two-shots of RZV given after transplantation [82]. Therefore, given that this preparation is not available, requires more injections, and lacks improved efficacy, most autologous transplant recipients can wait until after transplant to receive HZ immunization. Another area for research is the efficacy of immunization in the autologous transplant patient prior to stem-cell harvest. This method has shown efficacy in improving response to other immunizations given after transplant and should be considered for evaluation in the future [87, 88].

Posttransplant

Safety and efficacy of HZ vaccination has been shown in two randomized trials in autologous transplant patients. The first, mentioned previously, showed 64%

reduction in HZ events with the use of a irradiated virus vaccine [86]. The second, a double-blind randomized placebo-controlled study of RZV demonstrated a 68% reduction in HZ events posttransplant (ZOE-HSCT) in patients ≥18 years old [82]. In this trial, the first vaccine was given between days 50 and 70 posttransplant, with the second dose 1–2 months after. In addition to the reduction in HZ episodes, this study showed a marked improvement quality of life in patients who developed HZ [89]. Therefore, it should be given to all autologus patients ≥18 years old (though insurance may not cover those <50 years old at this time).

There has been no study of RZV in VZV seropositive allogeneic HSCT patients. Therefore, it is not currently recommended until 2 years post HSCT, greater than 8 months off immunosuppressive therapy and no flare-up of GVHD. However, other adjuvanted vaccines with strong adjuvants have been used safely in allogeneic HSCT, so in time, it is likely that RZV may be used sooner after transplant. It is currently recommended to continue VZV prophylaxis until one month after first vaccination.

Due to the low cost and safety of acyclovir for extended courses, this remains the standard of care until there are more data to support the use of HZ immunization without antiviral medication after transplant. Additionally, given the lack of good markers of VZV-specific immunity and the lack of complete protection by the vaccine, further study is needed to elucidate which patients may be able to stop antiviral medications earlier than usual.

Clinical Pearls

- RZV has replaced Zostavax to prevent HZ reactivation.
- It will be important to keep abreast of the latest research in the use of RZV in transplant patients.
- Until further research is done, patients receiving RZV in the peritransplant period should take the recommended antiviral prophylaxis.

Special Considerations

Infection Control

HSV has not been shown to be transmitted via the airborne route, so patients with active lesions do not need to be on airborne precautions in the healthcare setting. Usual contact precautions are recommended when dealing with active lesions.

For VZV, all immunosuppressed patients admitted to the hospital with primary or reactivation disease should be placed on airborne and contact isolation precautions until all lesions are crusted. Patients with localized zoster lesions should also have them covered to decrease transmission risk.

Susceptible patients (seronegative, highly immunocompromised) who are exposed to VZV should remain in airborne and contact precautions from day 10 to 21 while in the hospital after exposure to the index patient. Patients who receive passive immunoprophylaxis (VARIZIG, IVIG) should remain in precautions

until day 28 [31]. Consideration should be given to vaccination of seronegative close contacts and household members of patients with primary or disseminated disease [90].

Pregnancy

With increasing numbers of transplant patients pursing pregnancy, this population deserves special mention. Unfortunately, there has been no targeted research regarding HSV and VZV in pregnant transplant recipients; therefore, most recommendations are similar to those in nonpregnant adults with the exception that VZV seronegative women considering pregnancy are not currently candidates for the primary live-attenuated vaccine series. Transplant patients may have more frequent and severe HSV recurrences, so may need to be on suppressive therapy for delivery. CDC and ACOG have excellent recommendations for management of these infections in pregnancy [91, 92]. Acyclovir and valacyclovir are safe in pregnancy, though doses may need modification due to organ dysfunction or level of immune compromise.

IV Acyclovir Shortage

Unfortunately, there have been a number of recent shortages of intravenous acyclovir. When IV acyclovir is not available, alternative agents may be necessary to use. In less severe disease, consideration can be given for high-dose oral valacyclovir therapy, particularly in HSV where the IC50 is lower for acyclovir. Nevertheless, in transplant recipients where disease may progress rapidly, an initial course with an alternative IV treatment may be necessary to stabilize the infection, with subsequent transition to an oral regimen (Table 10). In patients with severe mucositis or esophageal HSV disease who may have odynophagia, oral acyclovir suspension may be attempted, but IV therapy with alternative agents may be necessary.

Cutting Edge Dreams

- Next-Generation sequencing may become a promising diagnostic technique to rapidly detect virus in tissue and determine virus susceptibility [58].
- CRISPR/Cas9 could potentially be used to cure herpes virus infections [93].

Table 10 Treatments for disseminated, visceral or CNS HSV and VZV during an IV acyclovir shortage

Medication	Dose
Ganciclovir	5 mg/kg iv every 12 h
Foscarnet	Infants and children: 60 mg/kg q 12 h Adults: 90 mg/kg q 12 h or 60 mg/kg q 8 h
Cidofovir	Typically, not used, does not cross blood-brain barrier

- In vitro HSV may be used improve outcomes from transplantation. The HSV-TK suicide gene has been inserted into donor stem cells for transplant as a way to provide a mechanism to downregulate the donor cells. By inserting the HSV-TK mechanism into donor cells, they become susceptible to ganciclovir so that if donor cells cause problems (e.g., GvHD), ganciclovir can be used to down-regulate the activity donor cells that have the HSV-TK to decrease the immunologic damage caused by these cells [94].

Conclusion

The alpha-herpesviruses have the potential to produce significant morbidity and mortality in transplant patients. With attention to pre- and posttransplant diagnostic and prevention techniques, these effects can be minimized. Thankfully, there are many new and exciting diagnostic, therapeutic, and preventive modalities in development that will likely change clinical practice considerably in the coming years.

Key Points

- HSV and VZV continue to cause significant disease in transplant recipients.
- Diagnosis is often made on clinical grounds, with laboratory support.
- As treatment options expand, novel therapies will need to be evaluated in transplant patients.
- Vaccination has the potential to become a cornerstone of prevention.
- Care providers are encouraged to incorporate the ongoing advances in diagnosis, treatment and prevention into their practice.

References

1. Grose C. Pangaea and the out-of-Africa model of varicella-zoster virus evolution and phylogeography. J Virol. 2012;86(18):9558–65.
2. van Delden C, Stampf S, Hirsch HH, Manuel O, Meylan P, Cusini A, et al. Burden and timeline of infectious diseases in the first year after solid organ transplantation in the Swiss Transplant Cohort Study. Clin Infect Dis. 2020.
3. QuickStats: Age-Adjusted* Trends in the Prevalence of Herpes Simplex Virus Type 1 (HSV-1) and Herpes Simplex Virus Type 2 (HSV-2) Among Adolescents and Adults Aged 14–49 Years – United States, 1999–2000 Through 2015–2016. MMWR Morb Mortal Wkly Rep [Internet]. 2018;67(6):203. Available from: http://www.cdc.gov/mmwr/volumes/67/wr/mm6706a7.htm?s_cid=mm6706a7_w
4. Globally, an estimated two-thirds of the population under 50 are infected with herpes simplex virus type 1 [Internet]. Available from: https://www.who.int/news-room/detail/28-10-2015-globally-an-estimated-two-thirds-of-the-population-under-50-are-infected-with-herpes-simplex-virus-type-1

5. Seale L, Jones CJ, Kathpalia S, Jackson GG, Mozes M, Maddux MS, et al. Prevention of herpesvirus infections in renal allograft recipients by low-dose oral acyclovir. JAMA. 1985;254(24):3435–8.
6. Meyers JD, Flournoy N, Thomas ED. Infection with herpes simplex virus and cell-mediated immunity after marrow transplant. J Infect Dis. 1980;142(3):338–346.
7. Martin-Gandul C, Stampf S, Héquet D, Mueller NJ, Cusini A, van Delden C, et al. Preventive strategies against cytomegalovirus and incidence of α-herpesvirus infections in solid organ transplant recipients: a nationwide cohort study. Am J Transplant [Internet]. 2017;17(7):1813–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28039960.
8. Carrega G, Castagnola E, Canessa A, Argenta P, Haupt R, Dini G, et al. Herpes simplex virus and oral mucositis in children with cancer. Support Care Cancer [Internet]. 1994; 2(4):266–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8087447.
9. Chickenpox | For Healthcare Professionals | Varicella | CDC [Internet]. Available from: https://www.cdc.gov/chickenpox/hcp/index.html#vaccination-impact
10. Reynolds MA, Kruszon-Moran D, Jumaan A, Schmid DS, McQuillan GM. Varicella seroprevalence in the U.S.: data from the National Health and Nutrition Examination Survey, 1999–2004. Public Health Rep. 2010;125(6):860–9.
11. Geel A, Zuidema W, Van Gelder T, Van Doornum G, Weimar W. Successful vaccination against varicella zoster virus prior to kidney transplantation. In: Transplantation Proceedings. Elsevier USA; 2005. p. 952–3.
12. Pergam SA, Forsberg CW, Boeckh MJ, Maynard C, Limaye AP, Wald A, et al. Herpes zoster incidence in a multicenter cohort of solid organ transplant recipients. Transpl Infect Dis [Internet]. 2011;13(1):15–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20636480
13. Sahoo F, Hill JA, Xie H, Leisenring W, Yi J, Goyal S, et al. Herpes zoster in autologous hematopoietic cell Transplant recipients in the era of acyclovir or valacyclovir prophylaxis and novel treatment and maintenance therapies. Biol Blood Marrow Transplant [Internet]. 2017;23:505–11. https://doi.org/10.1016/j.bbmt.2016.12.620.
14. Thomson KJ, Hart DP, Banerjee L, Ward KN, Peggs KS, Mackinnon S. The effect of low-dose aciclovir on reactivation of varicella zoster virus after allogeneic haemopoietic stem cell transplantation. Bone Marrow Transplant [Internet]. 2005;35(11):1065–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15806119
15. Weinmann S, Chun C, Schmid DS, Roberts M, Vandermeer M, Riedlinger K, et al. Incidence and clinical characteristics of herpes zoster among children in the varicella vaccine era, 2005–2009. J Infect Dis [Internet]. 2013;208(11):1859–68. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/jit405
16. Surveillance Manual | Varicella | Vaccine Preventable Diseases | CDC [Internet]. Available from: https://www.cdc.gov/vaccines/pubs/surv-manual/chpt17-varicella.html
17. Greenberg MS, Friedman H, Cohen SG, Oh SH, Laster L, Starr S. A comparative study of herpes simplex infections in renal transplant and leukemic patients. J Infect Dis [Internet]. 1987;156. Available from: https://academic.oup.com/jid/article-abstract/156/2/280/817280
18. Wald A, Zeh J, Selke S, Warren T, Ryncarz AJ, Ashley R, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. N Engl J Med [Internet]. 2000;342(12):844–50. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM200003233421203
19. Naraqi S, Jackson GG, Jonasson O, Yamashiroya HM. Prospective study of prevalence, incidence, and source of herpesvirus infections in patients with renal allografts. J Infect Dis [Internet]. 1977;136(4):531–40. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/136.4.531
20. Uhlin M, Stikvoort A, Sundin M, Mattsson J, Remberger M. Risk factors and clinical outcome for herpes simplex virus reactivation in patients after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant [Internet]. 2015;21(2):S170. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879114009574

21. Garib G, Hughey LC, Elmets CA, Cafardi JA, Andea AA. Atypical presentation of exophytic herpes simplex virus type 2 with concurrent cytomegalovirus infection: a significant pitfall in diagnosis. Am J Dermatopathol. 2013;35(3):371–6.
22. Burke EM, Karp DL, Wu TC, Corio RL. Atypical oral presentation of herpes simplex virus infection in a patient after orthotopic liver transplantation. Eur Arch Oto-Rhino-Laryngology. 1994;251(5):301–3.
23. Redding SW. Role of herpes simplex virus reactivation in chemotherapy-induced oral mucositis. NCI Monogr [Internet]. 1990;(9):103–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2160612.
24. Danve-Szatanek C, Aymard M, Thouvenot D, Morfin F, Agius G, Bertin I, et al. Surveillance network for herpes simplex virus resistance to antiviral drugs: 3-year follow-up. J Clin Microbiol. 2004;42:242.
25. Cunha BA, Eisenstein LE, Dillard T, Krol V. Herpes simplex virus (HSV) pneumonia in a heart transplant: diagnosis and therapy. Hear Lung J Acute Crit Care. 2007;36(1):72–8.
26. Smyth RL, Higenbottam TW, Scott JP, Wreghitt TG, Stewart S, Clelland CA, et al. Herpes simplex virus infection in heart-lung transplant recipients. Transplantation. 1990;49(4):735–9.
27. Macesic N, Abbott IJ, Kaye M, Druce J, Glanville AR, Gow PJ, et al. Herpes simplex virus-2 transmission following solid organ transplantation: donor-derived infection and transplantation from prior organ recipients. Transpl Infect Dis [Internet]. 2017;19(5):e12739. Available from: http://doi.wiley.com/10.1111/tid.12739
28. Onozawa M, Hashino S, Haseyama Y, Hirayama Y, Iizuka S, Ishida T, et al. Incidence and risk of postherpetic neuralgia after varicella zoster virus infection in hematopoietic cell transplantation recipients: Hokkaido Hematology Study Group. Biol Blood Marrow Transplant. 2009;15(6):724–9.
29. Yawn BP, Saddier P, Wollan PC, St Sauver JL, Kurland MJ, Sy LS. A population-based study of the incidence and complication rates of herpes zoster before zoster vaccine introduction. Mayo Clin Proc [Internet]. 2007;82. Available from: www.mayoclinicproceedings.com
30. Fehr T, Bossart W, Wahl C, Binswanger U. Disseminated varicella infection in adult renal allograft recipients: four cases and a review of the literature. Transplantation. 2002;73(4):608–11.
31. American Academy of Pediatrics. Committee on Infectious Diseases, Red Book: Report of the Committee on Infectious Diseases. Kimberlin DW, Brady MT, Jackson MA LS, editor. Committee on Infectious Diseases. 2018.
32. Renoult E, Lanthier S, Rouleau D, Jodoin N, Letourneau-Guillon L, Pâquet M, et al. Varicella zoster virus vasculopathy after kidney transplantation. Transplantation [Internet]. 2016;100(4):e19–20. Available from: http://insights.ovid.com/crossref?an=00007890-201604000-00041
33. Fall AJ, Aitchison JD, Krause A, Hasan A, Hamilton JR, Gould FK. Donor organ transmission of varicella zoster due to cardiac transplantation. Transplantation [Internet]. 2000;70(1):211–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10919605
34. Gnann JW Jr. Varicella-zoster virus: atypical presentations and unusual complications. J Infect Dis. 2002;186(s1):S91–8.
35. Zuckerman RA, Limaye AP. Varicella zoster virus (VZV) and herpes simplex virus (HSV) in solid organ transplant patients. Am J Transplant [Internet]. 2013;13(Suppl 3):55–66; quiz 66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23347214
36. Landry ML. Immunoglobulin M for acute infection: True or false? Clin Vaccine Immunol. 2016;23(7):540–5.
37. Nichols WG, Boeckh M, Carter RA, Wald A, Corey L. Transferred herpes simplex virus immunity after stem-cell transplantation: clinical implications. J Infect Dis. 2003;187:801.
38. Caviness AC, Oelze LL, Saz UE, Greer JM, Demmler-Harrison GJ. Direct immunofluorescence assay compared to cell culture for the diagnosis of mucocutaneous herpes simplex virus infections in children. J Clin Virol. 2010;49(1):58–60.
39. Legoff J, Péré H, Bélec L. Diagnosis of genital herpes simplex virus infection in the clinical laboratory. Virol J. 2014;11. BioMed Central Ltd.

40. Lakeman FD, Whitley RJ, Whitley RJ, Alford C, Cobbs CG, Morawetz R, et al. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. J Infect Dis. 1995; 171(4):857–63.
41. Weil AA, Glaser CA, Amad Z, Forghani B. Patients with suspected herpes simplex encephalitis: rethinking an initial negative polymerase chain reaction result. Clin Infect Dis [Internet]. 2002;34(8):1154–7. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/339550
42. Berrington WR, Jerome KR, Cook L, Wald A, Corey L, Casper C. Clinical correlates of herpes simplex virus viremia among hospitalized adults. Clin Infect Dis. 2009;49(9):1295–301.
43. Zuckerman RA. The clinical spectrum of herpes simplex viremia. Clin Infect Dis [Internet]. 2009;49(9):1302–4. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/606052
44. Morrow R, Friedrich D. Performance of a novel test for IgM and IgG antibodies in subjects with culture-documented genital herpes simplex virus-1 or -2 infection. Clin Microbiol Infect. 2006;12(5):463–9.
45. Leung J, Harpaz R, Baughman AL, Heath K, Loparev V, Vázquez M, et al. Evaluation of laboratory methods for diagnosis of Varicella. Clin Infect Dis. 2010;51(1):23–32.
46. Nagel MA, Forghani B, Mahalingam R, Wellish MC, Cohrs RJ, Russman AN, et al. The value of detecting anti-VZV IgG antibody in CSF to diagnose VZV vasculopathy. Neurology. 2007;68(13):1069–73.
47. Park SY, Kim JY, Kim JA, Kwon JS, Kim SM, Jeon NY, et al. Diagnostic usefulness of varicella-zoster virus real-time polymerase chain reaction analysis of DNA in saliva and plasma specimens from patients with herpes zoster. J Infect Dis. 2018;217(1):51–7.
48. Wilson DA, Yen-Lieberman B, Schindler S, Asamoto K, Schold JD, Procop GW. Should varicella-zoster virus culture be eliminated? A comparison of direct immunofluorescence antigen detection, culture, and PCR, with a historical review. J Clin Microbiol. 2012;50(12):4120–2.
49. van der Beek MT, Vermont CL, Bredius RGM, Marijt EWA, van der Blij-de Brouwer CS, Kroes ACM, et al. Persistence and antiviral resistance of varicella zoster virus in hematological patients. Clin Infect Dis [Internet]. 2013;56(3):335–43. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1093/cid/cis879
50. Saint-Léger E, Caumes E, Breton G, Douard D, Saiag P, Huraux J, et al. Clinical and virologic characterization of acyclovir-resistant varicella-zoster viruses isolated from 11 patients with acquired immunodeficiency syndrome. Clin Infect Dis [Internet]. 2001;33(12): 2061–7. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/324503
51. Sauerbrei A, Wutzler P. Serological detection of varicella-zoster virus-specific immunoglobulin G by an enzyme-linked immunosorbent assay using glycoprotein antigen. J Clin Microbiol. 2006;44(9):3094–7.
52. McLachlan E, Scholz H, Bolotin S, Crowcroft NS, Hatchette TF, Jackson C, et al. Calibration and evaluation of quantitative antibody titers for varicella-zoster virus by use of the BIOPLEX 2200. J Clin Microbiol. 2019;57(8)
53. Lee DH, Zuckerman RA. Herpes simplex virus infections in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9).
54. Setyapranata S, Holt SG, Wiggins KJ, Mulley WR, Kerr PG, Landgren AJ, et al. Renal allograft re-use and herpetic re-infection. Nephrology. 2015;20(1):17–21.
55. Longerich T, Eisenbach C, Penzel R, Kremer T, Flechtenmacher C, Helmke B, et al. Recurrent herpes simplex virus hepatitis after liver retransplantation despite acyclovir therapy. Liver Transplant [Internet]. 2005;11(10):1289–94. Available from: http://doi.wiley.com/10.1002/lt.20567
56. Wilhelmus KR. The treatment of herpes simplex virus epithelial keratitis. Trans Am Ophthalmol Soc [Internet]. 2000;98:505–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11190039

57. Erard V, Wald A, Corey L, Leisenring WM, Boeckh M. Use of long-term suppressive acyclovir after hematopoietic stem-cell transplantation: impact on herpes simplex virus (HSV) disease and drug-resistant HSV disease. J Infect Dis. 2007;196(2):266–70.
58. Fujii H, Kakiuchi S, Tsuji M, Nishimura H, Yoshikawa T, Yamada S, et al. Application of next-generation sequencing to detect acyclovir-resistant herpes simplex virus type 1 variants at low frequency in thymidine kinase gene of the isolates recovered from patients with hematopoietic stem cell transplantation. J Virol Methods. 2018;251:123–8.
59. Adalsteinsson JA, Pan M, Kaushik S, Ungar J. Foscarnet-induced genital lesions: an overview with a case report. Dermatol Rep. 2018;10(1):12–13.
60. Bacon TH, Levin MJ, Leary JJ, Sarisky RT, Sutton D. Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. Clin Microbiol Rev [Internet]. 2003;16(1):114–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12525428
61. Prelog M, Schönlaub J, Zimmerhackl LB. Aciclovir and Varicella-zoster-immunoglobulin in solid-organ transplant recipients. Pediatr Nephrol. 2011;26(5):663–73.
62. Pergam SA, Limaye AP, AST Infectious Diseases Community of Practice. Varicella zoster virus (VZV) in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant [Internet]. 2019;4:e13622. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31162727
63. Nagel MA, Bubak AN. Varicella zoster virus vasculopathy. J Infect Dis [Internet]. 2018;218(suppl_2):S107–12. Available from: https://academic.oup.com/jid/article/218/suppl_2/S107/5105958
64. Marin M, Güris D, Chaves SS, Schmid S, Seward JF. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2007;56(RR-4):1–40.
65. Corey L, Wald A, Patel R, Sacks SL, Tyring SK, Warren T, et al. Once-daily valacyclovir to reduce the risk of transmission of genital herpes. N Engl J Med. 2004;350(1):11–20.
66. Hardy I, Gershon AA, Steinberg SP, LaRussa P. The incidence of zoster after immunization with live attenuated varicella vaccine. N Engl J Med [Internet]. 1991;325(22):1545–50. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM199111283252204
67. Shiraki K, Yamanishi K, Takahashi M. Susceptibility to acyclovir of Oka-strain varicella vaccine and vaccine-derived viruses isolated from immunocompromised patients. J Infect Dis [Internet]. 1984;150(2):306–7. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/150.2.306
68. Levin MJ, Dahl KM, Weinberg A, Giller R, Patel A, Krause PR. Development of resistance to acyclovir during chronic infection with the Oka vaccine strain of varicella-zoster virus, in an immunosuppressed child. J Infect Dis. 2003;188(7):954–9.
69. Sartori AMC. A review of the varicella vaccine in immunocompromised individuals. Int J Infect Dis. 2004;8:259–70. Elsevier
70. Marin M, Leung J, Gershon AA. Transmission of vaccine-strain varicella-zoster virus: a systematic review. Pediatrics. 2019;144. American Academy of Pediatrics.
71. Levin MJ. Varicella vaccination of immunocompromised children. J Infect Dis [Internet]. 2008;197(s2):S200–6. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/522133
72. Harris AE, Styczynski J, Bodge M, Mohty M, Savani BN, Ljungman P. Pretransplant vaccinations in allogeneic stem cell transplantation donors and recipients: an often-missed opportunity for immunoprotection? Bone Marrow Transplant. 2015;50(7):899–903.
73. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–1238.
74. Updated recommendations for use of VariZIG-United States, 2013. Am J Transplant [Internet]. 2013;13(10):2765–7. Available from: http://doi.wiley.com/10.1111/ajt.12477
75. Ishida Y, Tauchi H, Higaki A, Yokota-Outou Y, Kida K. Postexposure prophylaxis of varicella in children with leukemia by oral acyclovir. Pediatrics. 1996;97(1):150–151.

76. Asano Y, Yoshikawa T, Suga S, Kobayashi I, Nakashima T, Yazaki T, et al. Postexposure prophylaxis of varicella in family contact by oral acyclovir. Pediatrics [Internet]. 1993;92(2):219–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8393173
77. Weinstock DM, Boeckh M, Boulad F, Eagan JA, Fraser VJ, Henderson DK, et al. Postexposure prophylaxis against varicella-zoster virus infection among recipients of hematopoietic stem cell transplant: unresolved issues. Infect Control Hosp Epidemiol. 2004;25(7):603–8.
78. Lee YJ, Neofytos D, Kim SJ, Cheteyan L, Huang Y-T, Papadopoulos EB, et al. Efficacy of brincidofovir as prophylaxis against HSV and VZV in hematopoietic cell transplant recipients. Transpl Infect Dis [Internet]. 2018;20(6):e12977. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30120866
79. Dvorak CC, Cowan MJ, Horn B, Weintrub PS. Development of herpes simplex virus stomatitis during receipt of cidofovir therapy. Clin Infect Dis [Internet]. 2009;49(8):e92–5. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/605678
80. Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med. 2005;352 (22):2271–2284.
81. Lal H, Cunningham AL, Godeaux O, Chlibek R, Diez-Domingo J, Hwang S-J, et al. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. N Engl J Med [Internet]. 2015;372(22):2087–96. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1501184
82. Bastidas A, De La Serna J, El Idrissi M, Oostvogels L, Quittet P, López-Jiménez J, et al. Effect of recombinant zoster vaccine on incidence of herpes zoster after autologous stem cell transplantation: a randomized clinical trial. JAMA. 2019;322(2):123–33.
83. Vink P, Ramon Torrell JM, Sanchez Fructuoso A, Kim S-J, Kim S, Zaltzman J, et al. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in chronically immunosuppressed adults following renal transplant: a phase III, randomized clinical trial. Clin Infect Dis. 2019;70(2):181–190.
84. Distler E, Schnürer E, Wagner E, von Auer C, Plachter B, Wehler D, et al. Recovery of varicella-zoster virus-specific T cell immunity after T cell-depleted allogeneic transplantation requires symptomatic virus reactivation. Biol Blood Marrow Transplant. 2008;14(12):1417–24.
85. Meyers JD, Flournoy N, Thomas ED. Cell-mediated immunity to varicella-zoster virus after allogeneic marrow transplant. J Infect Dis [Internet]. 1980;141(4):479–87. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/141.4.479
86. Winston DJ, Mullane KM, Cornely OA, Boeckh MJ, Brown JW, Pergam SA, et al. Inactivated varicella zoster vaccine in autologous haemopoietic stem-cell transplant recipients: an international, multicentre, randomised, double-blind, placebo-controlled trial. Lancet. 2018;391 (10135):2116–27.
87. Locke FL, Menges M, Nishihori T, Nwoga C, Alsina M, Anasetti C. Boosting humoral and cellular immunity to pneumococcus by vaccination before and just after autologous transplant for myeloma. Bone Marrow Transpl. 2016;51:291–4. Nature Publishing Group
88. Ljungman P, Engelhard D, de la Cámara R, Einsele H, Locasciulli A, Martino R, et al. Vaccination of stem cell transplant recipients: recommendations of the Infectious diseases Working Party of the EBMT. Bone Marrow Transplant. 2005;35(8):737–46.
89. Curran D, Matthews S, Rowley SD, Young J-AH, Bastidas A, Anagnostopoulos A, et al. Infectious disease recombinant zoster vaccine significantly reduces the impact on quality of life caused by herpes zoster in adult autologous hematopoietic stem cell transplant recipients: a randomized placebo-controlled trial (ZOE-HSCT) biology of blood. Biol Blood Marrow Transpl [Internet]. 2019;25:2474–81. Available from: https://doi.org/10.1016/j.bbmt.2019.07.036
90. Macartney K, Heywood A, Mcintyre P. Vaccines for post-exposure prophylaxis against varicella (chickenpox) in children and adults. Cochrane Database Syst Rev. 2014; 2014. John Wiley and Sons Ltd.
91. Genital HSV Infections – 2015 STD Treatment Guidelines [Internet]. Available from: https://www.cdc.gov/std/tg2015/herpes.htm

92. American College of Obstetricians and Gynecologists. Cytomegalovirus, parvovirus B19, varicella zoster, and toxoplasmosis in pregnancy. Practice Bulletin No. 151. American College of Obstetricians and Gynecologists. Obstet Gynecol [Internet]. 2015;125(6):1510–25. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84929934101&partnerID=tZOtx3y1
93. Chen YC, Sheng J, Trang P, Liu F. Potential application of the CRISPR/CAS9 system against herpesvirus infections. Viruses. 2018;10. MDPI AG.
94. Ciceri F, Bonini C, Labopin M, Oliveira G, Nagler A, Yannaki E, et al. Safety and efficacy of donor T cells engineered with herpes simplex virus thymidine-kinase suicide gene (TK cells) given after T-cell depleted (TCD) haploidentical hematopoietic transplantation (Haplo-HSCT): results of a 14-year follow-up in 45 patients. Biol Blood Marrow Transpl [Internet]. 2017;23(3):S54–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879117301040

Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorders

27

Masaki Yamada, Andrew Nowalk, and Michael Green

Contents

Introduction 656
Disease Epidemiology 656
EBV Infection and PTLD After SOT 657
Risk Factors for PTLD After SOT 658
EBV Infection and PTLD After HSCT 660
Clinical Impacts on Transplantation 661
Disease Presentation and Spectrum 661
Outcome of PTLD After SOT and HSCT 662
Diagnosis of EBV Disease and PTLD 663
Serologic Testing 663
Nucleic Acid Detection 663
Cell-Mediated Immune Responses 664
Tissue Histopathology 665
Radiographic Imaging 665
Management and Prevention of EBV Disease and PTLD 666
The Management of EBV Disease Including PTLD 666
EBV Disease and PTLD Treatment in SOT 668

M. Yamada
Division for Advanced Medicine for Virus Infections, National Center Child Health and Development, Tokyo, Japan

Department of Pediatrics, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

A. Nowalk
Department of Pediatrics, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

M. Green (✉)
Department of Pediatrics, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Department of Surgery, University of Pittsburgh School of Medicine and UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA
e-mail: Michael.Green@chp.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_27

EBV Disease and PTLD Treatment in HSCT 670
Prevention of EBV and PTLD 672
Conclusion 677
Key Points 677
References 678

Introduction

Transplantation of solid organs (SOT) or hematopoietic bone marrow and stem cells (HSCT) is a growing field with international impact. Refinements in both broad arms of transplant medicine in the past half-century have led to improved patient outcomes in previously fatal pediatric and adult diseases, enabling survival and return to function for countless patients. In both cases, the use of immune suppression is critical in the management of the transplanted tissue, whether solid or hematologic. Advancements in T-cell-targeted therapy – from steroids to cyclosporine to tacrolimus – have improved survival and patient outcomes. These improvements come at the cost of susceptibility to infection. Herpesviruses such as Epstein-Barr virus (EBV) remain major challenges in the posttransplant period, with both acute and chronic complications and mortality. The most extreme form of EBV infection results in the transformation of the host B cell into a lymphoproliferative condition, with many similarities to de novo neoplastic disease. Posttransplant lymphoproliferative disorder (PTLD) refers to this heterogeneous group of diseases that occur in the setting of SOT or HSCT, as described in the WHO classification (Table 1) [1]. While EBV-negative PTLD does occur, EBV is the most important pathogen, associated with the development of PTLD, accounting for more than 90% of cases of PTLD in these patient populations.

This chapter will focus on the epidemiology, diagnosis, and management of EBV-driven PTLD. While EBV-negative PTLD often occur late after transplant in adult recipients, they are beyond the focus of this discussion. While the current work will summarize prior and current understanding and management of EBV-driven PTLD, we acknowledge the work done on guidelines for patients with PTLD after bone marrow and stem cell transplant [2] as well as solid organ transplant [3]. Recommendations of the authors also reflect the recommendations of a 2019 consensus conference on the diagnosis, management and prevention of EBV-positive and EBV-negative PTLD in children undergoing SOT cosponsored by the International Pediatric Transplant Association whose final recommendations are expected in 2020.

Disease Epidemiology

Epstein-Barr virus ubiquitously infects more than 90% of the adult population worldwide. EBV is usually spread via infected saliva but is also less commonly spread via sexual intercourse, blood products, and passenger leukocytes

Table 1 Histological classification of posttransplant lymphoproliferative disorders (PTLD). (World Health Organization 2016 [1])

Architecture	Pathological type	Immunohistochemistry	EBV association	Genetic findings
Nondestructive	Plasmacytic hyperplasia	No diagnostic value	≈100%	None
	Infectious mononucleosis			
	Florid follicular hyperplasia			
Destructive	Polymorphic	B and T cells	>90%	Variable
	Monomorphic (B- and T/NK-cell types)	DLBCL	EBV+ and EBV−	Yes
	Classical Hodgkin lymphoma	CD20-CC30+	>90%	Yes

accompanying transplanted organ. Exposure to EBV begins early in infancy with peaks of EBV infection occurring between the ages of 2 and 4 and around the age of late adolescence and young adulthood. In developing countries, the majority of infants and young children are infected with EBV. In contrast, in developed countries, 50% of adolescents and young adults remain EBV seronegative. As many as 95% of middle-aged adults will be EBV seropositive in both developing and developed countries.

Among infants and young children, primary EBV infection usually is asymptomatic or results in nonspecific symptoms. In contrast, about 25% of adolescents and young adults experiencing primary EBV infection will develop the classical syndrome of infectious mononucleosis [4–6]. Subsequently, EBV establishes lifelong asymptomatic latent infection in majority of immunocompetent individuals after the resolution of primary infection. Among immunocompromised patients such as recipients of SOT or HSCT, clinical manifestations of EBV infection range from asymptomatic EBV DNAemia through classical syndrome of infectious mononucleosis and end-organ-specific disease to PTLD including true malignancies.

Terminologies to define conditions of EBV infection used in this chapter includes **EBV infection** defined by the presence of evidence of current or past EBV infection; **EBV disease defined by the presence of** symptomatic EBV infection typically with the presence of concurrent EBV DNAemia and/or EBV-infected cells in the tissue; **EBV positive (+) PTLD** with histopathological evidence of EBV, typically with positive EBV-encoded small RNAs (EBER) staining identified within PTLD specimen. **EBV-negative (−) PTLD** are those lesions without evidence of EBV.

EBV Infection and PTLD After SOT

In the SOT setting, EBV can frequently be transmitted through the graft, especially in EBV-mismatched (donor seropositive/recipient seronegative; D+/R−) organ recipients. Less frequently, transmission can also occur via non-leukoreduced

blood products. EBV can also be spread to EBV-naïve SOT recipients via saliva or sexual transmission, though this typically occurs beyond the first posttransplant year. Primary EBV infection after SOT is the major risk factor for EBV disease, persistent detection of EBV DNA in blood (DNAemia), and development of PTLD.

EBV disease not meeting the definition of PTLD may manifest as a nonspecific febrile illness, adenopathy/adenitis, hepatitis, or enteritis as well as involving other organs. Incidence of EBV disease exclusive of PTLD is reported as high as 15% after pediatric liver transplant in one study but has not been fully described in other organ recipients and adult cohorts potentially due to a lack of accepted definitions categorizing EBV disease not meeting the diagnostic criteria for PTLD.

The presence of EBV DNAemia typically precedes the development of EBV disease and PTLD; hence, measurement of EBV genome in blood by quantitative nucleic acid test (NAT) (e.g., PCR) has been the standard of care over last two decades [5]. The presence of a high or rising level of DNAemia in a patient with compatible symptoms is a sensitive predictor of the presence of EBV disease including PTLD; however, it cannot be used alone as a diagnostic marker for PTLD due to the lack of specificity [7].

The incidence of PTLD after SOT follows a bimodal distribution; early PTLD refers to PTLD occurring within the first 1 to 2 years after SOT; late-onset PTLD refers to PTLD occurring >1–2 years, typically between 5 and 15 years after SOT. The highest incidence of PTLD after SOT is seen in the first year after transplant, typically among EBV-mismatched (D+/R−) patients. The overall incidence of EBV + PTLD after SOT ranges from 1% to 20%. The majority of early PTLD have a polymorphic B-cell phenotype containing EBV genome, while late-onset PTLD may or may not be driven by EBV and are typically characterized by extra nodal disease, monomorphism, and worse prognosis. This is especially true among adults who were EBV seropositive prior to transplant [8–10]. The cumulative incidence of EBV-PTLD increases over time after transplantation [1]. Of note, recent studies have shown an approximately 50% decrease in the overall incidence of both EBV+ and EBV− PTLD in the 2000s as compared to the 1990s. This may be attributable at least in part to the EBV viral load monitoring to inform preemptive reduction in immunosuppression or preemptive use of the anti-CD20 monoclonal antibody rituximab and lowering the target levels of immunosuppressants [8, 11].

Risk Factors for PTLD After SOT

The risk of developing EBV-related disease is most significant in children. Pediatric SOT recipients, who are often EBV seronegative (R−) at the time of transplantation, are at least a tenfold higher risk of EBV-related complications including PTLD than their adult EBV seropositive counterparts. Of interest, some data suggests that young age (e.g., <1 year of age) adds to the risk in combination with EBV seronegativity though young age alone in EBV seropositive patients does not add to the risk for EBV-associated PTLD [12]. While at reduced risk, EBV seropositive SOT recipients (R+) experience 1–2% risk for PTLD at 10 years posttransplant [8]. While EBV-

Table 2 Cumulative 1-year and 5-year incidence of PTLD in pediatric and adult SOT recipients by transplanted organs, 2017 OPTN/SRTR annual report [13–16, 18]

Organ	Pediatric 1 year (%)	Pediatric 5 years (%)	Adult 1 year (%)	Adult 5 years (%)
Heart	1.2	4.0	0.2	1.1
Intestine	3.9[a]	6.9[a]	3.9[a]	6.9[a]
Kidney	0.9	1.8	0.2	0.6
Liver	1.8	3.8	0.3	1.0
Lung	2.7	4.1	0.8	2.0
Pancreas isolated	NA	NA	1.8	2.6

[a]Intestine only shows combined pediatric and adult data

PTLD account for the majority of these, EBV+ PTLD do occur in these seropositive SOT recipients, likely due to the reactivation of latently infected EBV or acquisition of a new EBV strain. Except for the receipt of an intestinal allograft, specific risk factors for the development of EBV+ PTLD in R+ patients have not been defined.

Besides EBV serostatus and recipient age, the incidence of PTLD varies according to several factors including the type of organ transplanted, which may, in fact, reflect differing levels of target immunosuppression and lymphoid load in different types of allograft. Generally, intestinal transplant recipients are at the highest risk (incidence up to 20%) for the development of PTLD, followed by lung recipients (6–10%). Recipients of pancreas (1–6%), heart (3–5%), and liver (2–3%) transplants are at moderate risk, while kidney transplant recipients are at relatively low risk (1–2%) (Table 2) [13–18]. PTLD lesions in SOT recipients are most often of recipient origin, though lesions that are limited to the graft occurring early after transplant are predominantly of donor origin.

Immunosuppression is an important risk factor for the development of PTLD. Among biologic therapies, lymphocyte-depleting agents such as OKT3 and rabbit anti-thymocyte globulin (ATG) have been historically associated with increased risk for PTLD, though some recent study, assessing the risk of usage of ATG, shows inconclusive results [19]. In contrast, the use of the anti-CD52 monoclonal antibody, alemtuzumab, has not appeared to change the risk of PTLD. Among other newer biologic agents, belatacept was associated with a higher incidence of EBV+ PTLD presenting predominantly as primary central nervous system (CNS) lymphoma observed in kidney recipients who were EBV seronegative (R−) prior to transplant [19]. This risk was significant enough to warrant a black box warning for its use in EBV-negative or EBV-unknown organ transplant recipients. The introduction of tacrolimus was associated with an increased incidence of PTLD which was felt to be attributable to its much higher potency compared to cyclosporine A. Despite the continued predominant use of tacrolimus, the incidence of PTLD, especially EBV+ and early PTLD, has been decreasing [11]. This may reflect learned experience with reducing tacrolimus dosing over time in response to lower maintenance targets or the presence of elevated or rising EBV load. The final class of immunosuppressants is the antiproliferative agents such as mycophenolate and sirolimus for which positive

effects against the development of PTLD have been observed for each of these agents in in vitro studies [20]. However, these potential beneficial effects have not been confirmed in clinical trials.

Attempts to quantify the risk associated with specific immunosuppressive agents used for induction or maintenance therapy have often led to inconsistent results though biologics as a class and the use of tacrolimus are of particular concern. As or more likely, the risk of PTLD attributable to these agents reflects their contributions to the net state of immunosuppression rather than a specific type of immunosuppressive agent that patients may be on. Indeed, the duration of immunosuppression is also an important risk factor for the development of late-onset PTLD as well as other malignancies, as demonstrated by the continuous increase of the cumulative incidence of PTLD over time. This observation highlights the need for studies to find ways to measure and ultimately optimize the intensity of long-term immunosuppression in individual patients.

The measurement of EBV DNA in the peripheral blood has been used as a marker of risk for EBV-associated PTLD since the 1990s. While the test typically performs well for its negative predictive value for EBV-associated PTLD, it lacks specificity. Sequential monitoring of SOT recipients has identified patients who develop persistently high degree of EBV DNAemia who have been defined as chronic high EBV load carriers. Retrospective studies have demonstrated a significantly high rate of late-onset EBV+ PTLD among these high-load carriers after pediatric heart transplantation although this was not observed in EBV high-load carriers who were recipients of other organs [21–24]. High-load carriers were more likely to be EBV seronegative prior to and experience primary EBV infection after transplant. They may develop their persistent high-load carrier state after either asymptomatic or symptomatic infection including PTLD. For the latter, the persistent EBV load may persist or recur after the resolution of clinical symptoms and findings of EBV and PTLD. Recognition of chronic high-load carriers with a potentially higher risk for PTLD has led physicians to decrease immunosuppression in response to high levels of EBV DNAemia. This may contribute to the recently decreased incidence of EBV+ PTLD among pediatric heart, liver as well as in other pediatric and adult transplant recipients [8, 11, 25].

EBV Infection and PTLD After HSCT

EBV disease and PTLD is a complication seen in allogeneic but not autologous stem cell recipients. The epidemiology of EBV disease and PTLD after allogeneic HSCT differs from that of SOT, reflecting the different biology associated with immunological reconstitution with donor blood cells following HSCT. EBV disease manifestations are similar to those seen in SOT recipients though the disease may progress rapidly [26]. In most cases, both the virus and the EBV-infected B cells in PTLD developing after HSCT originate from the donor. This is likely explained by the intense conditioning regimens used in HSCT recipients, which usually eradicates host immune cells including EBV-infected B cells. Therefore, in

contrast to SOT, EBV status of the recipient does not impact the risk for EBV+ PTLD after HSCT [2].

The reported incidence of EBV DNAemia ranges from 1% to 60%, while the overall incidence of PTLD after HSCT is less than 1%. However, the incidence may increase up to 8% among HSCT recipients with known risk factors such as T-cell depletion of the donor marrow, ATG use, unrelated or HLA-mismatched grafts, and age 50 years or older at HSCT and second HSCT [27]. As noted, PTLD is very rare following autologous BMT. Since cord blood does not contain EBV-infected B cells, it was first felt PTLD would not occur with this source of stem cells. However, the incidence of PTLD following cord blood transplant is similar to matched related donors, and may represent either EBV(−) PTLD or exposure and acquisition of EBV after the transplant [10].

Donor preparation strategies that specifically deplete T cells such as ATG confer a higher risk for PTLD compared to methods that deplete all lymphocyte types such as alemtuzumab and posttransplant cyclophosphamide. It is believed that depletion of both B and T lymphocytes from the donor lowers the risk of PTLD compared to those that only deplete T cells. The development of graft-versus-host disease (GVHD) does not appear to be a risk factor for PTLD, though the agents used to prevent and/or treat GVHD, such as anti-T cell antibodies do increase the risk of PTLD.

The time period of risk for PTLD after HSCT is relatively limited. EBV DNAemia may be initially recognized 3–4 weeks after HSCT; the majority of PTLD cases are diagnosed within 6 months with the peak incidence occurring in the third month posttransplant [27]. Elevation of EBV loads occurs when EBV-infected B cell proliferation precedes recovery of EBV-specific immunity. Elevation of EBV loads may persist while under immunosuppressive therapy which is often used up to 1 year after HSCT to suppress T-cell proliferation and their function. The risk of EBV disease and PTLD becomes lower once engraftment and functional recovery of immune cells are achieved.

Clinical Impacts on Transplantation

Disease Presentation and Spectrum

EBV infection and disease are caused by either primary infection, reactivation, or acquisition of a new EBV strain. The latter would only be known in the context of research studies. In SOT recipients, primary EBV infection is often derived from the donor allograft and tends to present with clinically significant symptoms, compared to frequently asymptomatic reactivation occurring among EBV seropositive recipients (R+). On the other hand, the majority of EBV disease and PTLD after HSCT is due to reactivation of donor-derived EBV transmitted by EBV-infected B cells. As noted earlier, the clinical spectrum of EBV infection ranges from asymptomatic infection to clinically significant and potentially life-threatening disease in recipients of SOT or HSCT. This includes the spectrum of PTLD ranging from an indolent self-

limited lymphoproliferation in a localized nodule to fulminant, disseminated disease [28].

Clinical symptoms attributed to EBV include nonspecific febrile illnesses seen with CMV or other viruses, the classic symptoms seen with infectious mononucleosis, those attributed to end-organ involvement, and symptoms associated with malignancy such as weight loss, night sweat, and prolonged low-grade fever. Infection with EBV has been associated with hemophagocytic lymphohistiocytosis (rare), the development of allograft rejection, GVHD, and opportunistic infections. The symptoms and physical findings of EBV disease meeting and not meeting criteria for PTLD may overlap, and the differences between those meeting criteria versus not may reflect histologic but also biologic differences within the EBV-infected and transformed B cells over a continuum. Recipients with a range of EBV clinical syndromes may present with fever alone, or in combination with lymphadenopathy (both superficial and deep), tonsillitis, splenomegaly, enteritis (bloody diarrhea), hepatitis (elevated liver function test and cholestasis), or pneumonitis (cough, increased work of breath). The CNS may also be involved as an isolated site (meningoencephalitis or PTLD) or as part of disseminated disease and is generally associated with very poor outcomes. From a laboratory perspective, patients may present with anemia, leukopenia, and thrombocytopenia as well as the markers of end-organ involvement noted above. While the presence of these findings in association with an elevated EBV load raises the suspicion for EBV disease and PTLD, their nonspecific nature requires definitive tissue diagnosis to confirm these diagnoses (see section "Diagnosis of EBV Disease and PTLD").

Outcome of PTLD After SOT and HSCT

PTLD has been a significant cause of mortality after SOT. Historically, the 1-year survival rate after diagnosis of PTLD was about 70% with 5-year survival rates as low as 50%. However, in more recent reports published using standardized therapies for PTLD including reduction of immunosuppression, rituximab (anti-CD20 monoclonal antibody), and chemotherapy, the 5-year overall survival rate is as high as 80% [29]. In addition to the direct effects of PTLD on patient survival, the presence of PTLD can also have a negative impact on graft survival. This is partially explained by factors resulting in an increased risk for graft damage including reduction of immunosuppression leading to rejection and toxicity from chemotherapeutic agents (see section "Management and Prevention of EBV Disease and PTLD").

Outcome of EBV+ PTLD after HSCT has been significantly improved over the last two decades. Mortality rates of PTLD were historically as high as 80% after HSCT. However, the use of preemptive rituximab and access to adoptive T-cell therapy significantly improved the overall survival rate, with rates as high as 90% now being reported [30]. However, it is important to note that unlike PTLD occurring in SOT recipients, the practice of diagnosing "probable" PTLD

(significant lymphadenopathy, hepatosplenomegaly, or other end-organ manifestations without tissue biopsy, together with significant EBV DNAemia) is more widely accepted and reflected in the management of PTLD after HSCT including the use of rituximab and/or adoptive T-cell therapy. While the survival rate reaches 90% for HSCT recipients with probable PTLD, rates of 60–70% are reported for those with proven PTLD.

Diagnosis of EBV Disease and PTLD

Approaches to the diagnosis of EBV infection and the classification of disease (from asymptomatic DNAemia to confirmed PTLD) involves several different tools, including serology, nucleic acid detection, cell-mediated immune responses, histologic examination, and radiographic imaging.

Serologic Testing

Assessment of EBV infection using antibody response is the oldest modality for detection. While useful in earlier decades, issues with sensitivity and specificity limit this diagnostic. It remains a critical part of the pretransplant assessment, allowing providers to determine risk based on the serologic status of donor and recipient. Antibody testing of recipient after transplant can be complicated by exposure to passive antibody from blood products including the administration of intravenous immunoglobulin, loss of titers in the face of protein-losing conditions, or blunted immune responses due to antilymphocyte-targeted therapies. Thus interpretation of EBV serology remains challenging, with some correlations (such as lack of anti-EBNA and the development of PTLD) not validated in larger cohorts [31]. Nucleic acid detection has replaced serology as the primary method for diagnosis and monitoring posttransplant.

Nucleic Acid Detection

The routine use of nucleic acid amplification and detection in the last two decades has led to dramatic improvement in the understanding and diagnosis of EBV infection and PTLD. While the initial description focused on pediatric SOT recipients [32], subsequent work has identified viral load monitoring as a key tool for HSCT, SOT, and other immunocompromised patient populations [33]. As data has accumulated on the correlation between elevated EBV loads and the development of PTLD, one of the primary challenges has been the interpretation of varying loads across different centers and reports. Rigorous study of standard samples revealed large differences between absolute values reported by various laboratories, as high as 3.3-fold in one study [34]. While these same data identified good reliability of loads

over time within single centers, the need for more standardization was clear. The establishment of the 1st WHO International Standard for Epstein-Barr virus for nucleic acid amplification has led to reduced interlaboratory variation, but the challenge of true international standard measurements is ongoing, and the implementation of the use of the standard is far from universal [35, 36]. Additional controversy remains regarding the appropriate sample for evaluation, as different studies have examined whole blood, peripheral blood cells, or plasma. While whole blood samples may have higher loads and become detectable earlier, plasma values may have more utility for detection of disease states like PTLD [7]. Examination of this dilemma continues. At a minimum, clinicians should understand what fluid compartment their lab is testing to ensure accurate comparisons over time.

A major challenge of viral load monitoring continues to be relevant breakpoints for the diagnosis of disease given diverse patient states and transplant types. While EBV loads have shown promise as a guide for preemptive therapies against PTLD [3], the performance of assays across centers and the standardization of these approaches continues to develop. Interpretation of loads in diagnosis is influenced strongly by serostatus prior to transplant, type of organ or HSCT, immune suppression, and prior EBV loads. There is clearly no universally applicable EBV load for the accurate prediction of EBV disease including PTLD in solid organ and stem cell transplant recipients. While patients with active disease and PTLD generally have elevated loads compared to those without disease, the sensitivity and specificity of this assertion have areas of weakness. Low or even negative serum loads do not rule out PTLD [33], highlighting that any EBV load measurement must be considered in the clinical context of the specific patient. At the other end of the spectrum, interpreting disease risk in specific patients with persistently elevated loads also is a challenge. These high-load carriers experience a dramatically different risk of PTLD across organ type and in HSCT versus SOT recipients. As an example, pediatric heart transplant high-load carriers have a relatively high rate of PTLD progression, while pediatric kidney recipients do not [21, 24]. As data accumulates regarding the risk of progression of these patients, more effective preventive therapies may be available.

Questions remain regarding adjunctive EBV loads in non-blood specimens. While prior studies had showed promise for the use of BAL and CSF specimens for diagnosis and monitoring of PTLD, further studies have yielded no clear evidence supporting their use by consensus guidelines [3].

Cell-Mediated Immune Responses

While EBV load measurement is a critical tool in diagnosis, data exists to suggest that simultaneous measurement of EBV-specific immune response would be a valuable parallel measure for the severity of infection and risk of PTLD. Smets and colleagues showed that the use of an EBV-specific T-cell ELISPOT assay correlated with EBV loads had a high predictive index for PTLD in pediatric liver transplant [37], suggesting that with the development of a standardized measure of

cellular immunity, PTLD prediction might be improved. While other reports on other laboratory techniques for assessing EBV immunity have been published [38], none have proved to be scalable to an international level. One of the simplest candidate measures was CD4+ T-cell numbers, which were shown to predict PTLD occurrence in a single center study [39]. However, few studies have examined individual markers in a multicenter trial or across SOT types or simultaneously with HSCT patients, so the utility of these observations remains low. Nonetheless further work on identifying adjunct biomarkers of cell-mediated immunity has promise for improving further diagnostics [40].

Tissue Histopathology

Histopathological examination remains the gold standard for PTLD diagnosis. Diagnostic endoscopy, bronchoscopy, cardiac catheterization, or interventional radiology biopsies are critical for the diagnosis of PTLD, and the importance of obtaining adequate specimen from suspicious masses cannot be overstated. Biopsy often helps to distinguish between rejection and PTLD, as well as infectious conditions and PTLD. Pathologic specimens also clarify the form of PTLD; whether the PTLD is polymorphic or monomorphic; which cell type is present within the PTLD, such as malignant lymphoma, Burkitt lymphoma, T/NK-cell lymphoma, plasmacytic lymphoma, smooth muscle tumor, and other rare forms (Table 1). Treatment regimens, response, and outcomes differ according to these different classifications. The prognostic factors for poor survival includes, but not limited to, advanced age, high performance status, elevated lactose dehydrogenase level, multifocal disease, monomorphic PTLD, and non-B-cell PTLD [41]. A key aspect of examination of tissue samples is screening for the presence of EBV genetic material. The most commonly used probe is RNA in situ hybridization targeting EBER. Examination of EBER+ cells in samples can distinguish which lymphocytes may be involved, while flow cytometry and CD20+ staining provides information on cell phenotypes and potential sensitivity to treatment agents such as rituximab. Biopsy specimens should undergo review by pathology staff with experience in the evaluation of EBV disease and transplant pathology. The WHO consensus definitions for the definition of PTLD [1] are a useful reference for these studies and should be used to classify any PTLD to clarify standard treatment algorithms.

Radiographic Imaging

Patients with suspicion for PTLD – whether based on symptoms or EBV load determination in specific patients – warrant radiologic evaluation for assessment of disease. While sonography can play a role in identifying potential sites of involvement, body imaging with computed tomography (CT) or magnetic resonance imaging (MRI) is critical. Assessment of neck, chest, abdomen, and pelvis preferentially employs CT, and high-resolution thin-cut scans of the chest are the most useful

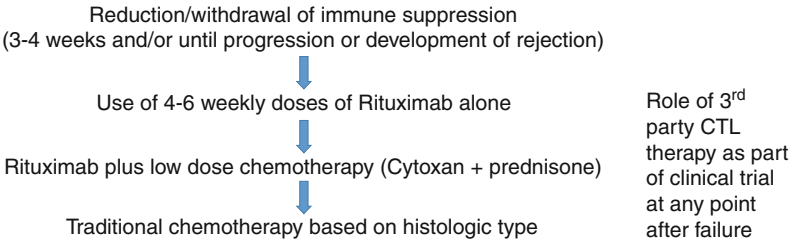
modality for identifying parenchymal lung nodules and mediastinal adenopathy. Examination of the central nervous system relies on MRI, and gadolinium enhancement can be useful in distinguishing between suppurative processes and PTLD. Many authors recommend evaluation of the brain along with the chest and abdomen in the staging and diagnosis of suspected PTLD, given the prognostic implications of CNS involvement. Musculoskeletal lesions – whether in the pelvis, spine, or extremities – are often best imaged with MRI as well.

The role of positron emission tomography (PET) scanning in the evaluation for EBV disease is evolving. The use of PET and PET/CT for the evaluation of solid tumors is well established and plays an increasing role in the diagnosis, staging, and follow-up of PTLD lesions [42]. The use of PET/CT has high specificity and sensitivity for diagnosis (estimated at ~90%) and can be useful for the follow-up evaluation of specific lesions. Given the overlap of infection and PTLD, care in the evaluation of PET images from patient should be taken to avoid confusion of presumed PTLD with sites of infection, as PET/CT is not as useful in diagnostic narrowing given the appearance of infectious states. PET as a guide for biopsy is a key use of this modality. Caution should always be exercised given the radiation exposure required for PET and PET/CT, which is higher than most other radiologic modalities.

Management and Prevention of EBV Disease and PTLD

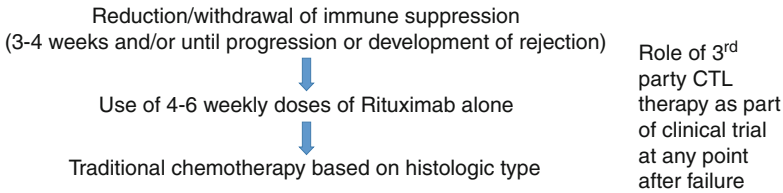
The Management of EBV Disease Including PTLD

The optimal treatments for each of the components of the spectrum of EBV disease including PTLD have not been fully established. While substantial literature is available describing potential treatments and outcomes of patients meeting diagnostic criteria for PTLD in recipients of both SOT and HSCT, little experience has been published describing the approach to patients with EBV disease not meeting these criteria. While reduction of immunosuppression is generally accepted as the first step for the entire spectrum of EBV-associated disease, the role of additional therapeutic options is less clear. Potential treatment strategies vary according to factors including histological features, disease stage, localization of the tumor, and comorbid conditions. Even for patients with biopsy-confirmed EBV+ PTLD, much of the published literature is based on case series drawn from individual centers, though recent multicenter studies in adults have begun to better define optimal strategies and chemotherapeutic regimens for those who fail to respond to the reduction of immune suppression as well as those with concerning histologic findings (e.g., Burkitt Lymphoma). Treatment approaches are different for recipients of SOT compared to HSCT. While detailed recommendations describing specific chemotherapeutic regimens are beyond the scope of this chapter, the following sections will provide an overview of generally accepted stepwise approach primarily focused on the management of EBV+ PTLD in the two patient populations. An algorithmic approach to the management of EBV+ PTLD in SOT (pediatric and adult) and HSCT recipients is shown in Figs. 1, 2, and 3.



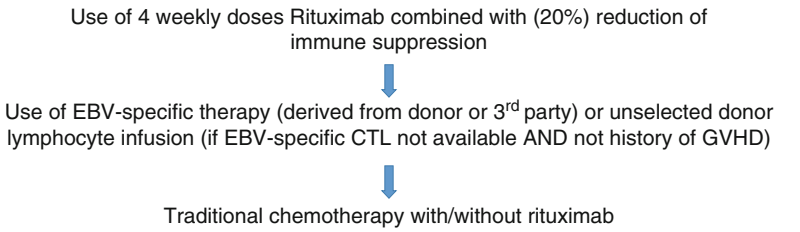
*Potential Exceptions to Standard Algorithm include: Burkitt PTLD, Hodgkin PTLD, T/NK cell lymphomas (monomorphic phenothpe), CNS PTLD and also plasmacytoma

Fig. 1 General algorithm for the treatment of EBV+ PTLD* in children post-SOT



*Potential Exceptions to Standard Algorithm include: Burkitt PTLD, Hodgkin PTLD, T/NK cell lymphomas (monomorphic phenothpe), CNS PTLD and also plasmacytoma

Fig. 2 General algorithm for the treatment of EBV+ PTLD* in adults post-SOT



*Presence of CNS EBV+ PTLD may require initial use of chemotherapy with or without rituximab in line with treatment recommendations for primary CNS lymphoma

Fig. 3 General algorithm for the treatment of EBV+ PTLD* in children and adults post-HSCT

EBV Disease and PTLD Treatment in SOT

Reduction of Immunosuppression

With few exceptions, the initial approach to managing patients with EBV disease including PTLD after SOT is reduction of immunosuppression whenever the graft function allows, or biopsy-proven histologic criteria do not warrant immediate progression to alternative therapies (e.g., rituximab or chemotherapy). The goal of reducing immunosuppression is to allow the patient to develop sufficient EBV-specific CTL function to control the EBV-driven proliferative process. The EBV section of the AST ID Guidelines recommends potentially using this approach for patients with non-Hodgkin lymphoma (NHL), but the Guideline specifically states that it has not been validated for some pathologic subtypes such as Burkitt and Hodgkin lymphomas and accordingly does not recommend this for theses classifications [3]. Having said this, the Guidelines do not provide specific recommendations on how much or which agent to reduce for individual patients receiving various immunosuppressive agents after undergoing different types of SOT but rather recommend reducing immune suppression to the lowest tolerated level for nearly all (early and late) B-cell PTLD in patients who do not have rapidly progressive disease. For some recipients, this might include complete withdrawal of immune suppression with such decisions often decided by center-specific protocols. Reported response rates for reducing immunosuppression vary greatly, ranging from 20% to 89% [43, 44]. In the absence of disease progression or development of rejection, most experts would recommend at least a 2–4-week trial of reduction or withdrawal of immune suppression prior to moving to more aggressive interventions.

Rituximab

Both the EBV section of the AST ID Guidelines and the IPTA-sponsored consensus conference on EBV and PTLD recommend the use of the anti-CD20 monoclonal antibody rituximab as the next step in the sequential approach to the management of EBV-associated PTLD for patients failing to respond to the reduction of immunosuppression. The use of rituximab (typically given as six weekly infusions) in this setting has been associated with an overall survival of 60–75% in pediatric SOT recipients [45]. Assessing the experience in adult SOT recipients is more challenging as clinical trials often include patient with both EBV+ and EBV− PTLD in whom response to rituximab monotherapy may differ by EBV status. Reported rates of response of PTLD to rituximab alone in adult SOT recipients have ranged from 25% to 60% [46, 47]. More recently, Trappe and colleagues conducted a phase II trial where patients initially received a 4-week course of rituximab with those achieving complete remission given four additional doses of rituximab at 3-week cycles, while those with evidence of progression were treated with CHOP – 25% of enrolled participants experiencing long-lasting remission without the use of chemotherapy. Again, the interpretation of these results may be impacted by the inclusion of both EBV+ and EBV− PTLD [29].

Chemotherapy

For pediatric SOT recipients, Gross et al. carried out a multicenter, phase II study using low-dose cyclophosphamide and prednisone every 3 weeks for six cycles for children with PTLD who failed to respond to the reduction or withdrawal of immune suppression, achieving an overall survival of 75% [48]. This was followed by a subsequent phase II study where rituximab was added to the low-dose chemotherapy regimen, and an 85% overall survival was achieved with fewer relapses compared to their earlier protocol with chemotherapy alone [49]. An interesting observation was that 75% of patients with radiographic evidence of persistent disease at completion of therapy achieved a complete remission at 1 year without any further therapy, suggesting a delayed immunologic effect of the rituximab and or CTL recovery. Of interest, the IPTA EBV PTLD Consensus Conference recommended this as the next sequential step for children with EBV-associated PTLD failing rituximab alone with progression to standard-dose chemotherapy for those failing this approach. However, data supporting the use of rituximab in combination with low-dose chemotherapy with Burkitt lymphoma-like PTLD are lacking. The Consensus Conference recommended direct progression to appropriate, standard-dose chemotherapy used for immunocompetent patients diagnosed with Hodgkin, T-cell, or plasmacytoma-like PTLD. No data are available assessing this approach in adult SOT recipients.

For adults failing rituximab or with aggressive PTLD forms, treatment with standard-dose chemotherapy is recommended. However, what the best chemotherapeutic regimen for the various EBV-driven PTLD phenotypes is less clear. Published reports on the treatment of PTLD in adults report complete remission rates ranging from 42% to 92% using a wide-range chemotherapeutic agents and combinations [7]. Of interest, once again, these reports typically included patients with both EBV+ and EBV− PTLD though the EBV status of the PTLD did not appear to impact response rates when evaluated. The EBV section of the AST ID Guidelines recommends the use of R-CHOP 21 for adult SOT recipients with CD20+ PTLD failing rituximab. They recommend the use of chemotherapy regimens used in immunocompetent patients with similar tumor phenotypes for patients with CD20 negative B-cell (including plasmablastic, plasma cell myeloma, plasmacytoma-like PTLD), T-cell, Hodgkin, and Burkitt lymphoma-like PTLD. Of concern, the use of traditional chemotherapy regimens in SOT recipients is associated with high rates of treatment-related morbidity and mortality compared to immunocompetent patients, with infectious sequelae an important contributor to both of these.

Additional Strategies

Radiation and surgery. The use of surgical resection or radiation for the treatment of PTLD has usually been limited to cases of isolated lesions or for debulking tumor burden at the time of making a diagnosis. Surgical intervention may also be required in fulminant cases when airway compromise is present due to mass effect or if gastrointestinal perforation has occurred. Since PTLD is most often a systemic disease, surgery alone is not likely to be successful long term. Radiation therapy is

most likely to be considered in the setting of CNS PTLD. A case series of 34 patients with CNS PTLD found a 5-year survival of approximately 50% with a reasonably high response rate to radiotherapy with or without concomitant treatments [50]. Though response to chemotherapy was less successful than radiotherapy, the authors still recommended that systemic chemotherapy should be considered for patients with CNS PTLD in the absence of systemic contraindications [51].

Antiviral agents and intravenous immunoglobulin. Historically, the antiviral agents, acyclovir and ganciclovir, have sometimes been used, with or without intravenous immune globulin (IVIG), in the treatment of EBV-associated PTLD. The rationale for the use of antivirals is that while latent virus-driven lymphoproliferation predominates in PTLD lesions, evidences of lytic virus gene and protein expression have been seen in some cases. However, evidence to support the use of antiviral agents (with/without immune globulin) in the absence of other interventions (e.g., reduction of immunosuppression or anti-CD20 therapy) is lacking. While some investigators have begun to explore strategies that utilize agents to induce the lytic cycle of EBV in combination with antiviral agents (e.g., arginine butyrate or bortezomib) [52, 53], evidence to date has not confirmed the effectiveness of this approach.

EBV-specific CTL therapy. As with recipients of HSCT (see below), the use of EBV-specific CTL therapy has been considered as a potential treatment for SOT recipients. While the generation and use of EBV-specific CTL therapy have been well established for HSCT recipients, this strategy has not been easily translated to SOT. This may be explained by the fact that most PTLD in SOT recipients are of recipient origin who develop EBV+ PTLD because of the lack of effective EBV-specific immunity under the presence of extrinsic immunosuppression. While some efforts using recipient cells which underwent ex vivo stimulation prior to infusion have been undertaken, limited published results to date have generally been disappointing [54]. Of interest, the use of third-party class 1 matched generated allogenic T cells coming from a donor bank for treatment of PTLD in SOT [43] demonstrated a response rate greater than 50% in patients with refractory PTLD [55]. At present, it would seem that the use of EBV-specific CTL therapy would best be carried out as part of formal clinical trials to confirm their effectiveness in SOT recipients. To that end, the Children's Oncology Group as well as a number of third-party commercial providers is currently planning or conducting studies to assess the effectiveness of this approach. Refer to Fig. 4 for an example of the method for generating EBV-specific T cells.

EBV Disease and PTLD Treatment in HSCT

Treatment of EBV in HSCT recipients includes any interventions use for patients with probable or proven EBV disease including PTLD. This includes those patients who fail preventive strategies (see below). Treatment strategies include the use of rituximab, reduction of immune suppression, EBV-specific CTL therapy, and chemotherapy. Per the ECIL Guidelines, reduction of immune suppression is defined as

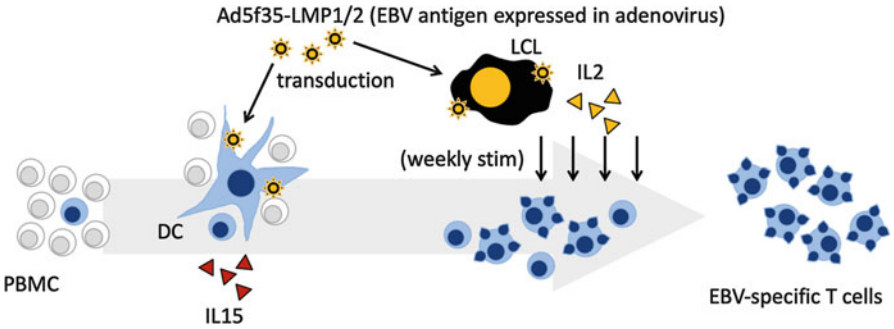


Fig. 4 Stepwise approach to cytotoxic EBV-specific T-cell creation, through the creation of lymphoblastoid cell lines (LCL) and dendritic cell (DC) stimulation of PBMC-harvested T cells, using a genetically modified adenovirus expressing EBV antigen (The authors acknowledge Dr. Catherine Bollard and Russell Cruz for developing and providing us with this figure)

a sustained decrease of at least 20% of the daily dose of immunosuppressants with the exception of low-dose corticosteroid therapy [2], and this strategy is combined with the other potential treatment options. The use of rituximab alone is associated with positive outcome in 65% of HSCT recipients treated for EBV+ PTLD. This rate rises to as high as 75% when rituximab is combined with reduced immune suppression, and the use of this combination is recommended by the ECIL when using rituximab to treat PTLD. The use of EBV-specific CTL therapy has positive outcomes in 68–85% of patients treated for EBV+ PTLD, with persistence of EBV-CTLs in vivo for up to 10 years with minimal toxicity including no significant GVHD [56]. While there are no published comparisons of these two treatment options, the limited availability of EBV-specific CTL therapy impacts the potential benefit of this approach compared to rituximab. The ESIL Guidelines recommend rituximab as the treatment of choice for probable or proven PTLD. Rituximab, in combination with reduced immune suppression, is given once weekly for up to four doses while monitoring EBV load in the peripheral blood. However, the presence of CNS involvement with EBV+ PTLD warrants consideration of initial use of chemotherapy with/without rituximab consistent with standard CNS lymphoma treatment protocols. For those failing to respond to rituximab plus reduction of immune suppression, the ESIL Guidelines recommend the use of EBV-specific CTL or, if not available, unselected donor lymphocyte infusions (DLI) to give a graft-versus-PTLD effect. Of note, a prior history of GVHD is typically considered as a contraindication to the use of DLI. More recently, the use of third-party EBV-specific CTLs may offer treatment options for HSCT recipients of cord blood transplants, with EBV-negative donors or when the donor is not available as a source of lymphocytes. Another potential use of third-party CTLs would be when there is not enough time to prepare EBV-specific CTLs from the donor due to rapid progression of EBV+ PTLD. Better response rates are noted with closer HLA matches, and no immediate or delayed toxicity using third-party EBV-CTLs has been described [55]. The use of chemotherapy with/without rituximab is recommended for those failing these second

options or if they are not available or if DLI is contraindicated. As with EBV+ PTLD after SOT, the use of antiviral therapy and/or IVIG is of unproven benefit and not recommended.

Prevention of EBV and PTLD

The impact of EBV-associated PTLD in children and adults undergoing SOT and HSCT has prompted interest in the prevention of EBV infection and its complication with a growing literature addressing the potential efficacy and impact of preventive strategies against EBV. However, published data derived from prospective randomized comparative trials are generally lacking, and most studies draw results and conclusions from either retrospective comparisons with historical controls or uncontrolled case series. While there is some overlap in the approach to prevention in SOT and HSCT recipients, major differences in the source of immune response (e.g., donor or recipient) and the competency of their immune systems to generate an adequate immune response over time lead to differing approaches and potential efficacies of strategies in the two populations. In general, potential prevention strategies against EBV can be categorized as immunoprophylaxis, chemoprophylaxis, or preemptive therapies initiated in response to the presence of or changes in quantitative measurement of EBV load in the peripheral blood (Table 3). In addition

Table 3 Preventive strategies against the development of EBV disease and PTLD for recipients of solid organ and stem cell transplantation

	SOT	HSCT
Chemoprophylaxis with antiviral therapy	Unproven	Unproven
Immunoprophylaxis		
Active (e.g., vaccine)	Not available	Not available
Passive		
IVIG	Unproven – Not recommended	Unproven – Not recommended
EBV-specific CTL	Unproven – Not recommended	Effective but limited availability
Rituximab	Unproven – Not recommended	Not shown to be superior to preemptive rituximab – Not recommended
Preemptive therapy		
Reduction of immune suppression	Appears effective and recommended	Ineffective alone – Recommended in combination with rituximab
		Unproven – Not recommended
Antiviral	Unproven – Not recommended	Effective and recommended
Rituximab	Limited data – Not recommended	Effective and recommended IF available
EBV-specific CTL	Unproven – Not recommended	

to these strategies, the use of leukocyte-reduced blood products also serves as an important barrier to the spread of EBV in both patient populations since the virus is typically carried in B lymphocytes which are eliminated through the process of leukoreduction.

Immunoprophylaxis. Immunoprophylaxis can be categorized as active or passive. Active immunoprophylaxis would be accomplished through the use of an EBV vaccine. While a number of investigators have worked to develop a vaccine against EBV in both the transplant and non-transplant setting, no vaccine is currently available or likely to be approved for use in the foreseeable future. Passive immunoprophylaxis is accomplished by providing anti-EBV antibodies through the infusion of IVIG or through infusion of EBV-specific cytotoxic T lymphocytes (CTL).

Initial interest in the use of IVIG to prevent EBV-associated PTLD was supported by the protective effect of IVIG on the development of EBV disease in a SCID mouse model [57]. The potential benefit of IVIG was further supported by a retrospective analysis of SOT recipients receiving anti-CMV immunoglobulins for CMV prophylaxis which found that PTLD did not develop in patients while receiving immune globulin during their first year posttransplant [12]. However, this benefit was not confirmed in a multicenter, randomized controlled trial using anti-CMV immunoglobulin prophylaxis vs. placebo in pediatric liver transplant recipients although a trend toward less EBV disease and PTLD was observed in patients receiving CMV IVIG [12, 58]. Similarly, the use of IVIG had no impact on time free from any EBV viral load, mean viral load, time free from achieving a "high" EBV viral load, or development of EBV-associated PTLD in a prospective study of IVIG versus placebo in EBV mismatched (donor EBV seropositive/recipient EBV seronegative) adult and pediatric organ recipients, all of whom received 12 months of a combination of IV and oral antiviral therapy (IV ganciclovir followed by oral acyclovir or ganciclovir) [59]. Evidence on the protective effect of IVIG in HSCT recipients against EBV is lacking.

The use of EBV-specific cytotoxic T lymphocytes (CTL) for treatment for EBV-associated PTLD has been proven to be efficacious in stem cell transplant recipients [60] though the limited availability and access to CTL therapy impacts the extent of its use. This approach has been primarily used preemptively (see below) in response to an elevated EBV load in the blood and not as prophylaxis. There are no studies describing the prophylactic use of EBV-specific CTLs in SOT recipients.

Another potential strategy is the use of prophylactic rituximab for those at increased risk of developing EBV disease including PTLD. This is exemplified by a study by Schachtner et al. which gave a single dose of rituximab to five EBV-negative kidney candidates 1 month prior to scheduled transplants from EBV-positive living donors [56]. None of these recipients developed a positive EBV load or PTLD, but the study was too small to confirm benefit or to assess the potential risks associated with the use of rituximab in this setting [60]. Larger trials comparing outcomes including both progression to EBV disease and PTLD but also documenting potential adverse events related to the use of rituximab are necessary to define the utility of this approach. Based on the absence of adequate data, the section

on EBV in the AST ID Guidelines neither recommends for or against the use of preemptive rituximab [3]. The potential use of prophylactic rituximab was addressed in ECIL Guidelines [2]. While the Guidelines recognize the potential beneficial effect of this approach for those at increased risk for developing EBV disease including PTLD, they concluded that available evidence did not demonstrate better outcomes compared to preemptive use of rituximab for those with a documented elevated or rising EBV load and favored its preemptive use over prophylaxis.

Chemoprophylaxis. Chemoprophylaxis using antiviral agents, such as acyclovir and ganciclovir, represents another possible approach to preventing EBV infection and disease including PTLD. Theoretically, ganciclovir or its prodrug valganciclovir may be the preferred drug for EBV prophylaxis because of its higher in vitro antiviral activity compared to acyclovir. However, these drugs are only effective against the lytic forms of EBV. This might translate into inefficiency in preventing latently driven disease (e.g., PTLD), providing a potential explanation for the lack of clear evidence of benefit for the use of antivirals to prevent EBV infection and development of disease.

Initial enthusiasm for the role of antiviral therapy was derived from several single-center retrospective studies that appeared supportive for a role for antiviral therapy, but these studies suffered from methodologic concerns including the lack of appropriate controls or concomitant changes in clinical practice that could have impacted the development of EBV and PTLD [61–63]. A US case-controlled study reported that the use of ganciclovir given for CMV prophylaxis reduced the PTLD incidence in kidney transplant recipients [64], but analysis of data from a similar European registry did not confirmed the efficacy of ganciclovir, valganciclovir, or acyclovir against EBV/PTLD in SOT recipients [58]. A randomized prospective trial of 2 weeks of ganciclovir compared to 2 weeks of ganciclovir followed by 50 weeks of oral acyclovir in EBV seronegative pediatric liver transplant patients did not establish any benefit to the extended use of antiviral therapy to prevent EBV disease [65]. Similarly, the previously described study of IVG and antiviral therapy with ganciclovir and or acyclovir also failed to show benefit of these therapies [59]. A small sub-analysis of a prospective trial in pediatric renal transplant recipients observed a significant reduction of the 1-year incidence of EBV primary infection in 20 EBV D+/R patients on ganciclovir or valganciclovir prophylaxis compared with 8 patients without prophylaxis [66]. However, one patient each developed monomorphic PTLD in both the treated and untreated cohorts, and the authors concluded that no significant impact of ganciclovir or valganciclovir prophylaxis on PTLD occurrence could be derived from this study. More recently, a 2016 meta-analysis showed that the use of antiviral drugs (ganciclovir, valganciclovir, acyclovir, and valacyclovir) in mismatched EBV transplant recipients (D+/R) had no effect on PTLD incidence [67]. No significant differences were seen across all types of solid organ transplants, age groups, or antiviral use as prophylaxis or preemptive strategy. The use of antivirals for the prevention of EBV disease and PTLD was not recommended at the IPTA EBV Consensus Conference and is also not currently recommended in the AST ID Guidelines [3]. While clinical evidence assessing the potential impact of antiviral therapy on preventing EBV disease and PTLD is drawn

from the SOT population, there is no reason to believe that its impact would be substantially different in HSCT recipients in whom the bone marrow suppression of these agents can have substantial impact on successful engraftment. Consistent with this, the ECIL Guidelines specifically state that there is no evidence to support effectiveness of antiviral in this population and accordingly do not recommend the use of these agents for this purpose [2]. While there are some antiviral agents in development that may have activity against EBV in vitro, these drugs have not been systematically studied in the treatment or prevention of EBV related-disease and will also suffer from the limitation of not impacting proliferation of EBV-transformed B cells driven by human replicative enzymes.

Finally, since transmission of EBV from the donor to the EBV-naïve recipient is a major risk factor for EBV disease and PTLD, the role of antiviral chemoprophylaxis given to the donor to limit transmission has also been recently explored. A small pilot study of randomized treatment of living kidney donors with 2 weeks of valganciclovir provides encouraging preliminary results that treatment of the donor or recipient pretransplant might impact EBV transmission and potentially PTLD [68]. Additional studies are necessary to fully evaluate this novel approach to prevent EBV-associated disease.

Viral load monitoring and preemptive strategies of prevention. Despite limited published data, surveillance monitoring of EBV loads to inform a preemptive intervention has been widely implemented for both SOT and HSCT recipients. Among SOT recipients, the use of monitoring the EBV load in the peripheral blood to inform reductions in immune suppression in patients who have developed primary EBV after SOT has resulted in a decreased incidence of EBV/PTLD compared to historical controls. McDiarmid reported a decreased incidence of PTLD from 10% to 5% using EB viral load monitoring to guide the combined use of reduced immune suppression and intravenous ganciclovir in pediatric liver transplant recipients with rising EBV loads [69]. Two other studies demonstrated decreased incidences of PTLD using decreased immunosuppression alone without ganciclovir in response to elevated EBV loads [70, 71]. An important limitation of this approach is that the development of a detectable load does not necessarily predict progression to EBV disease, and additional risk factors that enhance the predictive value of an elevated load as a marker of risk of developing EBV disease including PTLD have not been identified. Patients experiencing initial recognition of a positive load in the setting of primary EBV infection after SOT may both be at increased risk and represent an opportunity for the newly infected recipient to optimize their chance to develop an appropriate immune response. However, even in this setting, many such patients may not progress to EBV disease or PTLD at this time or in the future. Assigning the risk of progression to symptomatic EBV disease in patients who develop recurrent or persistent asymptomatic elevations in their EBV viral loads remains challenging at best. Specific criteria relating to a specific height or rate of rise of EBV load are not available, in part due to the heterogeneity of available EBV load assays and differences in compartments of blood (e.g., whole blood or serum) being sampled. Accordingly, reductions in immune suppression in both published studies and clinical practice have been carried out based on elevated

load alone. Due to the risk of rejection, these reductions have been done carefully with ongoing attention for the presence of findings suggestive of early rejection. For HSCT recipients who develop an elevated EBV load early transplant, the absence of donor T-cell engraftment and function is the source of immune impairment predisposing to progression to EBV disease. Accordingly, reduction of immune suppression is more likely to play a therapeutic role for those presenting later after transplant with some T-cell engraftment receiving prophylaxis or treatment against GVHD. Despite the less clear impact of reduction of immune suppression in HSCT, the ECIL Guidelines do recommend this but in combination with preemptive rituximab or CTL therapy.

The anti-CD20 monoclonal antibody rituximab has been considered as preemptive therapy in both SOT and HSCT recipients. Little data is available to inform the use of rituximab for SOT recipients. Martin reported encouraging results using EBV load monitoring to inform the preemptive use of rituximab in EBV D+/R− adult kidney transplant recipients [72]. However, the majority of treated patients actually had clinical evidence of EBV disease at the time of treatment. Accordingly, these data speak more to the use of rituximab for early treatment and not prevention of EBV disease. Additional experience is needed to confirm efficacy and long-term safety of rituximab in a prevention/preemption model against EBV in the SOT setting. While its potential use as preemptive therapy was discussed at both the IPTA Consensus Conference and in the EBV section of the AST ID Guidelines, neither of these recommended general use of preemptive rituximab in SOT recipients at this time.

Among HSCT recipients, rituximab is more often employed than among SOT recipients. Because HSCT recipients at high risk for PTLD often develop EBV infection prior to reestablishment of adequate cell-mediated immunity, reduction of immune suppression is frequently not an option. Since the period of risk is much more predictable and relative early after HSCT, monitoring with EBV viral load PCR and preemptive therapy with rituximab appears to reduce the incidence of PTLD [73]. Although no randomized trials have been published to date, this approach of serially monitoring high-risk patients (e.g., patients that receive TCD) by weekly EBV blood PCR and giving rituximab for patients with elevated EBV DNA levels or persistently increasing levels has become standard of care at many institutions, especially for centers without access to adoptive immunotherapy against EBV (see above). Consistent with this, the ECIL Guidelines strongly recommend the preemptive use of rituximab along with reduction of immune suppression where possible. Rituximab is given as a weekly dose of 350 mg/m^2 until the load is no longer detectable (typically 1–4 doses) [2]. While preemptive use of CTL therapy is likely efficacious and may actually be the preferred therapy (see below), its limited availability makes rituximab the most frequently used preventive strategy in the HSCT population.

Finally, the preemptive use of EBV-specific CTL from either the allograft donor or an HLA partially matched third-party donor has been shown to be highly effective in HSCT recipients. Rates of prevention approaching 100% have been reported by centers with access to EBV-specific T-cell therapy [74]. Unfortunately, at present,

access to EBV-specific CTL for preemptive therapy is limited to a relatively small number of centers limiting its impact in this patient population. However, if available, the ECIL Guidelines recommended their use in this setting. In contrast to the successful use of this approach in HSCT recipients, efforts to translate these benefits to the prevention of EBV/PTLD in SOT recipients have been limited, and clear evidence of efficacy for preemptive use in SOT recipients is lacking at this time [75].

To summarize the approach to SOT recipients, available data supports the use of the strategy of using EBV load monitoring for patients who are EBV negative prior to transplant from an EBV-positive donor, to inform preemptive reduction in immune suppression to prevent EBV/PTLD as the optimal currently available preventive strategy. This approach was endorsed by the IPTA Guidelines and is supported in the EBV section of the AST ID Guidelines. With few exceptions (i.e., intestinal transplant recipients, those retransplanted for history of PTLD), this should be limited to the patients who is EBV seronegative prior to transplant, and this approach should not be used for those who were EBV seropositive prior to transplant. For HSCT recipients, consistent with the ECIL Guidelines, preemptive use of rituximab combined with reduction of immune suppression where feasible is strongly recommended. However, centers with access to EBV-specific CTL for preemptive therapy should consider this as a potentially preferred option.

Conclusion

Epstein-Barr virus is an important potential cause of morbidity and mortality in children and adults undergoing SOT and HSCT. These immunocompromised patients are at risk for uncontrolled lymphoproliferation because of the EBV virus' ability transform and immortalize B lymphocytes leading to viral-driven proliferation of infected B cells causing a wide spectrum of disease including true malignancies. Careful observations and sustained research over the last 30 years have led to an understanding of key risk factors associated with development of these complications as well as enhanced diagnostic studies to optimize early diagnosis and even inform preventative and preemptive interventions. Individual and collaborative efforts have identified potential options that differ for SOT HSCT recipients leading to improved outcomes. Evidence-based guidelines now provide recommendations on their use for both prevention and treatment of the complications of EBV. Going forward, larger, prospective, and ideally randomized trials are necessary to define the best treatment strategies for the varying clinical scenarios seen in both the SOT and HSCT patient populations.

Key Points

1. EBV (Epstein-Barr virus) impacts morbidity and mortality in children and adults after solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT).

2. EBV is the most important pathogen causing posttransplant lymphoproliferative disorder (PTLD).
3. Pretransplant EBV serology, type of organ, and age differentiate the risks for EBV-associated PTLD in SOT.
4. PTLD in HSCT is of donor origin, and the risk increases with T-cell depletion therapy.
5. Antivirals do not prevent PTLD.
6. EBV viral load monitoring is useful to monitor the EBV activity after SOT and HSCT though limitations with both sensitivity and specificity must be considered in interpreting results.
7. EBV load monitoring to inform reduction of immunosuppressants appears to be a generally effective strategy to prevent PTLD in SOT recipients.
8. Prophylactic rituximab in response to elevated or rising EBV load has been effective in the prevention of EBV-associated PTLD in HSCT but controversial in SOT.
9. EBV-specific CTL therapy in response to elevated or rising EBV load is also effective for prevention of EBV-associated PTLD in HSCT but not SOT; this strategy is limited by its availability.
10. Tissue diagnosis is required and determines the characteristics of PTLD and the prognosis.
11. Non-B-cell PTLD has worse outcome.
12. For SOT recipients, a stepwise approach to the treatment of EBV+ PTLD is generally recommended with initial reduction of immunosuppressants followed by rituximab for those who do not respond or develop rejection.
13. Chemotherapy is required if the PTLD does not respond to the first- or second-line therapies.
14. For recipients of HSCT, adoptive T-cell therapy is effective for PTLD if available though rituximab might be used if not. Chemotherapy is poorly tolerated in the HSCT setting.

References

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
2. Styczynski J, van der Velden W, Fox CP, Engelhard D, de la Camara R, Cordonnier C, et al. Management of Epstein-Barr Virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: sixth European conference on infections in leukemia (ECIL-6) guidelines. Haematologica. 2016;101(7):803–11.
3. Allen UD, Preiksaitis JK, Practice ASTIDCo. Post-transplant lymphoproliferative disorders, EBV infection and disease in solid organ transplantation: guidelines from the American Society of transplantation infectious diseases community of practice. Clin Transplant. 2019;33:e13652.
4. Fields BN, Knipe DM, Howley PM. Fields virology. Philadelphia: Wolters Kluwer Health/ Lippincott Williams & Wilkins; 2013.
5. Green M, Michaels MG. Epstein-Barr virus infection and posttransplant lymphoproliferative disorder. Am J Transplant. 2013;13(Suppl 3):41–54; quiz.

6. Balfour HH Jr, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W. Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. J Infect Dis. 2013;208(8):1286–93.
7. Kanakry JA, Hegde AM, Durand CM, Massie AB, Greer AE, Ambinder RF, et al. The clinical significance of EBV DNA in the plasma and peripheral blood mononuclear cells of patients with or without EBV diseases. Blood. 2016;127(16):2007–17.
8. Peters AC, Akinwumi MS, Cervera C, Mabilangan C, Ghosh S, Lai R, et al. The changing epidemiology of posttransplant lymphoproliferative disorder in adult solid organ transplant recipients over 30 years: a single-center experience. Transplantation. 2018;102(9):1553–62.
9. Dierickx D, Habermann TM. Post-transplantation lymphoproliferative disorders in adults. N Engl J Med. 2018;378(6):549–62.
10. Dharnidharka VR, Green M, Webber SA. Post-transplant lymphoproliferative disorders. Heidelberg: Springer; 2010. xii, 190p.
11. Narkewicz MR, Green M, Dunn S, Millis M, McDiarmid S, Mazariegos G, et al. Decreasing incidence of symptomatic Epstein-Barr virus disease and posttransplant lymphoproliferative disorder in pediatric liver transplant recipients: report of the studies of pediatric liver transplantation experience. Liver Transpl. 2013;19(7):730–40.
12. Green M, Michaels MG, Katz BZ, Burroughs M, Gerber D, Shneider BL, et al. CMV-IVIG for prevention of Epstein Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. Am J Transplant. 2006;6(8):1906–12.
13. Smith JM, Weaver T, Skeans MA, Horslen SP, Noreen SM, Snyder JJ, et al. OPTN/SRTR 2017 annual data report: intestine. Am J Transplant. 2019;19(Suppl 2):284–322.
14. Kandaswamy R, Stock PG, Gustafson SK, Skeans MA, Urban R, Fox A, et al. OPTN/SRTR 2017 annual data report: pancreas. Am J Transplant. 2019;19(Suppl 2):124–83.
15. Colvin M, Smith JM, Hadley N, Skeans MA, Uccellini K, Lehman R, et al. OPTN/SRTR 2017 annual data report: heart. Am J Transplant. 2019;19(Suppl 2):323–403.
16. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Castro S, et al. OPTN/SRTR 2017 annual data report: kidney. Am J Transplant. 2019;19(Suppl 2):19–123.
17. Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, Noreen SM, et al. OPTN/SRTR 2017 annual data report: liver. Am J Transplant. 2019;19(Suppl 2):184–283.
18. Valapour M, Lehr CJ, Skeans MA, Smith JM, Uccellini K, Lehman R, et al. OPTN/SRTR 2017 annual data report: lung. Am J Transplant. 2019;19(Suppl 2):404–84.
19. Hertig A, Zuckermann A. Rabbit antithymocyte globulin induction and risk of post-transplant lymphoproliferative disease in adult and pediatric solid organ transplantation: an update. Transpl Immunol. 2015;32(3):179–87.
20. Sang AX, McPherson MC, Ivison GT, Qu X, Rigdon J, Esquivel CO, et al. Dual blockade of the PI3K/Akt/mTOR pathway inhibits posttransplant Epstein-Barr virus B cell lymphomas and promotes allograft survival. Am J Transplant. 2019;19(5):1305–14.
21. Bingler MA, Feingold B, Miller SA, Quivers E, Michaels MG, Green M, et al. Chronic high Epstein-Barr viral load state and risk for late-onset posttransplant lymphoproliferative disease/lymphoma in children. Am J Transplant. 2008;8(2):442–5.
22. Green M, Soltys K, Rowe DT, Webber SA, Mazareigos G. Chronic high Epstein-Barr viral load carriage in pediatric liver transplant recipients. Pediatr Transplant. 2009;13(3):319–23.
23. Lau AH, Soltys K, Sindhi RK, Bond G, Mazariegos GV, Green M. Chronic high Epstein-Barr viral load carriage in pediatric small bowel transplant recipients. Pediatr Transplant. 2010;14(4):549–53.
24. Yamada M, Nguyen C, Fadakar P, Ganoza A, Humar A, Shapiro R, et al. Epidemiology and outcome of chronic high Epstein-Barr viral load carriage in pediatric kidney transplant recipients. Pediatr Transplant. 2018;22:e13147.
25. Chinnock R, Webber SA, Dipchand AI, Brown RN, George JF, Pediatric Heart Transplant Study. A 16-year multi-institutional study of the role of age and EBV status on PTLD incidence among pediatric heart transplant recipients. Am J Transplant. 2012;12(11):3061–8.
26. Rouce RH, Louis CU, Heslop HE. Epstein-Barr virus lymphoproliferative disease after hematopoietic stem cell transplant. Curr Opin Hematol. 2014;21(6):476–81.

27. Landgren O, Gilbert ES, Rizzo JD, Socie G, Banks PM, Sobocinski KA, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood. 2009;113(20):4992–5001.
28. Bowden RA, Ljungman P, Snydman DR. Transplant infections. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2010. xii, 788p
29. Trappe RU, Dierickx D, Zimmermann H, Morschhauser F, Mollee P, Zaucha JM, et al. Response to rituximab induction is a predictive marker in B-cell post-transplant lymphoproliferative disorder and allows successful stratification into rituximab or R-CHOP consolidation in an international, prospective, multicenter phase II trial. J Clin Oncol. 2017;35(5):536–43.
30. Styczynski J, Einsele H, Gil L, Ljungman P. Outcome of treatment of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in hematopoietic stem cell recipients: a comprehensive review of reported cases. Transpl Infect Dis. 2009;11(5):383–92.
31. Preiksaitis JK, Diaz-Mitoma F, Mirzayans F, Roberts S, Tyrrell DL. Quantitative oropharyngeal Epstein-Barr virus shedding in renal and cardiac transplant recipients: relationship to immunosuppressive therapy, serologic responses, and the risk of posttransplant lymphoproliferative disorder. J Infect Dis. 1992;166(5):986–94.
32. Rowe DT, Qu L, Reyes J, Jabbour N, Yunis E, Putnam P, et al. Use of quantitative competitive PCR to measure Epstein-Barr virus genome load in the peripheral blood of pediatric transplant patients with lymphoproliferative disorders. J Clin Microbiol. 1997;35(6):1612–5.
33. Nowalk A, Green M. Epstein-Barr virus. Microbiol Spectr. 2016;4(3) https://doi.org/10.1128/microbiolspec.DMIH2-0011-2015.
34. Preiksaitis JK, Pang XL, Fox JD, Fenton JM, Caliendo AM, Miller GG, et al. Interlaboratory comparison of Epstein-Barr virus viral load assays. Am J Transplant. 2009;9(2):269–79.
35. Hayden RT, Sun Y, Tang L, Procop GW, Hillyard DR, Pinsky BA, et al. Progress in quantitative viral load testing: variability and impact of the WHO quantitative international standards. J Clin Microbiol. 2017;55(2):423–30.
36. Fryer JF, Heath AB, Wilkinson DE, Minor PD. The Collaborative study Group. Collaborative study to evaluate the proposed 1st WHO international standardfor EpsteineBarr virus (EBV) for nucleic acid amplification (NAT)-based assays. WHO ECBS Report. Geneva, Switzerland: World Health Organization 2011; WHO/BS/2011.2172, http://whqlibdoc.who.int/hq/2011/WHO_BS_2011.2172_eng.pdf
37. Smets F, Latinne D, Bazin H, Reding R, Otte JB, Buts JP, et al. Ratio between Epstein-Barr viral load and anti-Epstein-Barr virus specific T-cell response as a predictive marker of posttransplant lymphoproliferative disease. Transplantation. 2002;73(10):1603–10.
38. Martinez OM, Krams SM. The immune response to Epstein Barr virus and implications for posttransplant lymphoproliferative disorder. Transplantation. 2017;101(9):2009–16.
39. Sebelin-Wulf K, Nguyen TD, Oertel S, Papp-Vary M, Trappe RU, Schulzki A, et al. Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PLTD. Transpl Immunol. 2007;17(3):203–10.
40. Yamada M, Nowalk AJ. Two sides of the Epstein-Barr virus (EBV) coin: Long-term control of EBV after liver transplantation? Pediatr Transplant. 2017;21(5):e12943.
41. Dharnidharka VR, Webster AC, Martinez OM, Preiksaitis JK, Leblond V, Choquet S. Post-transplant lymphoproliferative disorders. Nat Rev Dis Primers. 2016;2:15088.
42. Panagiotidis E, Quigley AM, Pencharz D, Ardeshna K, Syed R, Sajjan R, et al. (18)F-fluorodeoxyglucose positron emission tomography/computed tomography in diagnosis of post-transplant lymphoproliferative disorder. Leuk Lymphoma. 2014;55(3):515–9.
43. Hayashi RJ, Kraus MD, Patel AL, Canter C, Cohen AH, Hmiel P, et al. Posttransplant lymphoproliferative disease in children: correlation of histology to clinical behavior. J Pediatr Hematol Oncol. 2001;23(1):14–8.
44. Webber SA, Naftel DC, Fricker FJ, Olesnevich P, Blume ED, Addonizio L, et al. Lymphoproliferative disorders after paediatric heart transplantation: a multi-institutional study. Lancet. 2006;367(9506):233–9.

45. Messahel B, Taj MM, Hobson R, Hadzic N, Ramsay A, Hann I, et al. Single agent efficacy of rituximab in childhood immunosuppression related lymphoproliferative disease: a United Kingdom Children's Cancer Study Group (UKCCSG) retrospective review. Leuk Lymphoma. 2006;47(12):2584–9.
46. Choquet S, Trappe R, Leblond V, Jager U, Davi F, Oertel S. CHOP-21 for the treatment of post-transplant lymphoproliferative disorders (PTLD) following solid organ transplantation. Haematologica. 2007;92(2):273–4.
47. Gonzalez-Barca E, Domingo-Domenech E, Capote FJ, Gomez-Codina J, Salar A, Bailen A, et al. Prospective phase II trial of extended treatment with rituximab in patients with B-cell post-transplant lymphoproliferative disease. Haematologica. 2007;92(11):1489–94.
48. Gross TG, Bucuvalas JC, Park JR, Greiner TC, Hinrich SH, Kaufman SS, et al. Low-dose chemotherapy for Epstein-Barr virus-positive post-transplantation lymphoproliferative disease in children after solid organ transplantation. J Clin Oncol. 2005;23(27):6481–8.
49. Gross TG, Orjuela MA, Perkins SL, Park JR, Lynch JC, Cairo MS, et al. Low-dose chemotherapy and rituximab for posttransplant lymphoproliferative disease (PTLD): a Children's Oncology Group Report. Am J Transplant. 2012;12(11):3069–75.
50. Cavaliere R, Petroni G, Lopes MB, Schiff D, International Primary Central Nervous System Lymphoma Collaborative Group. Primary central nervous system post-transplantation lymphoproliferative disorder: an International Primary Central Nervous System Lymphoma Collaborative Group Report. Cancer. 2010;116(4):863–70.
51. Evens AM, Choquet S, Kroll-Desrosiers AR, Jagadeesh D, Smith SM, Morschhauser F, et al. Primary CNS posttransplant lymphoproliferative disease (PTLD): an international report of 84 cases in the modern era. Am J Transplant. 2013;13(6):1512–22.
52. Perrine SP, Hermine O, Small T, Suarez F, O'Reilly R, Boulad F, et al. A phase 1/2 trial of arginine butyrate and ganciclovir in patients with Epstein-Barr virus-associated lymphoid malignancies. Blood. 2007;109(6):2571–8.
53. Shirley CM, Chen J, Shamay M, Li H, Zahnow CA, Hayward SD, et al. Bortezomib induction of C/EBPbeta mediates Epstein-Barr virus lytic activation in Burkitt lymphoma. Blood. 2011;117(23):6297–303.
54. Gottschalk S, Rooney CM. Adoptive T-cell immunotherapy. Curr Top Microbiol Immunol. 2015;391:427–54.
55. Haque T, Wilkie GM, Jones MM, Higgins CD, Urquhart G, Wingate P, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007;110(4):1123–31.
56. Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010;115(5):925–35.
57. Abedi MR, Linde A, Christensson B, Mackett M, Hammarstrom L, Smith CI. Preventive effect of IgG from EBV-seropositive donors on the development of human lympho-proliferative disease in SCID mice. Int J Cancer. 1997;71(4):624–9.
58. Opelz G, Daniel V, Naujokat C, Fickenscher H, Dohler B. Effect of cytomegalovirus prophylaxis with immunoglobulin or with antiviral drugs on post-transplant non-Hodgkin lymphoma: a multicentre retrospective analysis. Lancet Oncol. 2007;8(3):212–8.
59. Humar A, Hebert D, Davies HD, Humar A, Stephens D, O'Doherty B, et al. A randomized trial of ganciclovir versus ganciclovir plus immune globulin for prophylaxis against Epstein-Barr virus related posttransplant lymphoproliferative disorder. Transplantation. 2006;81(6):856–61.
60. Schachtner T, Reinke P. Pretransplant prophylactic rituximab to prevent Epstein-Barr virus (EBV) viremia in EBV-seronegative kidney transplant recipients from EBV-seropositive donors: results of a pilot study. Transpl Infect Dis. 2016;18(6):881–8.
61. Malouf MA, Chhajed PN, Hopkins P, Plit M, Turner J, Glanville AR. Anti-viral prophylaxis reduces the incidence of lymphoproliferative disease in lung transplant recipients. J Heart Lung Transplant. 2002;21(5):547–54.

62. Darenkov IA, Marcarelli MA, Basadonna GP, Friedman AL, Lorber KM, Howe JG, et al. Reduced incidence of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. Transplantation. 1997;64(6):848–52.
63. Davis CL, Harrison KL, McVicar JP, Forg PJ, Bronner MP, Marsh CL. Antiviral prophylaxis and the Epstein Barr virus-related post-transplant lymphoproliferative disorder. Clin Transpl. 1995;9(1):53–9.
64. Funch DP, Walker AM, Schneider G, Ziyadeh NJ, Pescovitz MD. Ganciclovir and acyclovir reduce the risk of post-transplant lymphoproliferative disorder in renal transplant recipients. Am J Transplant. 2005;5(12):2894–900.
65. Green M, Kaufmann M, Wilson J, Reyes J. Comparison of intravenous ganciclovir followed by oral acyclovir with intravenous ganciclovir alone for prevention of cytomegalovirus and Epstein-Barr virus disease after liver transplantation in children. Clin Infect Dis. 1997;25(6): 1344–9.
66. Hocker B, Fickenscher H, Delecluse HJ, Bohm S, Kusters U, Schnitzler P, et al. Epidemiology and morbidity of Epstein-Barr virus infection in pediatric renal transplant recipients: a multicenter, prospective study. Clin Infect Dis. 2013;56(1):84–92.
67. AlDabbagh MA, Gitman MR, Kumar D, Humar A, Rotstein C, Husain S. The role of antiviral prophylaxis for the prevention of Epstein-Barr virus-associated posttransplant lymphoproliferative disease in solid organ transplant recipients: a systematic review. Am J Transplant. 2017;17(3):770–81.
68. Hocker B, Bohm S, Fickenscher H, Kusters U, Schnitzler P, Pohl M, et al. (Val-)ganciclovir prophylaxis reduces Epstein-Barr virus primary infection in pediatric renal transplantation. Transpl Int. 2012;25(7):723–31.
69. McDiarmid SV, Jordan S, Kim GS, Toyoda M, Goss JA, Vargas JH, et al. Prevention and preemptive therapy of postransplant lymphoproliferative disease in pediatric liver recipients. Transplantation. 1998;66(12):1604–11.
70. Lee TC, Savoldo B, Rooney CM, Heslop HE, Gee AP, Caldwell Y, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. Am J Transplant. 2005;5(9):2222–8.
71. Ganschow R, Schulz T, Meyer T, Broering DC, Burdelski M. Low-dose immunosuppression reduces the incidence of post-transplant lymphoproliferative disease in pediatric liver graft recipients. J Pediatr Gastroenterol Nutr. 2004;38(2):198–203.
72. Martin SI, Dodson B, Wheeler C, Davis J, Pesavento T, Bumgardner GL. Monitoring infection with Epstein-Barr virus among seromismatch adult renal transplant recipients. Am J Transplant. 2011;11(5):1058–63.
73. van Esser JW, Niesters HG, van der Holt B, Meijer E, Osterhaus AD, Gratama JW, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood. 2002;99(12):4364–9.
74. Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998;92(5):1549–55.
75. Savoldo B, Goss JA, Hammer MM, Zhang L, Lopez T, Gee AP, et al. Treatment of solid organ transplant recipients with autologous Epstein Barr virus-specific cytotoxic T lymphocytes (CTLs). Blood. 2006;108(9):2942–9.

HHV-6, HHV-7, and HHV-8: Forgotten Viruses in Transplantation

28

Ghady Haidar

Contents

Introduction 683
Human Herpesvirus-6 684
Overview 684
HHV-6 Infection in HSCT Recipients 685
HHV-6 Infection in SOT Recipients 689
HHV-6 in HSCT and SOT: Challenges and Future Directions 691
Human Herpesvirus-7 692
Overview 692
HHV-7 Infection in HSCT Recipients 692
HHV-7 Infection in SOT Recipients 693
HHV-7 in HSCT and SOT: Challenges and Future Directions 694
Human Herpesvirus-8 694
Overview 694
HHV-8 Infection in SOT Recipients 695
HHV-8 Infection in HSCT Recipients 698
HHV-8 in HSCT and SOT: Challenges and Future Directions 699
Conclusions and Future Directions 699
Key Points 700
References 701

Introduction

Human herpesviruses (HHV)-6, HHV-7, and HHV-8 are DNA viruses that have been recognized as pathogens among solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients for nearly three decades. However, despite advances in our understanding of their biology and natural history, the

G. Haidar (✉)
Department of Medicine, Division of Infectious Diseases, University of Pittsburgh and UPMC, Pittsburgh, PA, USA
e-mail: haidarg@upmc.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_28

precise roles of HHV-6 and HHV-7 in causing posttransplant infection and immune modulation remain incompletely defined. In contrast, and perhaps due its recognition as the cause of Kaposi sarcoma (KS) during the human immunodeficiency virus (HIV) epidemic in the 1980s, HHV-8-related syndromes after transplantation are well-described though they are much less common after HSCT compared to SOT. Herein, the biology, risk factors, clinical features, and management of HHV-6, HHV-7, and HHV-8 infections after transplantation are discussed, with an emphasis on knowledge gaps that require further investigation.

Human Herpesvirus-6

Overview

Background

HHV-6 was first isolated from patients with lymphoproliferative disorders in 1986 [1]. It belongs to the subfamily β-herpesviridae in the genus *Roseolovirus* and is most closely related to HHV-7 and cytomegalovirus (CMV) [2]. There are two HHV-6 variants, HHV-6A and HHV-6B, which share approximately 90% amino acid identity but are nonetheless classified as two HHV-6 species due to their distinct epidemiology, biology, genomes, cell tropism, and growth and antigenic properties [3]. HHV-6B causes most documented primary infections and reactivation events [4]. Little is known about HHV-6A, though both HHV-6A and HHV-6B have been recovered from peripheral blood mononuclear cells (PBMCs) of immunocompromised patients. For the purposes of this review, HHV-6A and HHV-6B will be collectively referred to as HHV-6 unless otherwise indicated.

Following acute infection, HHV-6 establishes latency in a wide variety of host cells, although it replicates most efficiently in CD4+ T lymphocytes by binding to its cell surface receptor CD134 [5, 6]. It has also been recovered from lymph nodes, PBMCs, CD8+ T lymphocytes, natural killer (NK) cells, renal tubular cells, salivary glands, and the central nervous system (CNS) [7]. HHV-6 is believed to have immunomodulatory characteristics and can impair cellular proliferation of infected PBMCs in vitro. It is transmitted via saliva and is the most common cause of roseola infantum in infants. Over 90% of adults are seropositive for HHV-6, with infection after HSCT or SOT almost always representing reactivation of latent disease, though donor transmission can occur as discussed below [2].

Chromosomally Integrated HHV-6

Population-based studies have estimated that vertical germ line inheritance of HHV-6 occurs in about 1% of the population [8]. This phenomenon is referred to as chromosomally integrated HHV-6 (ciHHV-6). These individuals are completely healthy, but the genome of HHV-6 is integrated into the telomere regions of a single chromosome in every nucleated cell of their body. They are therefore able to

pass this HHV-6 genome to their children. Since HHV-6 can essentially be thought of as being part of these individuals' genetic makeup as humans, patients with ciHHV-6 always exhibit sustained levels of extremely high HHV-6 viral loads (generally $>10^{5.5}$ copies/mL, though the exact threshold is unclear) that are stable over time and unaffected by any intervention [3, 9].

Since ciHHV-6 is not a pathological state, it almost never requires any specific treatment, even in transplant recipients. Proving the presence of ciHHV-6 is however difficult, as current laboratory testing cannot reliably differentiate between innocuous ciHHV-6 viremia and viremia due to HHV-6 reactivation. Therefore, detection of high HHV-6 viral loads in clinical specimens of transplant patients can confuse clinicians, who may not be able to prove that this is ciHHIV-6, resulting in the administration of antiviral therapy when none is warranted. The presence of ciHHV-6 should however be suspected in any patient with extremely elevated and stable viremia. Viral loads measured in whole blood are typically higher than those measured in plasma [3]. Polymerase chain reaction (PCR) or fluorescent in situ hybridization (FISH) testing of hair follicles or nails can confirm the diagnosis, but these are labor-intensive and impractical [3]. A novel technology called droplet digital PCR can assist with the diagnosis by determining the ratio of HHV-6 to cellular DNA, which is expected to be 1:1 in patients with ciHHV-6 due to the presence of HHV-6 DNA in every single nucleated cell [10].

Diagnosis and Antivirals

In the current era, PCR of peripheral blood or cerebrospinal fluid (CSF) is the diagnostic modality of choice [3]. These assays are however limited by lack of standardization and validation. Immunohistochemistry can also detect viral antigens from clinical specimens. While HHV-6 causes a self-limited illness in healthy children and its direct role as a pathogen after transplantation is debated, some situations – specifically in transplant recipients with encephalitis – often warrant antiviral therapy. Unfortunately, no antiviral is licensed for the treatment of HHV-6. Ganciclovir, foscarnet, cidofovir, and brincidofovir (which is not currently in development) all possess in vitro activity and have all been used to treat patients with HHV-6-related syndromes, with varying degrees of success [3]. Acyclovir appears to be inactive. Adoptive immunotherapy for HHV-6 infections is an area of active investigation [11].

HHV-6 Infection in HSCT Recipients

Epidemiology and Risk Factors

HHV-6 reactivation occurs in 30–80% of HSCT recipients within the first 2–6 weeks after transplant and is associated with significant morbidity and mortality [12–15]. The wide discrepancy in incidence rates is due to the pitfalls inherent to single-center retrospective studies, which have accounted for most of the published data evaluating HHV-6 infections. These studies have used different modalities to establish the presence of HHV-6 infection (such as various PCR assays

performed on whole blood, plasma, or other tissue fluid or brain imaging findings alone) and have inconsistently evaluated for the presence of ciHHV-6. While generally thought of as reactivation of latent infection, primary HHV-6 infection has also been described, potentially due to either donor stem cells or HHV6-positive blood products [13, 16].

A major risk factor for HHV-6 infection is the type of HSCT, with umbilical cord transplant recipients experiencing the highest rates (up to 92%), followed by allogeneic (bone marrow, peripheral blood) and autologous HSCT recipients (30–50% and 9%, respectively) [5, 13, 17, 18]. Peak plasma viral loads are also higher after umbilical cord HSCT compared to other types of transplants [5]. The increased incidence of HHV-6-related syndromes after umbilical cord transplantation is hypothesized to be due to the absence of primed HHV-6-specific memory T cells in cord blood and the immunologic immaturity after umbilical cord blood transplantation [13, 19]. Other risk factors for HHV-6 infection include receipt of human leukocyte antigen (HLA) or sex-mismatched HSCT, younger recipient age, CD4+-rich and NK cell-poor grafts, delayed CD8+ reconstitution, myeloablative conditioning, use of anti-CD3 monoclonal antibodies, and acute graft-versus-host disease (GVHD) [5, 13, 15, 17, 20]. Naïve T-cell depletion (which is performed to reduce the risk of GVHD) may be an emerging risk factor for HHV-6-related syndromes, though this warrants further investigation [21]. T-cell reconstitution has been shown to confer protection against HHV-6 infections [22].

Association with Infectious Syndromes

Clinical data regarding HHV-6 infection after HSCT are mostly limited to case reports, case series, and single-center retrospective observational studies. The literature suggests that HHV-6 infection should be considered in cord blood, allogeneic, or autologous HSCT recipients with sustained and unexplained rashes and fevers, diarrhea, pneumonia, and confusion, particularly in the early posttransplant period [14, 18, 23]. However, the ubiquitous and often self-limited nature of most post-engraftment fevers and rashes after HSCT calls into question the true role of HHV-6 in these patients. HHV-6 causes bone marrow suppression by infecting hematological progenitor cells, leading to a reduction in colony formation [24, 25]. In a recent study, HHV-6 infection was implicated in the pathogenesis of the idiopathic pneumonia syndrome, where HHV-6 DNA was found in 55% of BAL samples [26]. The authors speculated that the presence of HHV-6 in the lungs of HSCT recipients precipitated lung injury, predisposing to the idiopathic pneumonia syndrome through dysregulated host and alloimmune responses. HHV-6 viremia can precede or occur simultaneously with CMV infection and has been associated with BK virus infections, suggesting that it is a marker of overall immunosuppression [20, 27].

Encephalitis is one of the most well-described, debilitating, and potentially fatal syndromes attributed to HHV-6. It is associated with increased 100-day mortality and can lead to long-term neuropsychiatric sequela in up to 57% of patients [17]. Data from the Center for International Blood and Marrow Transplant

Research Database evaluating over 27,000 patients demonstrated that HHV-6 was the most common virus isolated from the CSF of HSCT recipients with encephalitis (52% of cases) and that HHV-6 encephalitis occurred earlier in the posttransplant course than did encephalitis caused by other viruses (median of 28 vs. 80 days, respectively) [28]. A well-recognized HHV-6-related CNS syndrome is posttransplant acute limbic encephalitis (HHV-6-PALE), which presents with altered mental status, anterograde amnesia, marked deficit in short-term memory recall, intermittent agitation, lethargy, mild CSF pleocytosis, temporal electroencephalogram abnormalities, and MRI changes in the limbic system [5]. Half of the patients in this study died, and most survivors unfortunately had residual fatigue and episodic memory impairment despite receiving foscarnet. Other HHV-6-related CNS syndromes after HSCT include hippocampal atrophy and optic neuritis [29, 30].

Overall, it remains uncertain whether HHV-6 causes these syndromes or whether it is merely incidentally detected in patients with fevers, rash, diarrhea, and confusion resulting from other causes.

Association with Transplant-Related Events

In addition to infectious syndromes, HHV-6 has also been implicated in the pathogenesis of adverse graft-related and immunological phenomena after HSCT. These include delayed monocyte and platelet engraftment, GVHD (which is also a risk factor for HHV-6 infection), and disease relapse [5, 15]. It is hypothesized that HHV-6 can trigger or exacerbate GVHD by interacting with and upregulating CD134, which is the HHV-6 cell entry receptor. CD134 is an immunomodulatory molecule that blocks regulatory T-cell (Treg) activity, which when activated releases anti-inflammatory cytokines such as interleukin-10, thereby reducing the functional activity of autoreactive cells and ultimately preventing the development of acute GVHD [31]. By upregulating CD134 expression, HHV-6B infection prevents Treg-mediated anti-inflammatory cascade, increases alloreactivity, and ultimately leads to GVHD. An alternative explanation for this observation is that by directly infecting and depleting CD4+ cells, HHV-6 may have detrimental effects on early T-cell reconstitution, resulting in uncontrolled alloreactivity secondary to Treg depletion and eventually leading to acute GVHD [32].

Despite these observations, detection of HHV-6 viremia may simply be an epiphenomenon. Indeed, whether HHV-6 is causative in the pathogenesis of GVHD, or whether its detection is simply a marker of immune dysregulation heralding the development of acute GVHD, is not currently known. Thus, based on current data, it cannot be recommended to screen patients for HHV-6 viremia as a marker for future GVHD, nor can it be recommended to treat asymptomatic HHV-6 viremia after HSCT. Further studies (which must evaluate for the presence of ciHHV-6) are required to fully understand the complex interplay between HHV-6 viremia and graft-related outcomes.

Chromosomally Integrated HHV-6 After HSCT

Roughly 2% of HSCT donors and/or recipients have ciHHV-6, which confounds molecular testing for HHV-6 viremia [12, 33]. Though data are limited, the rate of

ciHHV-6 among patients with syndromes that may be attributed to HHV-6 was around 4% in one study [20]. As previously mentioned, all patients (including transplant patients) with ciHHV-6 have persistently elevated viral loads (usually $>10^{5.5}$ copies/ml) in blood and CSF specimens, with no dynamic change on serial testing [5]. In contrast, transplant recipients with HHV-6 reactivation usually have transiently detectable HHV-6 DNA, typically at $<10^5$ copies/ml in blood samples. Only a few studies have investigated the role of ciHHV-6 after HSCT. In one study, seven patients diagnosed with HHV-6-PALE had plasma viral loads $>10^{5.5}$ copies/ml, suggesting that the HHV-6-PALE possibly occurred in the context of ciHHV-6 [5]. The presence of ciHHV-6 has also been described in patients with other CNS as well as pulmonary syndromes typically attributed to HHV-6 infections [12]. In addition, one study showed that acute GVHD and CMV viremia were more frequent when recipients or donors had ciHHV-6, though this did not confer an increased risk for chronic GVHD, delayed engraftment, or mortality [33]. Patients with ciHHV-6 viremia have been shown to clear their viremia after engraftment from a donor without ciHHV-6, and conversely, HSCT recipients with transplants from donors with ciHHV-6 develop HHV-6 viremia after engraftment, consistent with donor chimerism [20, 34]. Further studies are required to understand the complex biology of this phenomenon. The benefit of screening for ciHHV-6 to guide donor selection and post-HSCT risk stratification warrants additional investigation.

Utility of Treating HHV-6 After HSCT

Whether HHV-6 is even a treatable virus remains controversial, as antivirals with in vitro activity have never been conclusively shown to improve outcomes, and many patients with HHV-6-related syndromes after HSCT improve with no specific intervention [35, 36]. In addition, antivirals given to prevent CMV do not have an impact on the incidence of HHV-6 viremia [13]. Nonetheless, the use of antivirals (ganciclovir, foscarnet, or cidofovir) has been shown to result in successful eradication of HHV-6 viremia in some patients, though this practice had no impact on survival in one study [37]. In contrast, another study found that all haploidentical HSCT recipients with an HHV-6-related encephalitis improved after receiving foscarnet [38]. However, in a randomized trial of umbilical cord HSCT recipients evaluating the efficacy of foscarnet for treating HHV-6 viremia compared to historical controls who received no treatment, foscarnet significantly suppressed HHV-6 reactivation but did not prevent the development of HHV-6 encephalitis or GVHD [39]. There have even been reports of viremia attributed to ciHHV-6 that ostensibly responded to antivirals, which calls into question whether this was truly ciHHV-6 viremia [20].

While the data are conflicting, they demonstrate that in a subset of patients, HHV-6 may truly cause disease and respond to antivirals. It is difficult to justify withholding antivirals for patients with encephalitis who have evidence of HHV-6 infection. In this setting, treatment is warranted. However, a well-designed randomized trial of antivirals for the treatment of HHV-6-related syndromes needs to be conducted, with standardized HHV-6 monitoring and testing for the presence of ciHHV-6.

HHV-6 Infection in SOT Recipients

Epidemiology and Risk Factors

While initially reported in 1989 after a seronegative liver transplant recipient developed primary HHV-6 infection from a seropositive donor [40], there is a dearth of contemporary data describing HHV-6-related syndromes after SOT, with most published reports clustered between the 1990s and early 2000s. Nonetheless, the central concepts surrounding HHV-6 infection in SOT recipients appear to mirror those of HSCT recipients. HHV-6 infection occurs within the first few weeks after SOT, at a median of 36 days in one study [41]. Incidence rates vary by testing modality and the type of organ transplanted but have ranged from 25 to 82% in liver, 20 to 79% in kidney, 26 to 91% in lung, and up to 73% in heart transplant recipients [41–46]. Cases after pancreas and intestinal transplantation have also been described [34, 47]. Given the near universal seropositivity of adults, post-SOT HHV-6 infection usually represents viral reactivation, though primary infection (particularly after pediatric transplantation) has been described [48]. Antiviral prophylaxis (e.g., with ganciclovir) for other viruses like CMV does not appear to affect the incidence of HHV-6 viremia [45].

Risk factors for HHV-6 infection after SOT have not been fully defined. Receipt of OKT3 may increase the risk, though this agent is no longer clinically available in the United States [49]. Whether thymoglobulin, alemtuzumab, or basiliximab increase the rates of HHV-6 viremia or HHV-6-related syndromes is not known. Acute rejection has been inconsistently found to be a risk factor [2]. In pediatric liver transplant recipients, younger age and severity of liver failure were associated with higher rates of HHV-6 infection [50].

Association with Infectious Syndromes

As with HSCT recipients, it is unclear if the infectious syndromes that have been linked to HHV-6 after SOT are truly caused by HHV-6 or if HHV-6 is merely a bystander. While most patients with viremia are asymptomatic, HHV-6-related syndromes have included rashes, diarrhea, cytopenias, pneumonitis, confusion, and even shock. About 50% of cases of fever of unknown origin after liver transplant were attributed to HHV-6 [51]. In addition, 84% of kidney transplant recipients with HHV-6 reactivation developed fever of unknown origin in one study, compared to 30% of patients without HHV-6 viremia [52]. It is unknown if these findings would be replicated in the current era, given the improved diagnostics of fever of unknown origin. Some have called these early observations into question. For example, in a longitudinal study of kidney, liver, and kidney-liver transplant recipients, HHV-6 was detected in 31% of patients after transplant, but clinical syndromes were observed only in patients who had concomitant CMV infection [53].

More fulminant manifestations of HHV-6 infection have been described. For instance, HHV-6A was thought to be the cause of a severe disseminated donor-derived syndrome of colitis, fever, and cytopenias in two kidney transplant recipients and resulted in the death of one of them [54]. In addition, a case report

by Singh et al. describes an acute febrile illness characterized by life-threatening thrombocytopenia, progressive encephalopathy, and skin rash, which developed in association with invasive HHV-6 infection in a liver transplant recipient [55]. HHV-6 was isolated from blood and bone marrow, and the patient responded to ganciclovir.

CNS dysfunction of undetermined etiology remains the most well-recognized syndrome attributed to HHV-6 infection after SOT. The clinical presentation is nonspecific and variable but is generally characterized by confusion, involuntary movements, amnesia, decreased responsiveness, seizures, and visual hallucinations, often with fevers and rashes [47, 56]. The reported onset in the literature is between 17 days and 3 months after transplant, with a median of 25 days. Brain imaging can be normal or reveal abnormalities in the medial temporal lobes or hippocampus [24, 47]. CSF pleocytosis and elevated protein are inconsistently reported. Most patients with encephalitis occurring in the setting of detection of HHV-6 DNA have been treated with ganciclovir or foscarnet, though it is unclear if improvement or survival is related to antiviral therapy.

HHV-6 has been implicated in increasing the predisposition to and severity of other infections after SOT, including CMV, hepatitis C virus, and opportunistic fungi, though detection of HHV-6 in these patients may simply represent a heightened state of immunosuppression [57]. HHV-6 viremia has been frequently identified concurrently with or immediately preceding CMV viremia [45, 58]. Indeed, the presence of HHV-6 viremia heralded the development of CMV viremia in high-risk (CMV donor positive, recipient negative) kidney transplant recipients, all of whom were HHV-6 seropositive [42]. It is speculated that subclinical CMV infection may have led to immune dysregulation resulting in HHV-6 viremia prior to overt CMV viremia. Alternatively, a heightened state of immunosuppression may have led to viremia with both viruses, with the timing of viremia seen in these studies being a spurious observation. Whether HHV-6 viremia occurring prior to or concurrently with CMV viremia portends more severe CMV infections is unknown.

Association with Transplant-Related Events

The potentially immunomodulatory and immunosuppressive properties of HHV-6 have been described in the SOT population, in whom it is thought to facilitate allograft rejection and graft loss. However, these may simply be parallel phenomena, as the literature is rife with conflicting data. For instance, the presence of HHV-6 viremia was not associated with bronchiolitis obliterans syndrome (BOS) after lung transplantation in one study [44] but did predict stage 1 or greater BOS in another [59]. Some studies have found that HHV-6 viremia was associated with decreased liver allograft survival and the development of chronic allograft nephropathy [60, 61]. However, another study found no correlation between graft rejection and HHV-6 infection after kidney, liver, or liver-kidney transplant [53]. In addition, in one of the only randomized trials comparing preemptive monitoring of HHV-6 viremia (with reduction of immunosuppression and antiviral therapy upon the detection of viremia) to usual care with no monitoring

among 129 adult liver transplant recipients, no cases of symptomatic HHV-6 viremia were detected, nor did viremia have any impact on graft function or mortality [41]. The authors concluded that preemptive monitoring of HHV-6 viremia confers no benefit after liver transplant.

Chromosomally Integrated HHV-6 After SOT

Chromosomally integrated HHV-6 has rarely been reported after SOT and is estimated to occur in about 0.9% of patients [62]. A single-center study described the outcomes of seven liver transplant recipients with suspected ciHHV-6 (defined as persistent viremia of $>10^6$ genomes/mL of whole blood) in addition to nine SOT recipients from the literature (five liver, two liver-kidney, one small bowel, and one kidney transplant) [34]. None of the patients had fever, rash, or organ-invasive clinical syndromes that were attributed to HHV-6 by their clinicians. Notably, HHV-6 DNA was even detected at high levels in the CSF of the small bowel transplant recipient, though this was not believed to be pathogenic. Five patients unnecessarily received antivirals (ganciclovir, valganciclovir, or foscarnet), which had no effect on viremia, consistent with the notion that HHV-6 is integrated in every nucleated cell.

The phenomenon of donor-derived ciHHV-6 after SOT has not been thoroughly investigated. A case of fatal transmission of ciHHV-6A from donor to recipient through liver transplantation was recently reported, with HHV-6 DNA isolated from multiple compartments (CSF, liver tissue, saliva, respiratory fluid). This ultimately resulted in the recipient's death despite ganciclovir and foscarnet [63]. However, routine screening for ciHHV-6 cannot currently be recommended. Nonetheless, clinicians should be cognizant of this phenomenon to avoid the unnecessary use of antiviral drugs in SOT recipients with ciHHV-6, in whom antivirals may be safely discontinued.

HHV-6 in HSCT and SOT: Challenges and Future Directions

A major problem with interpreting studies of patients with HHV-6 viremia after transplantation is that testing for HHV-6 is imprecise, and the presence of viremia does not prove that HHV-6 is causing the patient's syndrome. Indeed, fevers, rashes, pneumonia, diarrhea, and confusion are extremely common after both HSCT and SOT due to polypharmacy (immunosuppression, antimicrobials, other medications), GVHD, mucositis, engraftment, rejection, and other infections. Thus, patients who are found to have HHV-6 viremia in the presence of these syndromes may be falsely labelled as having HHV-6 disease, when in fact the HHV-6 may simply be present in a complex background of unrelated events. The inconsistent efficacy of antivirals in published studies supports the notion that HHV-6 viremia on its own may not always be pathogenic. Indeed, the apparent response to antiviral therapy in some studies may be coincidental, representing the natural history of the underlying disease as opposed to cure of a viral infection. Nonetheless, there does appear to be a subset of patients in whom HHV-6 is truly pathogenic, particularly those with CNS infection or fulminant disease.

Taken together, the abundance of reports providing completely disparate points of view on the pathogenicity of HHV-6 suggests that both scenarios may be true: HHV-6 is a true pathogen capable of leading to significant morbidity and mortality in some patients but is also an innocent bystander in others. The immunologic milieu dictating the emergence of one phenotype over the other is unknown. Future studies must focus on host-pathogen signatures in syndromes thought to be caused by HHV-6, as well as the complex interactions between CMV and HHV-6. It is imperative that we better define the role of HHV-6 in posttransplant infectious and graft-related outcomes, to help us identify which patients would benefit from monitoring and therapy.

Human Herpesvirus-7

Overview

HHV-7 was first isolated from activated CD4+ T cells of a healthy individual in 1990 [64]. It belongs to the *Roseolovirus* family and bears genetic and antigenic resemblance to HHV-6. It is a lymphotropic virus that replicates in CD4+ T cells but has a much narrower host-cell range compared to HHV-6, which includes cord blood mononuclear cells and macrophages [65]. Unlike HHV-6, it utilizes CD4 as a cellular receptor for cell entry. It is ubiquitous, sheds through saliva, and leads to latent infection in adults, with seroprevalence rates of over 95% [2]. Chromosomal integration is not thought to occur. Primary infection in infants usually leads to a nonspecific febrile illness, including roseola. Antiviral agents with in vitro activity against HHV-7 are less well-defined than those for HHV-6. Foscarnet and cidofovir appear to be active; reports on the activity of ganciclovir are conflicting [2, 66, 67]. Acyclovir has no activity.

There is a dearth of literature describing HHV-7 after either HSCT or SOT, with most of the published studies limited to case reports, case series, or small, single-center retrospective reviews. While it does have cytopathic and immunomodulatory effects, no study has definitively proven that HHV-7 causes any syndrome, nor has any study shown that administering antivirals for syndromes attributed to HHV-7 affects outcomes. Therefore, even more so than HHV-6, the presence of HHV-7 in clinical specimens of transplant patients may simply be a marker of immune dysregulation.

HHV-7 Infection in HSCT Recipients

HHV-7 viremia has been described in 6–56% of HSCT recipients, occurring at a median of 18–21 days after HSCT [68–71]. HHV-7 viremia has been detected in patients with fever of unknown origin, rash, hepatitis, pneumonitis, and CNS infection [68–70, 72]. GVHD was associated with HHV-7 infection in a few studies [69, 71]. Allogeneic transplant was a risk factor in one study [71], though others

have surprisingly found a greater incidence of HHV-7 viremia in autologous compared to allogeneic HSCT and in peripheral blood HSCT compared to cord blood HSCT [70, 73]. These data are biologically implausible and appear to contradict the HHV-6 literature, which has established cord blood and allogeneic HSCT as the major risk factors for HHV-6 infections. Most patients in the literature have not received any specific treatment for HHV-7 viremia [69]. However, a case report of encephalitis attributed to HHV-7 after allogeneic HSCT described clinical improvement with foscarnet but not ganciclovir [74].

A few studies have evaluated the dynamics of CMV and HHV-7 infection. One such study found that HHV-7 viremia occurred simultaneously with CMV viremia in 26% of patients and may have contributed to increasing the severity of CMV disease [75]. In addition, patients with HHV-7 viremia had higher CMV viral loads compared to those without viremia (1986 vs. 432 copies/mL, respectively), though the duration of CMV was similar across both groups. In an older study, HSCT recipients with CMV disease were more likely to have concurrent HHV-7 DNA viremia prior to onset of disease than were patients with asymptomatic CMV infection, suggesting that HHV-7 may be a cofactor in the progression from CMV infection to CMV disease [76]. HHV-7 viremia has also been described in patients with other viral infections (adenovirus, parainfluenza) and bacterial and fungal opportunistic infections. Whether this is due to ill-defined HHV-7-related immune modulation, or whether the simultaneous occurrence of these infections is an indirect marker of the net state of immune dysfunction, is not currently known.

HHV-7 Infection in SOT Recipients

As is the case with HHV-7 after HSCT, most studies in the SOT population are limited to case reports, case series, and retrospective studies from the 1990s and early 2000s. Viremia has been reported in 6–88%, 41–48%, and 50% of kidney, liver, and lung or heart-lung transplant recipients, respectively [45, 77–81]. Valganciclovir prophylaxis for CMV has no impact on the incidence of HHV-7 viremia, which occurs within the first month after SOT. Most cases have described incidental asymptomatic viremia with absolutely no clinical consequences [45, 77, 79, 80], though HHV-7 may have been the cause of hepatitis and fever in some liver transplant recipients in one study [77].

HHV-7 viremia after SOT has been postulated to be a cofactor in the pathogenesis of CMV infection in a few limited studies [45, 58, 77, 82, 83]. In a study of 52 kidney transplant recipients, there was a significant increase in CMV disease occurring in patients with CMV and HHV-7 coinfection compared to those with CMV infection alone [81]. The risk of progression to CMV disease after kidney transplant was more pronounced in patients with CMV, HHV-6, and HHV-7 viremia detected simultaneously and in patients with CMV and HHV-7 together, but not in those with only CMV and HHV-6 [84].

Some studies have investigated the role of HHV-7 in graft-related outcomes. HHV-7 DNA was found in 9% of transbronchial samples of lung

transplant recipients and was implicated in the pathogenesis of ischemia reperfusion injury [85]. In addition, all lung transplant recipients with bronchiolitis obliterans with organizing pneumonia (BOOP) were found to have HHV-7 DNA in their bronchoalveolar lavage samples [86]. It has also been linked to acute rejection and chronic allograft nephropathy after kidney transplant [61, 81].

Unfortunately, the veracity of these observations has not been duplicated in larger, prospective, and more contemporary studies. Indeed, a randomized trial of 129 liver transplant recipients with HHV-7 viremia found no association between viremia and clinical disease, nor did it find any benefit of HHV-7-specific intervention (e.g., antivirals or reduction of immunosuppression) [41].

HHV-7 in HSCT and SOT: Challenges and Future Directions

Contemporary prospective studies are required to better understand the true risk factors, biology, host-pathogen interface, and clinical consequences of HHV-7 viremia after transplant. As is the case with detection of HHV-6 DNA in bodily fluids, the presence of HHV-7 after transplant is unlikely to represent true infection but rather a state of immune dysregulation. With the explosion of molecular diagnostics, clinicians need to be judicious in testing for HHV-7 DNA and consider refraining from such testing until additional data are available to elucidate the biology of this virus and the complex interplay between HHV-7, the graft, and other infections.

Human Herpesvirus-8

Overview

Biology

Until the HIV epidemic in the 1980s, Kaposi sarcoma (KS) had largely been a rare skin tumor of older Mediterranean or Ashkenazi Jewish men [87]. HHV-8 itself (also known as Kaposi sarcoma herpes virus (KSHV)), was not identified as the causative agent of KS until 1994, when it was isolated from KS lesions. It is a member of the subfamily *Gammaherpesvirinae* and belongs to the genus *Rhadinovirus* [2].

HHV-8 infects a wide variety of cell types, including B cells, endothelial cells (spindle cells in KS lesions), macrophages, and epithelial cells [88]. As with any herpesvirus, it leads to both lytic and latent infection and has evolved mechanisms to evade the immune system. Cytotoxic T lymphocytes are required for viral control, as evidenced by the increased incidence of KS in transplant patients and those with advanced HIV and by the regression of KS lesions upon immune reconstitution. HHV-8 also contains viral oncogenes that are important in the pathogenesis of tumor formation. For instance, it can transform primary human endothelial cells, which are the cells affected in KS. HHV-8 is unique in that it contains an unprecedented number of genes that were transduced from host

cellular genomes during its evolution, a phenomenon known as molecular piracy [89]. These genes encode for cellular homologs that induce angiogenesis, regulate antiviral immunity, and alter cellular growth. HHV-8 is also a potent inducer of angiogenic cytokines, such as interleukin-6, basic fibroblastic growth factor, and interferon gamma [2].

Overview of Well-Known Manifestations

While KS it the most well-described entity related to HHV-8, it can also cause multicentric Castleman disease and primary effusion lymphoma. Castleman disease is an uncommon lymphoproliferative disorder characterized by fever, splenomegaly, hepatomegaly, and massive lymphadenopathy. Primary effusion lymphoma is a rare subclass of non-Hodgkin lymphoma (NHL) associated with HHV-8 infection and principally seen in persons living with HIV [90]. Other transplant-specific manifestations are discussed in greater detail below. A full discussion of these syndromes is beyond the scope of this chapter.

Epidemiology

The incidence of HHV-8-related disease mirrors its seroprevalence rates, which are highest in sub-Saharan Africa, intermediate in Italy and other Mediterranean areas, and lowest in the United States, Northern Europe, and Asia [91]. Transmission is believed to be via saliva, though it has also been transmitted through sex, blood transfusion, and SOT. It is a predominantly male disease, with the male-to-female ratio of KS in different endemic populations ranging from 3:1 to 15:1 [92].

Diagnosis and Antiviral Therapy

As with HHV-6 and HHV-7, PCR is the gold standard for diagnosis of HHV-8 viremia [3]. KS and other associated syndromes can be diagnosed pathologically. Serology is useful for epidemiologic purposes, particularly donor-recipient risk stratification after SOT (see below). There are extremely limited reports evaluating the activity of antivirals against HHV-8. Ganciclovir, foscarnet, and cidofovir (but not acyclovir) are all active [93], as is the HIV protease inhibitor nelfinavir [94]. However, no clinical report has unequivocally shown the efficacy of antivirals. Mammalian target of rapamycin (mTOR) inhibitors such as everolimus and sirolimus have also been used. The efficacy of these agents is thought to be a result of the constitutive activation of the mTOR signaling pathway in KS and HHV-8-associated primary effusion lymphoma [95]. As discussed below, the mainstay of management is reduction of immunosuppression, often with chemotherapy for visceral KS and for other manifestations.

HHV-8 Infection in SOT Recipients

The first cases of KS occurring as complication of SOT were reported in the 1990s [96]. Since then, post-SOT HHV-8-related syndromes have been extensively described and include not only KS but also Castleman disease (aptly termed

iatrogenic Castleman disease in the post-SOT setting), primary effusion lymphoma, and severe non-neoplastic complications such as bone marrow failure or multiorgan dysfunction [97–100]. Most of these studies have been conducted in areas of the world in which HHV-8 is endemic, such as Italy, the Mediterranean, and the Middle East.

Epidemiology, Risk Factors, and Clinical Manifestations

HHV-8 infection after SOT represents either reactivation of latent infection or donor-derived transmission [96, 98, 101, 102]. Seroprevalence studies have suggested that primary infection from an exogenous source can also occur [103]. Risk factors other than the presence of HHV-8 antibodies include male gender, African or Middle Eastern origin, older age at transplant, and use of lymphocyte depleting induction therapy [104, 105]. HHV-8 usually presents within the first year of transplant [99], though late cases occurring nearly a decade later have been reported [90]. It is most common in adults and rarely occurs in the pediatric population [106].

Overall, KS after SOT is relatively uncommon, with incidence rates of 0.5% in the United States, 0.6% in France, 1.6% in Italy, 2.4% in Israel, 4% in South Africa, and 5.3% in Saudi Arabia [2]. KS represented 16% of all transplant malignancies in a registry of kidney transplant patients from Greece [107]. Cutaneous disease predominates, with visceral and lymph node involvement being less common. Allograft involvement with KS tumors has been described, particularly in donor-derived cases [108–110]. Prognosis of cutaneous disease is excellent, with lesions typically regressing simply with reduction of immunosuppression. Visceral involvement however is associated with a high risk of mortality. HIV infection does not impact the natural history of post-SOT KS [111], which is not surprising given the good graft, patient, and infection-related outcomes of HIV-infected SOT recipients in the current era [112]. Valganciclovir prophylaxis to prevent CMV is ineffective in preventing HHV-8 infections [113].

Some very late cases of HHV-8 infection have been described, such as a patient who succumbed to HHV-8-related primary effusion lymphoma 10 years after undergoing a liver transplant [90]. Other atypical cases have also been reported, such as a heart transplant recipient with both multicentric Castleman disease and KS [114], hemophagocytic lymphohistiocytosis (HLH) [115], Castleman disease occurring concurrently with Epstein-Barr virus-associated posttransplant lymphoproliferative disease [116], and a fatal case of a liver transplant recipient with three distinct donor-derived HHV-8-related entities occurring simultaneously: KS, multicentric Castleman disease, and HLH [117].

An emerging entity after SOT is the Kaposi sarcoma-associated inflammatory cytokine syndrome (KICS) [118]. First reported in HIV-infected individuals, it was recently described in a liver-kidney transplant recipient. This is a severe, non-neoplastic complication driven by lytic HHV-8 reactivation and the ensuing hyper-inflammatory host responses. KICS is characterized by markedly elevated fevers, high HHV-8 viral loads, elevated levels of pro-inflammatory cytokines, cytopenia, and organ failure. A high index of suspicion is required to establish the diagnosis and test for HHV-8 DNA in the blood. In this case, the patient survived after being

managed by reduction of immunosuppression followed by a switch from calcineurin to mammalian target of rapamycin (mTOR) inhibitors, weekly cidofovir followed by foscarnet, as well as rituximab, dexamethasone, and intravenous immunoglobulin.

In summary, HHV-8-related illness should be suspected not only in transplant patients with characteristic KS lesions but also in those who present with an acute febrile illness, systemic symptoms, lymphadenopathy, and/or multiorgan failure.

Donor and Recipient Screening

Whether organ donors and recipients residing in endemic areas would benefit from (a) screening for HHV-8 antibodies prior to transplant and (b) preemptive monitoring of HHV-8 viral loads (with reduction of immunosuppression at the onset of viremia) is unknown but is an area of active investigation. This is not yet standard of care in part because whether this strategy will improve outcomes is unclear and because no reliable HHV-8 screening antibody test exists [119]. In one study from Italy, HHV-8 seroprevalence was 4% and 18% in SOT donors and recipient, respectively [98]. After transplant, 3 (25%) of 12 HHV-8-mismatched SOT recipients developed primary HHV-8 infection, one of whom developed a lethal non-malignant illness characterized by cytopenias and multiorgan system failure. Two of 93 HHV-8-seropositive recipients (2.1%) had evidence of HHV-8 viremia after transplant, but only one of these patients developed KS. None of the 20 HHV-8 low-risk (donor negative, recipient negative) patients developed any HHV-8-related syndrome. In another study, only 4 out of 89 liver transplant recipients developed HHV-8 viremia, with KS lesions developing in only 2 liver recipients and 1 kidney transplant recipient [101].

Thus, even though HHV-8 transmission, reactivation, and attributable morbidity and mortality after SOT are infrequent, HHV-8 infection can be dramatic and life-threatening in some patients. Indeed, it may be inadvisable to wait for clinical manifestations of HHV-8 to emerge, as some patients with visceral KS or KICS can rapidly succumb to their illness. In addition, HHV-8 viremia may precede overt KS after kidney and liver transplant [120, 121]. However, systematic monitoring of HHV8 viremia may lead to increased cost with no added clinical benefit, as the optimal threshold of viremia at which to intervene (e.g., reduce immunosuppression, switch to an mTOR inhibitor, etc.) and the frequency and duration of monitoring have not been defined. Additional work is needed in the form of a randomized trial comparing preemptive HHV-8 monitoring and watchful waiting, particularly in endemic areas.

Management

Reduction of immunosuppression is the mainstay of treatment, though this can result in graft rejection. In this setting, salvage chemotherapy (e.g., with doxorubicin) has been used [108]. A switch to an mTOR inhibitor may be a reasonable option for patients in whom reduction of immunosuppression is unsafe, though primary effusion lymphoma has been described in patients already receiving rapamycin [95]. Cidofovir and foscarnet have been used [98, 118, 122], but their efficacy is questionable, especially given their toxic side effect profiles.

Castleman disease and primary effusion lymphoma should be managed as per standard of care for these entities in non-SOT recipients (e.g., rituximab, chemotherapy) [123]. A kidney transplant recipient with multicentric Castleman disease experienced complete remission after only graft nephrectomy, with no other intervention required [124].

HHV-8 Infection in HSCT Recipients

There are only a handful of case reports of HHV-8-related syndromes after HSCT. Most of these have described KS or a syndrome of fever and bone marrow failure, though atypical presentations such as gingival and respiratory tract disease have also occurred [125]. Post-HSCT KS is thought to represent reactivation of latent disease after HSCT, which develops when cytotoxic T lymphocytes are unable to respond to HHV-8 proteins as immunodeficiency worsens. It is therefore not surprising that KS coincides with CD4 lymphopenia and iatrogenic immunosuppression to treat GVHD [91, 125–127]. Most cases of KS appear to be cutaneous. In many instances, the KS itself requires no specific intervention, with complete regression of lesions after T-cell recovery and discontinuation of immunosuppression. However, some patients do require KS-specific therapy such as thalidomide, topical retinoids, radiation, and even chemotherapy [91, 128]. A waxing and waning course coinciding with the reintroduction of immunosuppression for GVHD flares has been described [91]. An extensive literature search did not reveal any cases of Castleman syndrome or primary effusion lymphoma after HSCT.

In a comprehensive review of 14 cases of post-HSCT KS, 71% (10/14) of the patients were male (ratio of around 3:1), and the median age at diagnosis was 46 (range 7–69 years) [91]. The median interval from transplant to diagnosis of KS was 8.5 months (range 3–27 months). Forty three percent of patients were of Italian descent ($n = 6$), followed by Spanish (21%, $n = 3$), French, Albanian, Israeli, Jamaican, and Chinese (7%, $n = 1$ each). Underlying diseases were acute myeloid leukemia (36%, $n = 5$), chronic myelogenous leukemia (14%, $n = 2$), multiple myeloma (14%, $n = 2$), sickle cell anemia (14%, $n = 2$), NHL, primary immunodeficiency, and aplastic anemia (7%, $n = 1$ each). All but one patient had undergone allogeneic HSCT, with two patients each having received reduced intensity conditioning or T-cell depleted grafts. Eighty six percent of patients had skin involvement ($n = 12$). Fifty percent of patients had localized disease ($n = 7$ total; 5 with cutaneous disease and 2 with lymph node involvement), and the other 50% had diffuse disease (> 1 site or multiple sites: lymph nodes, oral cavity, gastrointestinal tract, and pulmonary or hepatic involvement). Only 22% of patients had bone marrow failure ($n = 3$). In total, 76% of patients had GVHD ($n = 11$, 2 acute and 9 chronic), and all of these patients were on some form of iatrogenic immunosuppression, which typically included a combination of steroids, calcineurin inhibitors, antimetabolites, and anti-thymocyte globulin. Withdrawal of iatrogenic immunosuppression was the sole initial intervention for six patients and was successful in the three who had localized disease. The other three patients had diffuse disease and

underwent various treatments, including thalidomide with topical retinoids, radiation therapy, and interferon with doxorubicin ($n = 1$ each). Other treatments included interferon with surgery and radiation, interferon with radiation and donor lymphocyte infusion, bleomycin and interferon, surgery alone, and doxorubicin ($n = 1$ each). In one case, KS regressed after the patient was switched from a calcineurin inhibitor to sirolimus [129]. Overall, KS-related mortality was 36% ($n = 5$) and only occurred in patients with diffuse involvement.

A nonmalignant syndrome of HHV-8-related bone marrow failure, rash, and fever has been sporadically described after allogeneic HSCT [102, 130, 131]. This shares some features with KICS, which is encountered after SOT and in persons living with HIV. In one such case, the patient had a progressively deteriorating course and refractory marrow failure, ultimately succumbing to respiratory failure [130]. HHV-8 DNA was documented in her peripheral blood and bone marrow. Another patient who had undergone an allogenic HSCT presented with fever, diarrhea, and pancytopenia and died of related complications [102]. Post-mortem, HHV-8 DNA was detected in serial serum samples. Testing of archived pretransplant blood revealed the presence of anti-HHV-8 antibodies, suggesting that HHV-8 reactivated after HSCT. In addition, HHV-8-infected cells were detected by in situ hybridization and immunohistochemical analysis of the aplastic bone marrow but not in two previous normal bone marrow specimens, supporting the hypothesis that infection of bone marrow cells occurred as a result of viral reactivation. Thus, clinicians need to be cognizant of atypical HHV-8 infection as the cause of irreversible aplasia in HSCT recipients. There are no data however to indicate whether these patients should be managed similarly to cases of post-SOT KICS.

HHV-8 in HSCT and SOT: Challenges and Future Directions

Future research efforts from transplant centers in HHV-8-endemic regions should focus on determining the utility of protocolized donor and recipient screening for HHV-8 antibodies. Given the paucity of data in HSCT recipients, a multicenter study of HHV-8 infection after HSCT is warranted.

Conclusions and Future Directions

Despite advances in our understanding of the biology and clinical impact of HHV-6, HHV-7, and HHV-8, much remains completely unknown. It is imperative that future studies investigate the precise role of HHV-6 and HHV-7 in various direct and indirect clinical syndromes attributed to these viruses after transplantation. Defining the host-pathogen cytokine milieu will be crucial to fully understand the pathogenicity of these viruses, particularly HHV-7, which to this day remains a relatively poorly described pathogen with uncertain clinical significance. The role of these viruses in the pathogenesis of CMV needs to be more properly defined, particularly in the setting of late-onset (post-prophylaxis) CMV infection, as well

as CMV viremia and infection in patients being managed through a preemptive approach. Larger studies are needed to standardize the diagnostic criteria and management of KICS. In transplant centers located in HHV-8 endemic countries, the role of donor and recipient HHV-8 antibody testing needs to be more robustly explored, to help the transplant community formulate evidence-based recommendations for the monitoring and treatment of these patients.

Key Points

- HHV-6 infection after HSCT or SOT typically represents reactivation of latent disease, though donor-derived cases have been described.
- HHV-6 infection occurs early after transplant and has been associated with fever of unknown origin, diarrhea, rash, pneumonitis, and encephalitis.
- Posttransplant encephalitis attributed to HHV-6 typically has a protracted course, with most patients experiencing long-term neurological deficits despite the use of antivirals.
- HHV-6 infection may confer an increased risk of cytomegalovirus infection, allograft rejection, delayed engraftment, and graft-versus-host disease.
- Chromosomally integrated HHV-6 is found in around 1% of the population and may complicate the diagnosis of HHV-6 infection after transplant.
- Chromosomally integrated HHV-6 should be suspected in patients with sustained and markedly elevated viremia, but it may not have any clinical consequences.
- It remains unclear whether HHV-6 actually causes any of the diseases attributed to it, nor is it clear whether HHV-6 leads to immune dysregulation or is simply a marker of immunosuppression.
- Treating HHV-6 is usually not warranted, except in encephalitis or fulminant cases, where ganciclovir, foscarnet, or cidofovir have been used.
- Little is known about HHV-7 after transplant except that it is similar to HHV-6 and reactivates early after transplant.
- HHV-7 is likely not clinically significant, though further data are needed to determine its role in cytomegalovirus infection and graft survival.
- HHV-8 is endemic to sub-Saharan Africa, Italy, the Mediterranean, and the Middle East.
- HHV-8 infection after transplant can either be donor- or recipient-derived.
- HHV-8 infection after SOT causes Kaposi sarcoma, multicentric Castleman disease, primary effusion lymphoma, nonmalignant syndromes of bone marrow failure, and an inflammatory cytokine syndrome.
- Screening organ donors and recipients for HHV-8 antibodies to risk stratify patients for HHV-8 infection after SOT may be considered in endemic regions, though additional data are needed.
- HHV-8 has rarely been described after HSCT and is only known to cause KS and bone marrow failure.
- Increased immunosuppression is a risk factor for KS, and patients with cutaneous lesions recover with reduction in immunosuppression.

- mTOR inhibitors may play a role in treating KS after transplant.
- Antivirals should not be routinely used to treat HHV-8 infection.
- Routine monitoring for HHV-6, HHV-7, or HHV-8 should not be performed.
- Antiviral prophylaxis does not prevent HHV-6, HHV-7, or HHV-8 infection.

References

1. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science. 1986;234(4776):596–601.
2. Singh N. Infections with human herpesvirus-6, -7, and -8 after hematopoietic stem cell or solid organ transplantation. In: Bowden RA, Ljungman P, Snydman DR, editors. Transplant infections. 3rd ed; 2010. p. 411–20.
3. Pellett Madan R, Hand J, Practice ASTIDCo. Human herpesvirus 6, 7, and 8 in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13518.
4. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, et al. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. N Engl J Med. 1994;331(7):432–8.
5. Hill JA, Koo S, Guzman Suarez BB, Ho VT, Cutler C, Koreth J, et al. Cord-blood hematopoietic stem cell transplant confers an increased risk for human herpesvirus-6-associated acute limbic encephalitis: a cohort analysis. Biol Blood Marrow Transplant. 2012;18(11):1638–48.
6. Nagamata S, Nagasaka M, Kawabata A, Kishimoto K, Hasegawa D, Kosaka Y, et al. Human CD134 (OX40) expressed on T cells plays a key role for human herpesvirus 6B replication after allogeneic hematopoietic stem cell transplantation. J Clin Virol. 2018;102:50–5.
7. Cecile Tremblay. Virology, pathogenesis, and epidemiology of human herpesvirus 6 infection. In: Post TW, editor. UpToDate. Waltham: UpToDate. https://www.uptodate.com. Accessed 23 Apr 2019.
8. Zerr DM. Human herpesvirus 6 infection in hematopoietic cell transplant recipients. In: Post TW, editor. UpToDate. Waltham: UpToDate. https://www.uptodate.com. Accessed 23 Apr 2019.
9. Rashidi A, Obeid K, Hill J, Warlick ED, Weisdorf DJ. Multiple transmissions of chromosomally integrated human herpesvirus-6 in one family. Transpl Infect Dis. 2018;20(1):e12816
10. Sedlak RH, Cook L, Huang ML, Magaret A, Zerr DM, Boeckh M, et al. Identification of chromosomally integrated human herpesvirus 6 by droplet digital PCR. Clin Chem. 2014;60(5):765–72.
11. Tzannou I, Papadopoulou A, Naik S, Leung K, Martinez CA, Ramos CA, et al. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, Epstein-Barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2017;35(31):3547–57.
12. Sedlak RH, Hill JA, Nguyen T, Cho M, Levin G, Cook L, et al. Detection of human herpesvirus 6B (HHV-6B) reactivation in hematopoietic cell transplant recipients with inherited chromosomally integrated HHV-6A by droplet digital PCR. J Clin Microbiol. 2016;54(5):1223–7.
13. Yamane A, Mori T, Suzuki S, Mihara A, Yamazaki R, Aisa Y, et al. Risk factors for developing human herpesvirus 6 (HHV-6) reactivation after allogeneic hematopoietic stem cell transplantation and its association with central nervous system disorders. Biol Blood Marrow Transplant. 2007;13(1):100–6.
14. Hanson DJ, Hill JA, Koelle DM. Advances in the characterization of the T-cell response to human Herpesvirus-6. Front Immunol. 2018;9:1454.

15. Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. Clin Infect Dis. 2005;40(7):932–40.
16. Kuhn JE. Transfusion-associated infections with cytomegalovirus and other human herpesviruses. Infusionsther Transfusionsmed. 2000;27(3):138–43.
17. Ogata M, Oshima K, Ikebe T, Takano K, Kanamori H, Kondo T, et al. Clinical characteristics and outcome of human herpesvirus-6 encephalitis after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2017;52(11):1563–70.
18. Shargian-Alon L, Yahav D, Rozovski U, Dovrat S, Amitai I, Sela-Navon M, et al. Human herpes virus 6 reactivation following autologous hematopoietic cell transplantation – a single-center experience. Leuk Lymphoma. 2019;60:1–7
19. Tamaki H, Ikegame K, Yoshihara S, Kaida K, Yoshihara K, Inoue T, et al. Low incidence of HHV-6 reactivation in haploidentical hematopoietic stem cell transplantation with corticosteroid as graft-vs-host disease prophylaxis compared with cord blood transplantation. Transpl Infect Dis. 2019;21:e13073.
20. Quintela A, Escuret V, Roux S, Bonnafous P, Gilis L, Barraco F, et al. HHV-6 infection after allogeneic hematopoietic stem cell transplantation: from chromosomal integration to viral co-infections and T-cell reconstitution patterns. J Infect. 2016;72(2):214–22.
21. Sisinni L, Gasior M, de Paz R, Querol S, Bueno D, Fernandez L, et al. Unexpected high incidence of human Herpesvirus-6 encephalitis after naive T cell-depleted graft of haploidentical stem cell transplantation in pediatric patients. Biol Blood Marrow Transplant. 2018;24(11):2316–23.
22. Greco R, Crucitti L, Noviello M, Racca S, Mannina D, Forcina A, et al. Human herpesvirus 6 infection following haploidentical transplantation: immune recovery and outcome. Biol Blood Marrow Transplant. 2016;22(12):2250–5.
23. Yoshikawa T. Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. Br J Haematol. 2004;124(4):421–32.
24. Ljungman P, Singh N. Human herpesvirus-6 infection in solid organ and stem cell transplant recipients. J Clin Virol. 2006;37(Suppl 1):S87–91.
25. Epstein DJ, Tan SK, Deresinski S. HHV-6 and septic shock: tenuous proof of causation. Am J Transplant. 2019;19(1):303.
26. Zhou X, O'Dwyer DN, Xia M, Miller HK, Chan PR, Trulik K, et al. First onset herpesviral infection and lung injury in allogeneic hematopoietic cell transplantation. Am J Respir Crit Care Med. 2019;200:63.
27. Crocchiolo R, Giordano L, Rimondo A, Bologna M, Sarina B, Morabito L, et al. Human Herpesvirus 6 replication predicts Cytomegalovirus reactivation after allogeneic stem cell transplantation from haploidentical donor. J Clin Virol. 2016;84:24–6.
28. Abidi MZ, Hari P, Chen M, Kim S, Battiwala M, Dahi PB, et al. Virus detection in the cerebrospinal fluid of hematopoietic stem cell transplant recipients is associated with poor patient outcomes: a CIBMTR contemporary longitudinal study. Bone Marrow Transplant. 2019;54:1354.
29. Hwang J, Kim JE, Roh JH, Lee JH. Human herpes virus 6 encephalitis following bone marrow transplantation with uncommon magnetic resonance imaging findings. Dement Neurocogn Disord. 2016;15(3):88–91.
30. Hino Y, Doki N, Sekiya N, Takaki Y, Ohashi K. Optic neuritis as an initial manifestation of human herpesvirus 6 reactivation after unrelated bone marrow transplantation. Br J Haematol. 2016;172(5):654.
31. Phan TL, Carlin K, Ljungman P, Politikos I, Boussiotis V, Boeckh M, et al. Human herpesvirus-6B reactivation is a risk factor for grades II to IV acute graft-versus-host disease after hematopoietic stem cell transplantation: a systematic review and meta-analysis. Biol Blood Marrow Transplant. 2018;24(11):2324–36.
32. de Koning C, Admiraal R, Nierkens S, Boelens JJ. Human herpesvirus 6 viremia affects T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. Blood Adv. 2018;2(4):428–32.

33. Hill JA, Magaret AS, Hall-Sedlak R, Mikhaylova A, Huang ML, Sandmaier BM, et al. Outcomes of hematopoietic cell transplantation using donors or recipients with inherited chromosomally integrated HHV-6. Blood. 2017;130(8):1062–9.
34. Lee SO, Brown RA, Razonable RR. Chromosomally integrated human herpesvirus-6 in transplant recipients. Transpl Infect Dis. 2012;14(4):346–54.
35. Ogata M, Phan TL, Fukuda T. Antiviral therapy for the treatment of HHV-6-associated syndromes after transplant. Am J Transplant. 2019;19(1):306–7.
36. Miura H, Kawamura Y, Hattori F, Tanaka M, Kudo K, Ihira M, et al. Late-phase human herpesvirus 6B reactivation in hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2018;20(4):e12916.
37. Betts BC, Young JA, Ustun C, Cao Q, Weisdorf DJ. Human herpesvirus 6 infection after hematopoietic cell transplantation: is routine surveillance necessary? Biol Blood Marrow Transplant. 2011;17(10):1562–8.
38. Perruccio K, Sisinni L, Perez-Martinez A, Valentin J, Capolsini I, Massei MS, et al. High incidence of early human herpesvirus-6 infection in children undergoing haploidentical manipulated stem cell transplantation for hematologic malignancies. Biol Blood Marrow Transplant. 2018;24(12):2549–57.
39. Ogata M, Takano K, Moriuchi Y, Kondo T, Ueki T, Nakano N, et al. Effects of prophylactic foscarnet on human herpesvirus-6 reactivation and encephalitis in cord blood transplant recipients: a prospective multicenter trial with an historical control group. Biol Blood Marrow Transplant. 2018;24(6):1264–73.
40. Ward KN, Gray JJ, Efstathiou S. Brief report: primary human herpesvirus 6 infection in a patient following liver transplantation from a seropositive donor. J Med Virol. 1989;28(2):69–72.
41. Fernandez-Ruiz M, Kumar D, Husain S, Lilly L, Renner E, Mazzulli T, et al. Utility of a monitoring strategy for human herpesviruses 6 and 7 viremia after liver transplantation: a randomized clinical trial. Transplantation. 2015;99(1):106–13.
42. Van Leer-Buter CC, Sanders JS, Vroom HE, Riezebos-Brilman A, Niesters HG. Human herpesvirus-6 DNAemia is a sign of impending primary CMV infection in CMV sero-discordant renal transplantations. J Clin Virol. 2013;58(2):422–6.
43. Lautenschlager I, Razonable RR. Human herpesvirus-6 infections in kidney, liver, lung, and heart transplantation: review. Transpl Int. 2012;25(5):493–502.
44. Manuel O, Kumar D, Moussa G, Chen MH, Pilewski J, McCurry KR, et al. Lack of association between beta-herpesvirus infection and bronchiolitis obliterans syndrome in lung transplant recipients in the era of antiviral prophylaxis. Transplantation. 2009;87(5):719–25.
45. Lehto JT, Halme M, Tukiainen P, Harjula A, Sipponen J, Lautenschlager I. Human herpesvirus-6 and -7 after lung and heart-lung transplantation. J Heart Lung Transplant. 2007; 26(1):41–7.
46. de Ona M, Melon S, Rodriguez JL, Sanmartin JC, Bernardo MJ. Association between human herpesvirus type 6 and type 7, and cytomegalovirus disease in heart transplant recipients. Transplant Proc. 2002;34(1):75–6.
47. Yamamoto T, Watarai Y, Goto N, Horikoshi Y, Yamada S, Yasui K, et al. Encephalitis caused by human herpesvirus-6B in pancreas-after-kidney transplantation. Transpl Infect Dis. 2014;16(5):853–8.
48. Cervera C, Marcos MA, Linares L, Roig E, Benito N, Pumarola T, et al. A prospective survey of human herpesvirus-6 primary infection in solid organ transplant recipients. Transplantation. 2006;82(7):979–82.
49. Jacobs U, Ferber J, Klehr HU. Severe allograft dysfunction after OKT3-induced human herpes virus-6 reactivation. Transplant Proc. 1994;26(6):3121.
50. Yasui T, Suzuki T, Yoshikawa T, Yatsuya H, Kawamura Y, Miura H, et al. Clinical course of human herpesvirus 6 infection in pediatric living donor liver transplantation. Pediatr Transplant. 2018;22(7):e13239.
51. Chang FY, Singh N, Gayowski T, Wagener MM, Marino IR. Fever in liver transplant recipients: changing spectrum of etiologic agents. Clin Infect Dis. 1998;26(1):59–65.

52. Singh N. Infections in solid organ transplant recipients. Curr Opin Infect Dis. 1998;11(4):411–7.
53. Herbein G, Strasswimmer J, Altieri M, Woehl-Jaegle ML, Wolf P, Obert G. Longitudinal study of human herpesvirus 6 infection in organ transplant recipients. Clin Infect Dis. 1996;22(1):171–3.
54. Pilmore H, Collins J, Dittmer I, Williams L, Carpenter L, Thomas S, et al. Fatal human herpesvirus-6 infection after renal transplantation. Transplantation. 2009;88(6):762–5.
55. Singh N, Carrigan DR, Gayowski T, Singh J, Marino IR. Variant B human herpesvirus-6 associated febrile dermatosis with thrombocytopenia and encephalopathy in a liver transplant recipient. Transplantation. 1995;60(11):1355–7.
56. Rogers J, Rohal S, Carrigan DR, Kusne S, Knox KK, Gayowski T, et al. Human herpesvirus-6 in liver transplant recipients: role in pathogenesis of fungal infections, neurologic complications, and outcome. Transplantation. 2000;69(12):2566–73.
57. Razonable RR, Lautenschlager I. Impact of human herpes virus 6 in liver transplantation. World J Hepatol. 2010;2(9):345–53.
58. Harma M, Hockerstedt K, Lyytikainen O, Lautenschlager I. HHV-6 and HHV-7 antigenemia related to CMV infection after liver transplantation. J Med Virol. 2006;78(6):800–5.
59. Neurohr C, Huppmann P, Leuchte H, Schwaiblmair M, Bittmann I, Jaeger G, et al. Human herpesvirus 6 in bronchalveolar lavage fluid after lung transplantation: a risk factor for bronchiolitis obliterans syndrome? Am J Transplant. 2005;5(12):2982–91.
60. Pischke S, Gosling J, Engelmann I, Schlue J, Wolk B, Jackel E, et al. High intrahepatic HHV-6 virus loads but neither CMV nor EBV are associated with decreased graft survival after diagnosis of graft hepatitis. J Hepatol. 2012;56(5):1063–9.
61. Chapenko S, Folkmane I, Ziedina I, Chistyakovs M, Rozentals R, Krumina A, et al. Association of HHV-6 and HHV-7 reactivation with the development of chronic allograft nephropathy. J Clin Virol. 2009;46(1):29–32.
62. Potenza L, Barozzi P, Masetti M, Pecorari M, Bresciani P, Gautheret-Dejean A, et al. Prevalence of human herpesvirus-6 chromosomal integration (CIHHV-6) in Italian solid organ and allogeneic stem cell transplant patients. Am J Transplant. 2009;9(7):1690–7.
63. Bonnafous P, Marlet J, Bouvet D, Salame E, Tellier AC, Guyetant S, et al. Fatal outcome after reactivation of inherited chromosomally integrated HHV-6A (iciHHV-6A) transmitted through liver transplantation. Am J Transplant. 2018;18(6):1548–51.
64. Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, Danovich RM, et al. Isolation of a new herpesvirus from human CD4+ T cells. Proc Natl Acad Sci USA. 1990;87(2):748–52.
65. Zhang Y, de Bolle L, Aquaro S, van Lommel A, De Clercq E, Schols D. Productive infection of primary macrophages with human herpesvirus 7. J Virol. 2001;75(21):10511–4.
66. Brennan DC, Storch GA, Singer GG, Lee L, Rueda J, Schnitzler MA. The prevalence of human herpesvirus-7 in renal transplant recipients is unaffected by oral or intravenous ganciclovir. J Infect Dis. 2000;181(5):1557–61.
67. Zhang Y, Schols D, De Clercq E. Selective activity of various antiviral compounds against HHV-7 infection. Antivir Res. 1999;43(1):23–35.
68. Dzieciatkowski T, Przybylski M, Basak GW, Torosian T, Jedrzejczak WW, Mlynarczyk G. Human herpesvirus 7 in allogeneic hemopoietic stem cell transplant recipients in the central clinical hospital in Warsaw: a three-year survey. Intervirology. 2011;54(1):25–9.
69. Fule Robles JD, Cheuk DK, Ha SY, Chiang AK, Chan GC. Human herpesvirus types 6 and 7 infection in pediatric hematopoietic stem cell transplant recipients. Ann Transplant. 2014;19:269–76.
70. Chan PK, Li CK, Chik KW, Lee V, Shing MM, Ng KC, et al. Risk factors and clinical consequences of human herpesvirus 7 infection in paediatric haematopoietic stem cell transplant recipients. J Med Virol. 2004;72(4):668–74.
71. Khanani M, Al-Ahmari A, Tellier R, Allen U, Richardson S, Doyle JJ, et al. Human herpesvirus 7 in pediatric hematopoietic stem cell transplantation. Pediatr Blood Cancer. 2007;48(5):567–70.

72. Chan PK, Chik KW, To KF, Li CK, Shing MM, Ng KC, et al. Case report: human herpesvirus 7 associated fatal encephalitis in a peripheral blood stem cell transplant recipient. J Med Virol. 2002;66(4):493–6.
73. Miyoshi H, Tanaka-Taya K, Hara J, Fujisaki H, Matsuda Y, Ohta H, et al. Inverse relationship between human herpesvirus-6 and -7 detection after allogeneic and autologous stem cell transplantation. Bone Marrow Transplant. 2001;27(10):1065–70.
74. Holden SR, Vas AL. Severe encephalitis in a haematopoietic stem cell transplant recipient caused by reactivation of human herpesvirus 6 and 7. J Clin Virol. 2007;40(3):245–7.
75. Tomaszewska A, Krysko A, Dzieciatkowski T, Przybylski M, Basak GW, Halaburda K, et al. Co-infections with cytomegalovirus and human herpesvirus type 7 in adult Polish allogeneic haematopoietic stem cell transplant recipients. Arch Immunol Ther Exp. 2014;62(1):77–80.
76. Chan PK, Peiris JS, Yuen KY, Liang RH, Lau YL, Chen FE, et al. Human herpesvirus-6 and human herpesvirus-7 infections in bone marrow transplant recipients. J Med Virol. 1997;53(3):295–305.
77. Thomasini RL, Sampaio AM, Bonon SH, Boin IF, Leonardi LS, Leonardi M, et al. Detection and monitoring of human herpesvirus 7 in adult liver transplant patients: impact on clinical course and association with cytomegalovirus. Transplant Proc. 2007;39(5):1537–9.
78. Peigo MF, Thomasini RL, Puglia AL, Costa SC, Bonon SH, Boin IF, et al. Human herpesvirus-7 in Brazilian liver transplant recipients: a follow-up comparison between molecular and immunological assays. Transpl Infect Dis. 2009;11(6):497–502.
79. Anton A, Cervera C, Pumarola T, Moreno A, Benito N, Linares L, et al. Human herpesvirus 7 primary infection in kidney transplant recipients. Transplantation. 2008;85(2):298–302.
80. Caiola D, Karras A, Flandre P, Boutolleau D, Scieux C, Agut H, et al. Confirmation of the low clinical effect of human herpesvirus-6 and -7 infections after renal transplantation. J Med Virol. 2012;84(3):450–6.
81. Kidd IM, Clark DA, Sabin CA, Andrew D, Hassan-Walker AF, Sweny P, et al. Prospective study of human betaherpesviruses after renal transplantation: association of human herpesvirus 7 and cytomegalovirus co-infection with cytomegalovirus disease and increased rejection. Transplantation. 2000;69(11):2400–4.
82. Galarraga MC, Gomez E, de Ona M, Rodriguez A, Laures A, Boga JA, et al. Influence of ganciclovir prophylaxis on citomegalovirus, human herpesvirus 6, and human herpesvirus 7 viremia in renal transplant recipients. Transplant Proc. 2005;37(5):2124–6.
83. Tong CY, Bakran A, Williams H, Cheung CY, Peiris JS. Association of human herpesvirus 7 with cytomegalovirus disease in renal transplant recipients. Transplantation. 2000; 70(1):213–6.
84. Osman HK, Peiris JS, Taylor CE, Warwicker P, Jarrett RF, Madeley CR. "Cytomegalovirus disease" in renal allograft recipients: is human herpesvirus 7 a co-factor for disease progression? J Med Virol. 1996;48(4):295–301.
85. Costa C, Curtoni A, Bergallo M, Solidoro P, Lorusso M, Delsedime L, et al. Quantitative detection of HHV-6 and HHV-7 in transbronchial biopsies from lung transplant recipients. New Microbiol. 2011;34(3):275–80.
86. Ross DJ, Chan RC, Kubak B, Ardehali A, Laks H, Nichols WS. Bronchiolitis obliterans with organizing pneumonia: possible association with human herpes virus-7 infection after lung transplantation. J Heart Lung Transplant. 2001;20(2):171.
87. Braun M. Classics in Oncology. Idiopathic multiple pigmented sarcoma of the skin by Kaposi. CA Cancer J Clin. 1982;32(6):340–7.
88. Casper C. Virology, epidemiology, and transmission of human herpesvirus 8 infection. In: Post TW, editor. UpToDate. Waltham: UpToDate. https://www.uptodate.com. Accessed 23 Apr 2019.
89. Boshoff C, Weiss RA. Aetiology of Kaposi's sarcoma: current understanding and implications for therapy. Mol Med Today. 1997;3(11):488–94.
90. Christenson ES, Teply B, Agrawal V, Illei P, Gurakar A, Kanakry JA. Human herpesvirus 8-related primary effusion lymphoma after liver transplantation. Am J Transplant. 2015;15(10):2762–6.

91. Deauna-Limayo D, Rajabi B, Qiu W, Htut M, Sweetenham J. Kaposi sarcoma after non-myeloablative hematopoietic stem cell transplant: response to withdrawal of immunosuppressant therapy correlated with whole blood human herpesvirus-8 reverse transcriptase-polymerase chain reaction levels. Leuk Lymphoma. 2013;54(10):2299–302.
92. Plancoulaine S, Abel L, van Beveren M, Gessain A. High titers of anti-human herpesvirus 8 antibodies in elderly males in an endemic population. J Natl Cancer Inst. 2002;94(17):1333–5.
93. Casper C, Wald A. The use of antiviral drugs in the prevention and treatment of Kaposi sarcoma, multicentric Castleman disease and primary effusion lymphoma. Curr Top Microbiol Immunol. 2007;312:289–307.
94. Gantt S, Carlsson J, Ikoma M, Gachelet E, Gray M, Geballe AP, et al. The HIV protease inhibitor nelfinavir inhibits Kaposi's sarcoma-associated herpesvirus replication in vitro. Antimicrob Agents Chemother. 2011;55(6):2696–703.
95. Boulanger E, Afonso PV, Yahiaoui Y, Adle-Biassette H, Gabarre J, Agbalika F. Human herpesvirus-8 (HHV-8)-associated primary effusion lymphoma in two renal transplant recipients receiving rapamycin. Am J Transplant. 2008;8(3):707–10.
96. Regamey N, Tamm M, Wernli M, Witschi A, Thiel G, Cathomas G, et al. Transmission of human herpesvirus 8 infection from renal-transplant donors to recipients. N Engl J Med. 1998;339(19):1358–63.
97. Borges-Costa J, Lopes L, Soares-Almeida L, Guerra J. Kaposi's sarcoma presenting as violaceous macules on the chest of a kidney transplanted patient. BMJ Case Rep. 2016. https://doi.org/10.1136/bcr-2015-210999
98. Chiereghin A, Barozzi P, Petrisli E, Piccirilli G, Gabrielli L, Riva G, et al. Multicenter prospective study for laboratory diagnosis of HHV8 infection in solid organ donors and transplant recipients and evaluation of the clinical impact after transplantation. Transplantation. 2017;101(8):1935–44.
99. Pietrosi G, Vizzini G, Pipitone L, Di Martino G, Minervini MI, Lo Iacono G, et al. Primary and reactivated HHV8 infection and disease after liver transplantation: a prospective study. Am J Transplant. 2011;11(12):2715–23.
100. Speicher DJ, Sehu MM, Mollee P, Shen L, Johnson NW, Faoagali JL. Successful treatment of iatrogenic multicentric Castleman's disease arising due to recrudescence of HHV-8 in a liver transplant patient. Am J Transplant. 2014;14(5):1207–13.
101. Lebbe C, Porcher R, Marcelin AG, Agbalika F, Dussaix E, Samuel D, et al. Human herpesvirus 8 (HHV8) transmission and related morbidity in organ recipients. Am J Transplant. 2013;13(1):207–13.
102. Luppi M, Barozzi P, Schulz TF, Setti G, Staskus K, Trovato R, et al. Bone marrow failure associated with human herpesvirus 8 infection after transplantation. N Engl J Med. 2000;343(19):1378–85.
103. Jenkins FJ, Hoffman LJ, Liegey-Dougall A. Reactivation of and primary infection with human herpesvirus 8 among solid-organ transplant recipients. J Infect Dis. 2002;185(9):1238–43.
104. Farge D, Lebbe C, Marjanovic Z, Tuppin P, Mouquet C, Peraldi MN, et al. Human herpes virus-8 and other risk factors for Kaposi's sarcoma in kidney transplant recipients. Groupe Cooperatif de transplantation d' Ile de France (GCIF). Transplantation. 1999;67(9):1236–42.
105. Cahoon EK, Linet MS, Clarke CA, Pawlish KS, Engels EA, Pfeiffer RM. Risk of Kaposi sarcoma after solid organ transplantation in the United States. Int J Cancer. 2018;143(11):2741–8.
106. Ocwieja KE, Vargas SO, Elisofon SA, Shulman DS, Lee CK, Fawaz R, et al. Pediatric post-transplant hepatic Kaposi sarcoma due to donor-derived human herpesvirus 8. Pediatr Transplant. 2019;23:e13384.
107. Zavos G, Moris D, Vernadakis S, Bokos J, Lionaki S, Mamarelis G, et al. Incidence and management of Kaposi sarcoma in renal transplant recipients: the Greek experience. Transplant Proc. 2014;46(9):3199–202.
108. Fu W, Merola J, Malinis M, Lacy J, Barbieri A, Liapakis AH, et al. Successful treatment of primary donor-derived human herpesvirus-8 infection and hepatic Kaposi sarcoma in an adult liver transplant recipient. Transpl Infect Dis. 2018;20(5):e12966.

109. Dudderidge TJ, Khalifa M, Jeffery R, Amlot P, Al-Akraa M, Sweny P. Donor-derived human herpes virus 8-related Kaposi's sarcoma in renal allograft ureter. Transpl Infect Dis. 2008;10(3):221–6.
110. Nannini N, Rebusso A, Lunardi F, Loy M, Calabrese F, Battistella L, et al. Asymptomatic pulmonary allograft Kaposi sarcoma: a case report. Exp Clin Transplant. 2017;15(4):477–9.
111. Charpentier C, Delyon J, Glotz D, Peraldi MN, Rerolle JP, Barrou B, et al. Kaposi sarcoma in HIV-positive solid-organ transplant recipients: a French multicentric national study and literature review. Transplantation. 2019;103(1):e22–e8.
112. Haidar G, Singh N. The times, they are a-changing: HOPE for HIV-to-HIV organ transplantation. Transplantation. 2017;101(9):1987–95.
113. Haidar G, Singh N. Viral infections in solid organ transplant recipients: novel updates and a review of the classics. Curr Opin Infect Dis. 2017;30(6):579–88.
114. Patel A, Bishburg E, Zucker M, Tsang P, Nagarakanti S, Sabnani I. Concomitant Kaposi sarcoma and multicentric Castleman's disease in a heart transplant recipient. Heart Lung. 2014;43(6):506–9.
115. Cohen GM, Langer AL, Sima H, Chang C, Troy K, Taimur S. Hemophagocytic lymphohzistiocytosis due to primary HHV-8 infection in a liver transplant recipient. Transplant Direct. 2018;4(12):e411.
116. Jha LK, Ulmer LL, Olivera-Martinez MA, McCashland TM, Fu K, Rochling FA. Castleman's disease and posttransplant lymphoproliferative disorder after liver transplant: 3-year follow-up. Case Reports Hepatol. 2018;2018:9324872.
117. Vijgen S, Wyss C, Meylan P, Bisig B, Letovanec I, Manuel O, et al. Fatal outcome of multiple clinical presentations of human herpesvirus 8-related disease after solid organ transplantation. Transplantation. 2016;100(1):134–40.
118. Mularoni A, Gallo A, Riva G, Barozzi P, Miele M, Cardinale G, et al. Successful treatment of Kaposi sarcoma-associated herpesvirus inflammatory cytokine syndrome after kidney-liver transplant: correlations with the human herpesvirus 8 miRNome and specific T cell response. Am J Transplant. 2017;17(11):2963–9.
119. Serraino D, Piselli P, Scuderi M, Gabbrielli F, Venettoni S, Grossi P, et al. Screening for human herpesvirus 8 antibodies in Italian organ transplantation centers. Clin Infect Dis. 2005;40(1):203–5.
120. Pozo F, Tenorio A, de la Mata M, de Ory F, Torre-Cisneros J. Persistent human herpesvirus 8 viremia before Kaposi's sarcoma development in a liver transplant recipient. Transplantation. 2000;70(2):395–7.
121. Sarid R, Pizov G, Rubinger D, Backenroth R, Friedlaender MM, Schwartz F, et al. Detection of human herpesvirus-8 DNA in kidney allografts prior to the development of Kaposi's sarcoma. Clin Infect Dis. 2001;32(10):1502–5.
122. Verucchi G, Calza L, Trevisani F, Zambruni A, Tadolini M, Giuliani R, et al. Human herpesvirus-8-related Kaposi's sarcoma after liver transplantation successfully treated with cidofovir and liposomal daunorubicin. Transpl Infect Dis. 2005;7(1):34–7.
123. Thaunat O, Mamzer-Bruneel MF, Agbalika F, Valensi F, Venditto M, Lebbe C, et al. Severe human herpesvirus-8 primary infection in a renal transplant patient successfully treated with anti-CD20 monoclonal antibody. Blood. 2006;107(7):3009–10.
124. Al Otaibi T, Al Sagheir A, Ludwin D, Meyer R. Post renal transplant Castleman's disease resolved after graft nephrectomy: a case report. Transplant Proc. 2007;39(4):1276–7.
125. Abbas AA, Jastaniah WA. Extensive gingival and respiratory tract Kaposi sarcoma in a child after allogenic hematopoietic stem cell transplantation. J Pediatr Hematol Oncol. 2012;34(2):e53–5.
126. Heyrman B, De Becker A, Schots R. A case report of immunosuppression-related Kaposi's sarcoma after autologous stem cell transplantation. BMC Res Notes. 2016;9:188.
127. Bruno B, Sorasio R, Barozzi P, Vieira J, Omede P, Giaretta F, et al. Kaposi's sarcoma triggered by endogenous HHV-8 reactivation after non-myeloablative allogeneic haematopoietic transplantation. Eur J Haematol. 2006;76(4):342–7.

128. de Medeiros BC, Rezuke WN, Ricci A Jr, Tsongalis G, Shen PU, Bona RD, et al. Kaposi's sarcoma following allogeneic hematopoietic stem cell transplantation for chronic myelogenous leukemia. Acta Haematol. 2000;104(2–3):115–8.
129. Marco de F, Infante B, Giovanni S, Gesualdo L. Rapamycin for Kaposi's sarcoma and graft-versus-host disease in bone marrow transplant recipient. Transplantation. 2010;89(5):633–4.
130. Cuzzola M, Irrera G, Iacopino O, Cuzzocrea A, Messina G, Console G, et al. Bone marrow failure associated with herpesvirus 8 infection in a patient undergoing autologous peripheral blood stem cell transplantation. Clin Infect Dis. 2003;37(7):e102–6.
131. Luppi M, Barozzi P, Schulz TF, Trovato R, Donelli A, Narni F, et al. Nonmalignant disease associated with human herpesvirus 8 reactivation in patients who have undergone autologous peripheral blood stem cell transplantation. Blood. 2000;96(7):2355–7.

Influenza Infections in Solid Organ and Stem Cell Transplant Recipients

29

Yoichiro Natori

Contents

Introduction 710
Influenza Incidence in SOT Recipients 710
Influenza Incidence in HSCT Recipients 711
Incidence of Hospital Admission and Intensive Care Unit (ICU) Admission After Influenza Infection in SOT and HSCT Recipients with Influenza Virus Infection 711
Hospital Admission 711
ICU Admission 712
Clinical Presentation of Influenza in SOT Recipients 712
Fever 713
Lower Respiratory Tract Infection 713
Graft Dysfunction 714
Clinical Presentation of Influenza in HSCT Recipients 714
Fever 714
Lower Respiratory Tract Infection 714
Diagnosis 715
Treatment and Outcomes 715
Adjunctive Therapy 716
Risk Factors for Mortality and ICU Admission 716
Prophylaxis 716
Chemoprophylaxis 716
Vaccination 717
Influenza Vaccination in SOT Recipients 717
Vaccine Strategy 717
Repeated Vaccination 718
High-Dose Vaccination 718
Intradermal Versus Intramuscular 718
Adjuvanted Vaccine 719
Influenza Vaccination Side Effects in SOT Recipients 719
Vaccination in HSCT Recipients 720

Y. Natori (✉)
Division of Infectious Disease, Department of Medicine, University of Miami Miller School of Medicine and Miami Transplant Institute, Miami, FL, USA
e-mail: yxn138@med.miami.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_30

Timing of Vaccination 720
Vaccine Strategy 720
Repeated Vaccination 720
High-Dose Vaccination 720
Adjuvanted 721
Donor Vaccination and Pre-HSCT Vaccination 721
Conclusion 721
Key Points 721
References 722

Introduction

In past decades, because of advances in surgical technique and development of new immunosuppressive medications, the outcome of solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) has been improved. However, even with development of antimicrobial prophylaxis and infection prevention methods, we still see a high incidence of bacterial, viral, and fungal infections in these immunocompromised hosts. Among the most common posttransplant infections are respiratory viral infections, including influenza virus, respiratory syncytial virus, parainfluenza virus, rhinovirus, adenovirus, and human metapneumovirus. Influenza virus infection causes high morbidity and mortality in this population. Influenza virus is a RNA virus which can cause acute febrile respiratory infection. Influenza virus is in the family of *Orthomyxovidae*. The clinical presentation of influenza infection in immunocompromised hosts varies and frequently differs from manifestations of influenza in the general population. The rate of lower respiratory tract infection is higher in transplant populations due to deficiencies of cellular and humoral immunity and may result in severe disease. Patients with lower respiratory airway disease, more aggressive immunosuppression, and late initiation of antivirals tend to have worse outcomes. Vaccination should be the major strategy to prevent infection, but immunogenicity after influenza vaccination is still suboptimal in transplant recipients.

This chapter focuses on influenza infection in both SOT and HSCT populations and will focus on disease epidemiology, clinical symptoms, treatment, and vaccination.

Influenza Incidence in SOT Recipients

The reported incidence of influenza in SOT recipients varies year to year and between seasonal influenza and pandemic influenza. A recent pandemic A(H1N1) influenza infection occurred in 2009.

For seasonal influenza, there have been several studies in transplant recipients. One single center retrospective study in Brazil demonstrated that the incidence of influenza virus infection in kidney transplant recipients was 26.5 cases/1,000 person-years [1]. A previous large study states the incidence of influenza in kidney, liver,

and lung transplant recipients was 4.3, 2.8, and 41.8 cases/1000 person-years, respectively [2].

During the influenza pandemic, an Australian multicenter retrospective study found a 3% incidence of infection in the lung transplant population in 2009 [3]. A influenza study from Singapore documented an incidence of approximately 20 cases/1000 person-years in kidney or liver transplant and 37 cases/1000 person-years for heart transplantation in 2009 [4]. There should be some reporting bias because the transplant population were more likely to be tested and might therefore have a higher reported incidence as compared to the general population.

The aforementioned reports focus on influenza A virus infection. There are fewer published reports regarding influenza B virus infection in SOT recipients.

Reports confirm that influenza can cause donor-derived infection [5]. Four recipients including two kidney, one liver, and one lung transplant recipient received organs from a single influenza B-positive donor [5]. One kidney and one lung transplant recipient developed influenza B virus infection soon after transplant, which was confirmed by polymerase chain reaction method [5]. Because of the timing early after transplant and infection in two recipients from the same donor, the authors concluded those infections were highly likely to have been donor derived [5].

Influenza Incidence in HSCT Recipients

A recent study from Australia showed that 9/382 recipients developed influenza infection within 100 days after HSCT [6]. Also, within 2 weeks of scheduled HSCT, one report from the USA documented that 10/458 (2%) patients tested positive for influenza virus with qualitative PCR method [7]. All of them had symptoms at the time of detection [6]. One patient out of 10 continued to show positivity even after transplant; influenza was thought to have ultimately contributed to their death [7].

Incidence of Hospital Admission and Intensive Care Unit (ICU) Admission After Influenza Infection in SOT and HSCT Recipients with Influenza Virus Infection

Hospital Admission

One retrospective cohort study done by Katzen et al. showed some benefit in shortened length of hospital stay when oseltamivir therapy was initiated soon after admission in the general population [8]. Oncology patients required longer duration of hospitalization [8]. In this study, around 5% of the study population were SOT recipients [8].

In an SOT population, hospital admission was required in 70% in a multicenter study of the A(H1N1) pandemic in 2009 [9]. For seasonal influenza, among SOT and HSCT recipients with positive influenza polymerase chain reaction (PCR) test, 373/561 (66.5%) were admitted with median of 6 days (interquartile range 3–14 days) in a multicenter prospective study [10].

ICU Admission

ICU admission in SOT recipients was seen in 16% of patients enrolled in a multicenter study of pandemic A(H1N1) in 2009 [9]. Early initiation of antiviral therapy was associated with a reduction in the incidence of ICU admission in SOT recipients [9]. Receipt of antilymphocyte globulin and diabetes were risk factors for ICU admission after multivariate analysis [9].

For seasonal influenza virus, a multicenter study showed that 68 out of 616 (11.0%) SOT and HSCT recipients required ICU admission with median duration of 5 days (interquartile range 2–17 days) [10]. Over the 5-year study period, the ICU admission rates varied from 8.1% to 14.3% [10]. In multivariate analysis, both early antiviral therapy and receipt of influenza vaccine in the same season were statistically significant factors lowering the incidence of ICU admission [10]. In the pediatric group, 3/25 (12.0%) of the recipients required ICU admission [10]. Vaccination and earlier initiation of antiviral agents were associated with reductions in admission to ICU and the need for ventilation for pandemic A(H1N1) [9, 11] in 2009 as well as seasonal influenza infection [10].

Clinical Presentation of Influenza in SOT Recipients

One study showed that the presentation of influenza infection is very similar in SOT and HSCT recipients [10]. This section will explain the clinical profile based on transplant type.

Immunocompromised hosts, including SOT and HSCT recipients, are less likely than the general population to develop symptoms after infection of influenza [12]. Of note, in immunocompromised hosts, symptoms of dry cough, chills, sweats, myalgia, and shortness of breath are less frequent, though they tend to have more severe disease and complications than the general population [12].

Another prospective study from Spain showed that 80% of SOT recipients developed cough at the time of presentation [11]. The biggest multicenter study conducted for pandemic A(H1N1) showed that cough was the most frequent symptom at presentation (91%) [9]. Other accompanying symptoms included myalgia in 51%, rhinorrhea in 43%, sore throat in 43%, and headache in 32% [9].

For seasonal influenza infection, a 5-year multicenter study of SOT recipients showed that more than 80% of the recipients developed cough and around 30–40% of the recipients developed sore throat, rhinorrhea, headache, myalgias, or

gastrointestinal symptoms [10]. Also, in 51 SOT recipients in multicenter prospective study in Spain for pandemic influenza A(H1N1) infection and cough continued for a median of 3 days [11]. Symptoms varied from report to report; the important thing is to suspect influenza infection and have a low threshold for testing in an immunocompromised host with atypical symptoms.

Fever

In a multicenter prospective study conducted in the USA, Canada, and Spain between 2010 and 2015 including 477 SOT and 139 HSCT recipients, 77 percent of the patients developed influenza A [10]. At the time of presentation, fever was absent in 36.2% in total; in SOT recipients around 60% had fever [10]. Fifty-one SOT recipients (including 24 kidney, 11 liver, 8 lung, 5 heart, and 3 combined organs) were identified in a multicenter prospective study in Spain of pandemic influenza A(H1N1) infection, and 94% of the recipients developed fever [11]. Fever lasted a median of 2 days [11]. In hospitalized patients, 80% SOT recipients similarly developed fever with pandemic A(H1N1) infection [13]. In summary, we cannot rule out influenza infection in SOT recipients due to absence of fever.

Lower Respiratory Tract Infection

Lower respiratory tract infection is commonly seen in this population, although the rate varies from study to study and year to year. In a multicenter prospective study of seasonal influenza enrolling 616 SOT and HSCT recipients, lower respiratory tract infection was documented in 22.1% (11.3–35.0% in year to year) [10]. During the A (H1N1) pandemic, lower respiratory tract infection was seen in 32% (73/230) patients in a multicenter study [9]. This study also indicated that the types of imaging abnormalities seen were predominantly either air space consolidation or interstitial infiltrate [9]. In an Australian cohort, lower respiratory tract infection occurred in more than 70% of a lung transplant population with influenza during the A(H1N1) pandemic [3].

In one prospective observational study, statistically significant factors to develop lower respiratory tract infection were older age and mycophenolate usage [10]. Preventive factors were same-season influenza vaccination, calcineurin inhibitor usage, and early antiviral therapy [10].

Only 6% of the recipients had bacterial pathogens identified in respiratory specimen at the time of diagnosis in a multicenter study [9]. Coinfection with other respiratory viruses was rare, and concomitant CMV infection was noted in 3% [9]. In a 5-year multicenter prospective study of seasonal influenza infection, bacterial and viral coinfections were noted in 25/134 (18.7%) and 15/134 (11.2%) of patients with lower respiratory tract infection recipients, respectively [10].

Graft Dysfunction

Especially in lung transplant recipients, we see graft dysfunction after influenza viral infection [11]; that is one of the reasons why it is important in SOT recipients to prevent influenza infection.

Clinical Presentation of Influenza in HSCT Recipients

As noted above, generally speaking, it is less likely to develop fever in immunocompromised hosts with influenza such as HSCT recipients. For seasonal influenza infection, a 5-year multicenter study of 139 HSCT recipients demonstrated that approximately 90% of the recipients developed cough and 30–50% of the recipients developed sore throat, rhinorrhea, headache, myalgias. or gastrointestinal symptoms [10]. In another multicenter prospective cohort study enrolling 289 HSCT recipients, cough was seen in 85% [14].

Fever

One multicenter retrospective cohort study from the UK collected 60 allogeneic HSCT patients with 2009 H1N1 in 2009 [15]. Of note, 35% of the patients did not develop fever [15]. In contrast, in a multicenter prospective cohort study from Europe enrolling 222 allogeneic and 64 autologous HSCT recipients, fever was seen in around 80% [14]. For seasonal influenza infection, fever was identified in 70% of 139 HSCT recipients [10]. In summary, more than 60% of HSCT recipients were febrile at the time of presentation [10, 14, 15].

Lower Respiratory Tract Infection

Several retrospective cohort studies from Jordan and Europe reported that approximately 20–35% of the patients developed lower respiratory disease [14–16]. One study showed that 15% of lower respiratory tract infection patients required ICU admission [15]. Risk factors for lower respiratory tract disease in hematological malignancy and HSCT recipients were a lymphocyte count of less than 200 cells/ml, lack or delay of influenza-directed therapy, and impaired kidney function [17]. A second study showed that older age and lymphocytopenia were independent risk factors for lower respiratory tract disease [14].

As documented above, symptoms may differ from the general population. The European Centre for Disease Prevention and Control provided a modified definition so that it could capture influenza like disease diagnoses earlier and easier [18]. Again, to detect influenza infection in immunocompromised hosts, a lower index of suspicion is necessary especially during influenza season, as they may lack typical symptoms.

Diagnosis

Currently, diagnosis is usually made with nucleic acid amplification testing. This technique is commercially available and has great specificity. Nasopharyngeal swab, wash, or aspirate specimens are widely used on initial presentation because they are easy to obtain. Bronchoalveolar lavage may sometimes provide a better specimen for diagnosis.

One multicenter prospective study showed that the duration of viral shedding was longer in an immunocompromised group [12]. The majority of participants in this study were hematologic malignancy patients [12].

A recent large multicenter prospective observational study of viral shedding in hematologic malignancy patients and SOT recipients showed that by day 3, 24% of all patients achieved viral load negativity and by day 6, more than half of all patients achieved viral load negativity of nasal swab specimens [10]. On the other hand, 25% of the patients at day 11 and 7.1% at day 18 still showed positivity [9]. However, in 4 weeks, no patients tested positive [10]. Interestingly, higher viral load at onset and delayed clearance were correlated with lower respiratory tract disease and ICU admission [10]. Also, this study showed that viral load was higher in the hematologic malignancy group as compared to the SOT group [10]. Although some reports showed resistance to oseltamivir [19, 20], oseltamivir should still be the first choice of treatment in immunocompromised patients. Also, the risk of resistance was correlated with immunosuppression and prior exposure to oseltamivir [21]. Resistance to neuraminidase inhibitors is uncommon in influenza A(H3N2) and influenza B viruses [21]. Guidelines recommend checking for antiviral resistance in recipients on appropriate antiviral therapy with prolonged viral shedding and/or prolonged symptomatic infection [21].

Treatment and Outcomes

Transplant populations are thought to be at higher risk after influenza infection. There are several mostly observational studies published. In some observational, retrospective, or prospective studies in either HSCT or SOT recipients, oseltamivir and zanamivir have been used for treatment [9, 10, 14, 15]. There are currently insufficient data about other antiviral therapies for influenza infection in the immunocompromised host.

The minimum recommended treatment duration is 5, days but therapy may be prolonged based on symptoms [21]. There are several observational studies of treatment duration. Median time of treatment duration in the biggest multicenter study for pandemic A(H1N1) in 2009 was 5 days [9]. This was also noted in another prospective study. The majority of the participants were treated with oseltamivir, either standard, high or low dose, with median of 5 days [10]. About 4% of the patients received more than 10 days [10]. Practically, some doctors recommend to repeat an influenza viral panel at the 5th day of the treatment in symptomatic

patients. If viral shedding is documented, some physicians prolong the therapy up to 10 days with either oseltamivir or zanamivir.

Adjunctive Therapy

Immunosuppression was reduced in 22% of patients in a multicenter study of pandemic A(H1N1) in 2009 [9]. This was also noted in another prospective observational multicenter study in which 17.4% of the patients had reductions in immunosuppressive medications for seasonal influenza [10]. In a multicenter study in Australia, 16/24 recipients received increased corticosteroid (prednisolone 1 mg/kg/day, at most 60 mg), and only 1/24 reduced immunosuppression for pandemic A (H1N1) influenza infection in lung transplant recipients [3]. Currently, there is no consensus about whether to reduce or increase immunosuppression when influenza is diagnosed though reduction of immunosuppressive medications is recommended in a current guideline for SOT recipients [21].

Risk Factors for Mortality and ICU Admission

In a multicenter study of pandemic A(H1N1) in 2009, even after treatment with oseltamivir, the mortality was 4% with death occurring a median of 15 (range 4–88) days after symptom onset [9]. In the other multicenter study for seasonal influenza infection in both HSCT and SOT recipients, death within 30 days after diagnosis was found in 2.9% [10]. In this multicenter study, authors did not perform risk factor analysis for mortality because the mortality was too low to perform the analysis. Vaccination and earlier initiation of antiviral agents were associated with reduced mortality especially in SOT recipients with pandemic A(H1N1) infection [9, 11]. One multicenter prospective observational study showed that neutropenia and older age were the risk factors for death of HSCT recipients with influenza virus infection [14].

Prophylaxis

Chemoprophylaxis

A randomized controlled trial showed approximately 80% efficacy with a good safety profile with oseltamivir prophylaxis in HSCT and SOT recipients [22]. Guidelines recommend postexposure prophylaxis in high-risk SOT patients such as lung transplant recipients, recipients receiving antirejection therapy, and recipients early post-transplant [21]. However, there is very little evidence regarding target population, duration, timing, prophylaxis medication, or dosage for either SOT or HSCT recipients currently.

Vaccination

Vaccination should be one of the best options to prevent influenza infection. However, the response to the vaccine is often insufficient or suboptimal in immunocompromised patients. Also, there is no study identifying a reduced incidence of influenza infection through vaccination though there are some data regarding immunogenicity after vaccination in this specific population. This section will discuss when, how, and what influenza vaccine should be used for SOT and HSCT recipients.

Influenza Vaccination in SOT Recipients

There have been many vaccine trials attempting to achieve better immunogenicity in the SOT population. The vaccine response improves with time after transplantation as the level of immunosuppression decreases; however influenza is a seasonal illness and might require vaccination at a specific time of year, based on the location where patients live. The American Society of Transplantation recommends initiating influenza vaccination as early as 3 months after transplantation in their current updated guideline [23]. In contrast, the Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommends starting 4 weeks after kidney transplantation [24]. There is no study which could determine the exact date to initiate influenza vaccination after SOT; this may even vary by organ transplanted. One report documented that with adjusting confounders, there was no correlation between time from transplant and response to influenza vaccination when compared from 1 month to 6 months after transplant and more than 6 months after transplant [25]. The limitation of this study is that one third of recipients received adjuvanted vaccine and the rest of the recipients received standard vaccine so the groups may not be comparable. In contrast, Baluch et al. documented that vaccine response was more likely to be seen in recipients more than 6 months post-transplant [26].

Two current guidelines state that there is no need to repeat influenza vaccination in the same season if the recipients received vaccination prior to transplant. [22, 23]. This may require further study. In summary, we may be able to initiate influenza vaccination 1 month after transplant, but the reaction to influenza vaccination might be suboptimal especially soon after transplant.

Vaccine Strategy

Previous reports documented insufficient immunogenicity with standard influenza vaccination. To overcome this issue, several methods have been tried to improve outcome.

Repeated Vaccination

There is a randomized controlled trial documenting this method in SOT recipients [27]. Cordero et al. published an open-label, phase 3 randomized controlled clinical trial evaluating the efficacy of repeated (5 weeks apart) single-dose standard vaccine, comparing to single standard vaccine administration in SOT recipients [27]. Two-dose vaccination was associated with a higher seroconversion rate only for A(H1N1) pandemic at 10 weeks, but not other included viral antigens, in the modified intention to treat analysis [27]. However, there was a statistically significant difference with per-protocol analysis, possibly because it was difficult for this population to get vaccinated twice [27]. Also, short-term postvaccination geometric mean titers were not significantly higher in the two-dose group than in the standard group [27]. At this point it appears that if recipients could receive the second dose, it might improve outcomes.

High-Dose Vaccination

GiaQuinta et al. conducted a randomized controlled trial enrolling 63 kidney and 16 liver transplant recipients to compare double-dose and standard-dose influenza vaccination [28]. An immune response was seen in 40% and 26% in double dose and standard dose, respectively ($p = 0.174$) [28]. In the double-dose arm, more patients developed seroprotection against all viral strains after vaccination (88% vs. 69%, $P = 0.048$) [28]. Natori et al. published a randomized controlled trial comparing high dose, which contained fourfold antigens and standard-dose influenza vaccine in SOT recipients [29]. They studied 84 high-dose and 77 standard-dose vaccine group patients [28]. Seroconversion to at least one vaccine containing antigen was statistically significantly higher in the high dose group (78.6% vs. 55.8%) [29]. Seroconversion to A(H1N1), A(H3N2), and B strains was also statistically significantly higher in the high-dose vaccine group (40.5% vs. 20.5%, 57.1% vs. 32.5%, and 58.3% vs. 41.6%, respectively) [29]. Postimmunization geometric mean titers of A(H1N1), A(H3N2), and B strains were also significantly higher in the high-dose group [29]. There was no statistically significant difference in side effects, but the sample size was too small to assess the rate of adverse events with this study [29].

Intradermal Versus Intramuscular

Manuel et al. reported that there was no statistically significant difference in immunogenicity between intradermal versus intramuscular vaccine administration in lung transplant recipients [30]. In this randomized controlled trial, patients received either 6 μg intradermally or 15 μg intramuscularly [30]. Forty-one patients received intradermal vaccine and 44 patients received intramuscular [30]. In the intradermal group, 14.6% of the recipients achieved response and 18.6% achieved in the

intramuscular group ($p = 0.77$) [30]. Seroprotection was statistically significantly higher in the intradermal group for H3N2 and B strains [30]. Morelon et al. reported higher seroconversion and seroprotection rates for influenza A(H1N1) and A(H3N2) but not influenza B in the intradermal group [31]. However, those were not statistically significant differences [31]. Baluch et al. compared high-dose intradermal vaccine against standard-dose intramuscular vaccine in a randomized controlled trial [26]. One hundred five recipients received intramuscular vaccine, and 107 recipient received intradermal [26]. Seroprotection to H1N1, H3N2, and B strains were not statistically significantly different between the groups [25]. Seroconversion to at least one antigen was also similar between groups (46.7% vs. 51.4%, $p = 0.49$) [26]. In summary, to date several randomized controlled trials found similar responses between intradermal and intramuscular influenza vaccination in SOT recipients.

Adjuvanted Vaccine

There are two adjuvanted vaccines available, which are MF59 and AS03. Kumar et al. conducted a randomized controlled trial comparing MF59-adjuvanted versus standard influenza vaccine for kidney transplant recipients [32]. Thirty-one recipients received adjuvanted vaccine, and 29 recipients received standard vaccine [32]. There were no statistically significant differences in seroconversion, seroprotection, and geometric mean titers seen between groups [32].

AS03-adjuvanted vaccine is generally used for pandemic A(H1N1) vaccine. One study enrolling only five recipients (one liver, two kidney, and two lung transplants) found only one recipient achieved seroprotection after vaccination. The number of patients was too small to conclude anything about vaccine performance [33].

Influenza Vaccination Side Effects in SOT Recipients

There have been some concerns with influenza vaccine causing rejection due to developing immunogenicity after vaccination. Some studies assessed HLA antibodies after immunization, and one of them showed that 11.9% of the recipients developed new anti-HLA antibodies.

A recently published systematic review and meta-analysis documented that there was no increased rate of rejection after vaccination when compared to the unvaccinated group [34]. In this systematic review, the incidence of de novo DSA was assessed in 14 studies and it was 1.85%, occurring between 21 and 94 days after vaccination [34]. The incidence of rejection was assessed in 83 studies and was 2.1%, presenting between 0.7 and 6 months after vaccination [34].

Also, there were no increases in HLA alloantibodies in patients who received adjuvanted vaccine [32]. In summary, there is high safety profile for influenza vaccine so far, and no statistically significant difference has been shown in the incidence of rejection in published data.

Vaccination in HSCT Recipients

Similar to the SOT population, there is no study indicating that vaccination will reduce the incidence of influenza infection in HSCT recipients, though one cross-sectional study documented that the rate of hospital admission may be reduced with influenza vaccination [35].

Timing of Vaccination

Recently, the European Conference on Infections in Leukemia (ECIL 7) updated their guideline for vaccination of HSCT recipients [36]. This guideline recommends one dose of seasonal vaccine annually at the beginning of influenza season [36]. Within 1 year after transplant, they also recommend to initiate vaccination 6 months after HSCT [36]. They also recommend an additional vaccine dose administered 3–4 weeks later in high-risk patients such as severe graft-versus-host disease patients or patients with low lymphocyte counts [36]. Even though there is insufficient evidence, they recommend vaccine initiation within 3 months after HSCT if there is a community outbreak.

Vaccine Strategy

Repeated Vaccination

One randomized trial compared immunogenicity between a regular influenza vaccination strategy of single dose versus two doses, 1 month apart [37]. Authors enrolled 65 patients whose transplant occurred more than 2 months previously [37]. The authors could not find any benefit with the two-dose vaccination strategy [37]. There were no documented side effects with repeated vaccination [37]. Again, current guidelines recommend repeated vaccination in the high-risk population.

High-Dose Vaccination

One study enrolled 44 recipients and 15 received standard dose and 29 received high dose. All recipients were vaccinated at least 6 months after transplantation [38]. More injection-site symptoms were noted in the high-dose group [38]. After vaccination, the high-dose group achieved a higher percentage of seroprotection and a higher geometric mean titer against the H3N2 strain [38]. Because of the small study population, it is very difficult to draw conclusions though high-dose vaccine might have some benefit [38].

Adjuvanted

Adjuvanted (MF59) versus standard influenza vaccine was tested by Natori et al. [39] Seroconversion to at least one of three influenza antigens was present in 62.9% versus 53.1% in adjuvanted versus non-adjuvanted vaccine, which was not statistically significant [39]. Factors associated with lower seroconversion rates with multivariate analysis were use of calcineurin inhibitors and shorter duration from transplantation [39]. AS03-adjuvanted vaccine was also studied by Engelhard et al. [40] Authors enrolled 55 allogeneic and 23 autologous HSCT recipients during the 2009 influenza pandemic season (21216315). Authors concluded that two doses of AS03-adjuvanted vaccination should be effective in this population [40]. Mohty et al. also compared 65 HSCT patients with AS03-adjuvanted vaccine group and 138 healthy control group, who received regular vaccine [41]. There was no difference between AS03-adjuvanted HSCT group and healthy controls with regular vaccine in that study although this is not a comparison between regular vaccine and adjuvanted vaccine in the same patient group [41].

Again, even with adjuvanted vaccine, published data could not show statistically significant differences between single-dose regular influenza vaccine and adjuvanted vaccine in HSCT recipients.

Donor Vaccination and Pre-HSCT Vaccination

One study conducted by Ambati et al. documented that pretransplant influenza vaccination could be beneficial even as compared to donor vaccination and no vaccination prior to transplant [42]. Authors concluded pre-HSCT vaccination could improve the seroprotection rate until day 180 after transplantation [42].

Conclusion

Influenza infection has significant morbidity and mortality in SOT and HSCT recipients. It is important to provide proper vaccination to these patients so to prevent infection. Also, when influenza virus infection is suspected in transplant recipients, antiviral medication should be initiated as soon as possible to optimize outcomes.

Key Points

- Influenza infection is commonly seen after transplantation.
- Symptoms of influenza infection may vary. It is important to suspect influenza with atypical presentations in the immunocompromised host.
- Fever may be absent in SOT or HSCT recipients with influenza infection.
- Nucleic acid amplification testing has become the main method of diagnosis.

- Oseltamivir and zanamivir are the main medications used to treat influenza virus infection in this population.
- Lower respiratory infection has been documented in 11.3–35% of transplant recipients with influenza; the incidence varies year to year.
- Lower respiratory tract infection, delayed initiation of antivirals, and infection early post-transplant are factors associated with poor outcomes in SOT and HSCT recipients.
- To prevent influenza infection, vaccination should be provided.
- Influenza vaccination may be administered as early as 1 month after transplant, but the immunologic response may be suboptimal early after transplant.
- In SOT recipients, either high-dose vaccine or repeated regular-dose vaccine should be recommended. In HSCT recipients, vaccination itself must be recommended, but it is still not known which type of inactivated influenza vaccine should be used.
- To achieve better outcomes, as soon as the diagnosis of influenza is suspected in SOT and HSCT recipients, neuraminidase inhibitor therapy should be initiated while waiting for the confirmatory test result.

References

1. Odongo FC, Azevedo LS, Neto ED, Yeh-Li H, Caiaffa H, Pierrotti LC. Clinical characteristics and outcomes of influenza A infection in kidney transplant recipients: a single-center experience. Transplant Proc. 2016;48(7):2315–8.
2. Manuel O, López-Medrano F, Keiser L, et al. Influenza and other respiratory virus infections in solid organ transplant recipients. Clin Microbiol Infect. 2014;20(Suppl 7):102–8.
3. Ng BJ, Glanville AR, Snell G, et al. The impact of pandemic influenza A H1N1 2009 on Australian lung transplant recipients. Am J Transplant. 2011;11(3):568–74.
4. Low CY, Kee T, Chan KP, et al. Pandemic (H1N1) 2009 infection in adult solid organ transplant recipients in Singapore. Transplantation. 2010;90(9):1016–21.
5. Le Page AK, Kainer G, Glanville AR, Tu E, Bhonagiri D, Rawlinson WD. Influenza B virus transmission in recipients of kidney and lung transplants from an infected donor. Transplantation. 2010;90(1):99–102.
6. Sim SA, Leung VKY, Ritchie D, Slavin MA, Sullivan SG, Teh BW. Viral respiratory tract infections in allogeneic hematopoietic stem cell transplantation recipients in the era of molecular testing. Biol Blood Marrow Transplant. 2018;24(7):1490–6.
7. Campbell AP, Guthrie KA, Englund JA, et al. Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. Clin Infect Dis. 2015;61(2):192–202.
8. Katzen J, Kohn R, Houk JL, Ison MG. Early oseltamivir after hospital admission is associated with shortened hospitalization: a five-year analysis of oseltamivir timing and clinical outcomes. Clin Infect Dis. 2019;69(1):52–58.
9. Kumar D, Michaels MG, Morris MI, et al. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. Lancet Infect Dis. 2010;10(8):521–6.
10. Kumar D, Ferreira VH, Blumberg E, et al. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67(9):1322–9.
11. Cordero E, Pérez-Romero P, Moreno A, et al. Pandemic influenza A(H1N1) virus infection in solid organ transplant recipients: impact of viral and non-viral co-infection. Clin Microbiol Infect. 2012;18(1):67–73.

12. Memoli MJ, Athota R, Reed S, et al. The natural history of influenza infection in the severely immunocompromised vs nonimmunocompromised hosts. Clin Infect Dis. 2014;58(2):214–24.
13. Minnema BJ, Patel M, Rotstein C, et al. Comparison of hospitalized solid organ transplant recipients and nonimmunocompromised patients with pandemic H1N1 infection: a retrospective cohort study. Transplantation. 2011;92(2):230–4.
14. Ljungman P, de la Camara R, Perez-Bercoff L, et al. Outcome of pandemic H1N1 infections in hematopoietic stem cell transplant recipients. Haematologica. 2011;96(8):1231–5.
15. Protheroe RE, Kirkland KE, Pearce RM, et al. The clinical features and outcome of 2009 H1N1 influenza infection in allo-SCT patients: a British Society of Blood and Marrow Transplantation study. Bone Marrow Transplant. 2012;47(1):88–94.
16. Rihani R, Hayajneh W, Sultan I, et al. Infections with the 2009 H1N1 influenza virus among hematopoietic SCT recipients: a single center experience. Bone Marrow Transplant. 2011;46(11):1430–6.
17. Paulsen GC, Danziger-Isakov L. Respiratory viral infections in solid organ and hematopoietic stem cell transplantation. Clin Chest Med. 2017;38(4):707–26.
18. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European conference on infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. Clin Infect Dis. 2013;56(2):258–66.
19. L'Huillier AG, Abed Y, Petty TJ, et al. E119D neuraminidase mutation conferring pan-resistance to neuraminidase inhibitors in an A(H1N1)pdm09 isolate from a stem-cell transplant recipient. J Infect Dis. 2015;212(11):1726–34.
20. Tamura D, DeBiasi RL, Okomo-Adhiambo M, et al. Emergence of multidrug-resistant influenza A(H1N1)pdm09 virus variants in an immunocompromised child treated with oseltamivir and zanamivir. J Infect Dis. 2015;212(8):1209–13.
21. Manuel O, Estabrook M, Practice ASoTIDCo. RNA respiratory viral infections in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13511.
22. Ison MG, Szakaly P, Shapira MY, Kriván G, Nist A, Dutkowski R. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. Antivir Ther. 2012;17(6):955–64.
23. Danziger-Isakov L, Kumar D, Practice AICo. Vaccination of solid organ transplant candidates and recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13563.
24. Group KDIGOKTW. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant. 2009;9(Suppl 3):S1–155.
25. Pérez-Romero P, Bulnes-Ramos A, Torre-Cisneros J, et al. Influenza vaccination during the first 6 months after solid organ transplantation is efficacious and safe. Clin Microbiol Infect. 2015;21(11):1040.e1011–48.
26. Baluch A, Humar A, Eurich D, et al. Randomized controlled trial of high-dose intradermal versus standard-dose intramuscular influenza vaccine in organ transplant recipients. Am J Transplant. 2013;13(4):1026–33.
27. Cordero E, Roca-Oporto C, Bulnes-Ramos A, et al. Two doses of inactivated influenza vaccine improve immune response in solid organ transplant recipients: results of TRANSGRIPE 1-2, a randomized controlled clinical trial. Clin Infect Dis. 2017;64(7):829–38.
28. GiaQuinta S, Michaels MG, McCullers JA, et al. Randomized, double-blind comparison of standard-dose vs. high-dose trivalent inactivated influenza vaccine in pediatric solid organ transplant patients. Pediatr Transplant. 2015;19(2):219–28.
29. Natori Y, Shiotsuka M, Slomovic J, et al. A double-blind, randomized trial of high-dose vs standard-dose influenza vaccine in adult solid-organ transplant recipients. Clin Infect Dis. 2018;66(11):1698–704.
30. Manuel O, Humar A, Berutto C, et al. Low-dose intradermal versus intramuscular trivalent inactivated seasonal influenza vaccine in lung transplant recipients. J Heart Lung Transplant. 2011;30(6):679–84.
31. Morelon E, Pouteil Noble C, Daoud S, et al. Immunogenicity and safety of intradermal influenza vaccination in renal transplant patients who were non-responders to conventional influenza vaccination. Vaccine. 2010;28(42):6885–90.

32. Kumar D, Campbell P, Hoschler K, et al. Randomized controlled trial of adjuvanted versus nonadjuvanted influenza vaccine in kidney transplant recipients. Transplantation. 2016;100(3): 662–9.
33. Resende MR, Husain S, Gubbay J, et al. Low seroconversion after one dose of AS03-adjuvanted H1N1 pandemic influenza vaccine in solid-organ transplant recipients. Can J Infect Dis Med Microbiol. 2013;24(1):e7–e10.
34. Mulley WR, Dendle C, Ling JEH, Knight SR. Does vaccination in solid-organ transplant recipients result in adverse immunologic sequelae? A systematic review and meta-analysis. J Heart Lung Transplant. 2018;37(7):844–52.
35. Piñana JL, Pérez A, Montoro J, et al. Clinical effectiveness of influenza vaccination after allogeneic hematopoietic stem cell transplantation: a cross-sectional prospective observational study. Clin Infect Dis. 2019;69(1):52–58.
36. Cordonnier C, Einarsdottir S, Cesaro S, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European conference on infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19:e200–12.
37. Karras NA, Weeres M, Sessions W, et al. A randomized trial of one versus two doses of influenza vaccine after allogeneic transplantation. Biol Blood Marrow Transplant. 2013;19(1): 109–16.
38. Halasa NB, Savani BN, Asokan I, et al. Randomized double-blind study of the safety and immunogenicity of standard-dose trivalent inactivated influenza vaccine versus high-dose trivalent inactivated influenza vaccine in adult hematopoietic stem cell transplantation patients. Biol Blood Marrow Transplant. 2016;22(3):528–35.
39. Natori Y, Humar A, Lipton J, et al. A pilot randomized trial of adjuvanted influenza vaccine in adult allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2017;52(7):1016–21.
40. Engelhard D, Zakay-Rones Z, Shapira MY, et al. The humoral immune response of hematopoietic stem cell transplantation recipients to AS03-adjuvanted A/California/7/2009 (H1N1)v-like virus vaccine during the 2009 pandemic. Vaccine. 2011;29(9):1777–82.
41. Mohty B, Bel M, Vukicevic M, et al. Graft-versus-host disease is the major determinant of humoral responses to the AS03-adjuvanted influenza A/09/H1N1 vaccine in allogeneic hematopoietic stem cell transplant recipients. Haematologica. 2011;96(6):896–904.
42. Ambati A, Boas LS, Ljungman P, et al. Evaluation of pretransplant influenza vaccination in hematopoietic SCT: a randomized prospective study. Bone Marrow Transplant. 2015;50(6): 858–64.

Respiratory Syncytial Virus in Transplant: Stopping the Short-Term Spread, Solving the Longer-Term Problems

30

A. J. Wright and A. Mah

Contents

Introduction 725
Disease Epidemiology 726
Clinical Impact in Transplant 728
Disease Diagnostics and Clinical Evaluation 729
Prevention 730
  Infection Control 730
  Vaccine 731
Treatment of Respiratory Syncytial Virus Infection 732
  Ribavirin 732
  Immunoglobulins 734
  New Treatments 735
Key Points 736
Conclusion 736
References 737

Introduction

Respiratory syncytial virus (RSV) is a nonsegmented RNA virus belonging to the family *Paramyxoviridae* with two major antigenic subgroups – A and B. It has a worldwide distribution and is a frequent cause of both upper and lower respiratory tract infections. It poses particularly high risk to patients who have undergone allogeneic hematopoeitic stem cell and lung transplantation. This review focuses on the most recent strategies aimed at preventing or treating RSV.

A. J. Wright (✉) · A. Mah
Division of Infectious Disease, University of British Columbia, Vancouver, BC, Canada
e-mail: alissa.wright@ubc.ca; allimah@mail.ubc.ca

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_31

Disease Epidemiology

Although the major morbidity and mortality occurs in children under 2 years of age, it has been increasingly recognized as a significant pathogen in adults with a disease burden similar to nonpandemic influenza A [1]. RSV typically occurs as annual or biennial seasonal epidemic in temperate climates, usually lasting from fall through spring [2, 3]. This is from October to May in the Northern Hemisphere and from May to September in the Southern Hemisphere. Disease in tropical climates can be more variable in onset and duration but often coincides with the rainy season [3, 4]. In the United States, the Centers for Disease Control and Prevention tracks RSV as part of the National Respiratory and Enteric Virus Surveillance System (NREVSS). Data is collected from participating laboratories around the country who submit information on the number of positive tests out of the total number of tests collected per week. Information on the current year can be found at https://www.cdc.gov/surveillance/nrevss/rsv/index.html. As detection methods have shifted to more sensitive molecular techniques such as polymerase chain reaction (PCR) testing, new statistical analysis has resulted in lengthening of the RSV season and changes in peak activity [2]. In addition, the availability of this technology has allowed documentation of nosocomial acquisition of infection and hospital outbreaks [5, 6].

RSV occurs in 3–7% of healthy elderly patients and is found in 1.3–9.2% of adults with a variety of medical conditions presenting to medical attention for acute respiratory tract infections [1, 7]. Within the immunocompromised patient population, the major groups at risk include hematological stem cell transplant recipients and lung transplant recipients. The periodic pattern of risk and onset of the season appears to match that of the general patient population [8]. RSV is estimated to affect 1–12% of hematopoietic stem cell transplant (HSCT) recipients and 2–16% of lung transplant recipients although incidence calculations vary depending on the cohort selected, season, testing method, and follow-up duration (see Table 1 for references and ranges). Some studies limit their patient population to hospitalized patients versus the entire cohort at risk. Studies that follow patients over several years result in the inclusion of patients with recurrent infections in different seasons, while studies confined to short time frames after transplant will usually isolate only the cases in a single season. Changes in testing methods to more sensitive techniques have led to increases in detection rates and higher rates over time [8]. In addition, outbreaks have been reported on high-risk units where vulnerable patients have prolonged stays such as the hematology-oncology ward [5, 6]. These can be challenging to manage, requiring intensified infection control precautions to break the cycle [9].

Incidence rates in other adult transplant groups are less well characterized. Given that children have the highest burden of disease overall, it is not surprising that there are multiple studies in pediatric solid organ transplant (SOT) and stem cell transplant recipients. Rates have varied depending on the group studied and year, but a recent review found that overall the incidence varied between 18 and 72 per 1000 children, with higher incidences in the first 2 years after HSCT or solid organ transplantation [10].

Table 1 RSV incidence in transplant populations

Reference	RSV Test	Location	Year	N	Incidence
HSCT					
Martino et al. [11]	DFA/ culture	Prospective cohort, single center (Spain)	1999–2003	172 allogeneic, 240 autologous	7.5% 2-year cumulative incidence (allo), 2.9% (auto)
Williams et al. [12]	DFA/ culture	Prospective cohort, single center (Spain)	1999–2004	198 allogeneic, 296 autologous	6.6% over 4 years (allo), 2.7% (auto)
Peck et al. [13]	PCR/ DFA/ culture	Prospective cohort, single center (Seattle, WA)	2000–2004	814 allogeneic and autologous transplants	5.8% cumulative incidence at day 100
Schiffer et al. [14]	DFA/ culture	Retrospective cohort, single center (Seattle, WA)	1997–2005	2,013 allogeneic, 751 autologous	1.4–2.1% cumulative incidence at day 100 (allo), 0.7% (auto)
Avetisyan et al. [15]	DFA/ culture	Retrospective cohort, single center (Sweden)	2000–2007	275 allogeneic	11.6% over 7 years
Nichols et al. [16]	Not specified	Retrospective cohort, single center (Seattle, WA)	1989–1999	3,897 total	4.5% overall attack rate, 10% during winter
Lung transplant					
Weinberg et al. [17]	PCR/ viral culture	Prospective cohort, single center (Denver, CO)	2005–2007	60	11.6%
Milstone et al. [18]	PCR/ serology/ viral culture	Prospective cohort, single center (Nashville, TN)	1999–2000	50	16%
Gottlieb et al. [19]	PCR/IF/ culture	Prospective cohort, single center (Germany)	2005–2006	388	1.8%
Pediatric					
Pohl et al. [20]	ELISA/ culture	Retrospective cohort, single center (Pittsburgh, PA)	1984–1991	493 liver recipients	3.7% over study period

(continued)

Table 1 (continued)

Reference	RSV Test	Location	Year	N	Incidence
Feldman et al. [21]	Not specified	Retrospective cohort, multiple centers (United States)	2004–2012	2554 liver recipients	5.3% of hospitalizations over study period
Rowan et al. [22]	PCR/ DFA/ culture	Retrospective cohort, multiple centers (United States)	2010–2014	1522 HSCT, both allogeneic and autologous	3.1% over study period
Hutspardol et al. [23]	Culture/ DFA/ PCR	Retrospective cohort, single center (Toronto, Ontario)	2000–2012	844 HSCT, both allogeneic and autologous	1.8% cumulative incidence at day 100

Clinical Impact in Transplant

The impact of RSV in the general inpatient population rivals that of influenza including the need for care in the intensive care unit (ICU), mechanical ventilation, and mortality [1]. Among the transplant population, there is a significant risk of morbidity and mortality. In both HSCT and SOT recipients, RSV infection can lead to the need for mechanical ventilation and ICU admission. In one study, 15% and 7% of HSCT recipients needed ICU admission and mechanical ventilation, respectively, compared with 18% and 14% of SOT recipients [8]. Overall, 5% and 11% of HSCT and SOT recipients died in that study. In another multicenter study, 67% and 83% of allogeneic HSCT and SOT recipients required hospital admission, respectively, and 17% and 10% required mechanical ventilation [24]. Sixty-day mortality was 8.3% and 13.3%, respectively. Not every paper has been consistent in these findings. A 2010 series from Switzerland reported a low number of complications in their lung transplant cohort despite the fact that patients were not specifically treated [25]. Conversely, higher mortality rates have been found in some HSCT cohorts with lower respiratory tract disease. In one study, 30- and 90-day mortality rates were 39% and 47% following the diagnosis of RSV [26].

Beyond ICU admission and death, RSV can either lead to secondary bacterial pneumonia or present along with bacterial, viral, or fungal coinfection. In the Andersen study, 52% of their transplant patients (both HSCT and SOT) received bacterial or fungal therapy following their RSV diagnosis although only 42% of those patients had microbiologically documented infection [24]. Unique to lung transplant recipients, RSV infection can lead to the development of chronic lung allograft dysfunction (CLAD) which is a leading cause of death in this patient population [27]. Avoidance of all community-associated respiratory viruses is

critical in this patient population, and RSV is particularly feared due to the severe inflammatory reaction induced by the patient's own immune system that is part of its pathogenesis [28]. In the HSCT patient population, early infection posttransplant has a high risk of progressing to lower tract disease and death [29]. As a result, patients with pretransplant RSV infection are in a difficult situation; delay in conditioning poses a risk of relapse, but continuing with therapy may result in RSV lower tract infection and death.

Disease Diagnostics and Clinical Evaluation

Detection of RSV can occur by viral culture, serology, direct fluorescent antibody (DFA) and immunofluorescent antibody assays, rapid antigen testing, and nucleic acid amplification tests (NAAT). Viral culture was once considered the gold standard but has now given way to more rapid, sensitive molecular tests [30]. Similarly, serological diagnosis relies on collection of both acute and convalescent specimens making it more of a historical or research method rather than a tool for diagnosis and management of individual patients [28]. There was a lot of interest in antigen assays, particularly because they did not necessarily require a laboratory and could be used for point-of-care testing with rapid turnaround times. However, antigen assays perform poorly in adults or during periods when the disease incidence is low; they are more reliable in pediatric populations [31]. As a result, molecular methods, particularly single and multiplex PCRs, have now become the new gold standard for respiratory viral detection. In the case of multiplex assays, they have the potential to detect coinfections. Moreover, certain molecular assays have been developed to function as point-of-care tests.

However, all diagnostic testing has limitations. Molecular assays may be commercially available or laboratory-developed with the potential for small variations in test characteristics between assays. In general, most molecular tests have a high sensitivity and negative predictive value. Nevertheless, they are not impervious to false negatives which can occur either from error during collection, transport, or processing of the specimen or from mutations in the virus [32]. In addition, site and collection method may impact whether the virus can be detected. Nasopharyngeal aspirates were the traditional gold standard, but depending on the patient population, this may not be feasible [30]. For example, nasal washes may be used if there is concern about bleeding; however, there can be difficulty in obtaining a good specimen with this technique. Patients with lower tract disease may have negative tests in their upper tract. Bronchoscopy with lavage may be necessary to obtain a specimen for testing. In addition, positive tests can also be difficult to interpret with respect to ongoing active infection and risk of transmission. Immunocompromised patients can have detectable PCR results for many weeks after acute infection, often longer than culture positivity [33]. On average, patients with hematological malignancies or solid organ transplants can have detectable virus for 2–4 weeks after acute infection [33–35]. Although there is interest in using the viral load and its relationship to severity of

illness or risk of transmission, there is no consistent data yet on how to use this clinically [30].

Transplant patients presenting with symptoms of a respiratory tract infection should be evaluated for RSV, particularly during the appropriate epidemiological season. Initial signs can be subtle and may be mistaken for symptoms of mucositis, anemia or deconditioning (dyspnea), or allergy. Asymptomatic carriage is also possible [6]. In cases of outbreaks, routine, periodic surveillance of the entire cohort may be necessary to terminate the outbreak in order to pick up these asymptomatic individuals.

Testing should include some form of sensitive viral assay – preferably a molecular test – as well as evaluation for lower tract disease as this has prognostic implications. Lower respiratory tract infection has a higher risk of complications including mortality [36]. Risk factors for progression to lower tract infection and poor outcome in HSCT patients include smoking, neutropenia, lymphopenia (especially <100 cells/mm^3), age >40, recent transplant, use of a myeloablative conditioning regimen, use of high-dose total body irradiation as conditioning, acute or chronic GVHD, and corticosteroid intake in 30 days before infection [37, 38]. Higher lymphocyte counts appear to be protective. A subset of these factors have been used to stratify patients for treatment (immunodeficiency scoring index) at one center, although validation of this still needs to be performed [37]. Detection of lower tract infection should include lower respiratory tract imaging and/or sampling. Chest X-ray is less sensitive, and patients may need computed tomography to detect disease [34]. Patients should also be evaluated for co-pathogens. Damaged lung tissue is susceptible to secondary bacterial infection although co-pathogens have been reported to be bacterial, viral, or fungal. Careful evaluation for these pathogens should be performed; however, more patients end up on antibacterial or antifungal therapy than have documented coinfections, so judicious use to avoid unnecessary therapy is recommended [24].

Prevention

Infection Control

Given the severity of disease and associated sequelae of RSV infection in HSCT and SOT recipients, and the relative lack of currently available therapeutic options, modalities to prevent disease are an essential component to the management of this infection in vulnerable patient populations. Due to the risk of spread, patients must be isolated ideally as soon as infection is suspected while workup is still pending. At minimum, patients should be placed in contact isolation [39]. National recommendations vary with both droplet and contact isolation precautions recommended by the Public Health Agency of Canada (PHAC). Institutions may want to implement more stringent droplet precautions given it can be transmitted by the respiratory route or even airborne precautions for ventilated patients [6]. Ideally, immunocompromised patients should not be cohorted together, and even patient and visitor activities may

need to be limited during outbreaks to prevent spread [9]. Transplant units should employ several strategies to prevent the acquisition of respiratory viral infections. It is recommended that good hand and respiratory hygiene be strongly encouraged, all visitors and healthcare providers with signs of respiratory illness be restricted from the patient environment, and young children be prevented from visiting transplant wards due to the high risk of respiratory viral transmission [40].

The duration of isolation precautions is unknown given the propensity for prolonged shedding. In immunocompromised hosts, prolonged respiratory viral shedding can occur on average for 2–4 weeks, but it has been reported for as long as 162 days [41]. Prolonged shedding potentially extends the period of infectivity and increases the opportunity for disease transmission. Some centers will require a negative repeat test as there is a potential continued risk of transmission as long as patients have detectable virus. However, it is clear that the sensitivity of the newer molecular tests may result in very long isolation requirements, and this can become very burdensome to manage during outbreaks when bed space is limited. Based on the 1998 guidelines, healthcare workers with febrile acute respiratory infections should be excluded from caring for high-risk patients [42]; an update is forthcoming. However, transmission is more likely to occur from immunocompetent healthcare workers with asymptomatic shedding, which has been found in some outbreak investigations [5]. For this reason, many hospital units caring for this vulnerable patient population have instituted the use of universal surgical masks in all individuals with direct contact with patients. Studies evaluating this intervention have demonstrated a reduction in respiratory viral infections as well as deaths attributable to respiratory viral infections in the post-implementation period [43, 44].

Experience with palivizumab for prevention of RSV in at-risk children has led some centers to utilize palivizumab in high-risk patients in an attempt to prevent transmission during outbreaks of RSV [45]. However, data are lacking to establish the efficacy and moreover the cost-effectiveness of this approach. When analyzed in pediatric populations undergoing HSCT, palivizumab has not been clearly shown to be effective in the prevention of RSV infection; furthermore, restrictive strategies for palivizumab use have not been shown to lead to worse clinical outcomes [46].

Vaccine

Although an area of active research, no vaccines against RSV are currently available. Natural infection does not confer long-lasting immunity to RSV, and repeated infections over a lifetime are common [47]. Therefore, much of vaccine research is focused on finding the appropriate targets which will confer a more durable immune response and protection. Initial RSV vaccine research was hampered by concerns regarding immune enhancement following a trial in the 1960s of a formalin-inactivated candidate vaccine which caused increased disease severity in vaccine recipients with a hospitalization rate of 80% and two deaths [48]. Much of the current vaccine research targeting RSV is focused on vaccination of pregnant women with a hope of conferring immunity via antibody transfer to neonates who are at

greatest risk for the infection [49]. The highest risk period for deleterious outcomes from RSV infection in immunocompromised hosts is during the period immediately post-stem cell transplant [37]. Vaccination strategies during this period are unlikely to provide protective benefit. However, vaccination could be an important adjunct measure in the prevention of RSV infection if it was able to effectively prevent infection in close contacts, healthcare workers, and family members of vulnerable patients. Vaccination of stem cell donors is also a possible consideration to try and enhance immunity prior to graft collection.

Vaccination may be a strategy to prevent infection in lung transplant recipients, who are the other high-risk group among transplant recipients. Likely candidate vaccines being investigated would target the F protein on the virus surface administered with an adjuvant [48]. As with any adjuvant vaccine in the posttransplant setting, studies would be required to ensure that enhanced immune stimulation did not result in an increased risk of allo-sensitization and graft rejection. Additional important end points for vaccine effectiveness in the lung transplant population would be the impact of vaccination on rates and progression of BOS.

Treatment of Respiratory Syncytial Virus Infection

Ribavirin

Ribavirin is a nucleoside analogue with in vitro activity against RSV. It can be administered orally, intravenously, or via aerosol. Practice guidelines recommend the use of ribavirin and/or immunoglobulin in the treatment of RSV infection in immunocompromised individuals [50]. However, there is no consensus as to what constitutes best practice, and surveys of transplant infectious disease clinicians demonstrate a wide range of approaches [40, 51, 52].

Several retrospective studies and case series have suggested that ribavirin therapy may be beneficial in HSCT and lung transplant recipients [36, 41, 53]. Unfortunately, the only randomized controlled study to assess the efficacy of ribavirin therapy for the treatment of RSV in HSCT transplant recipients was terminated due to poor patient accrual and consequently showed no treatment difference between the ribavirin and control arms [54]. In a review of the literature for ribavirin treatment in HSCT recipients with RSV infection, a pooled analysis of study subjects found decreased progression to lower respiratory tract infection (LRTI) and decreased mortality among those treated with any form of ribavirin compared to those not treated [36]. Studies of ribavirin therapy for RSV in lung transplant recipients are generally limited by their lack of comparator group. One single center study which looked at outcomes from a retrospective analysis of paramyxovirus infection in lung transplant recipients demonstrated a reduction in development of bronchiolitis obliterans syndrome in the cohort who received oral ribavirin compared to patients who could not receive antiviral therapy due to contraindications [55].

Aerosolized ribavirin can be either given as a continuous regimen of 6 g over 18 h per day or split into doses of 2 g administered over 2–3 h every 8 h. Both treatment

schedules have been demonstrated to be equally effective at prevention of progression from upper respiratory tract infection (URTI) to LRTI [56]. The largest retrospective study of aerosolized ribavirin for treatment of RSV in HSCT recipients demonstrated that administration of aerosolized ribavirin at the URTI stage was a protective factor in preventing progression to LRTI and mortality [57]. Looking specifically at the effect of aerosolized ribavirin in HSCT patients receiving treatment at the LRTI stage, investigators found that treatment with aerosolized ribavirin was protective against mortality [26]. At least two subsequent retrospective studies in similar patient populations failed to demonstrate a benefit from aerosolized or oral ribavirin although the authors postulate that this may be related to smaller numbers and selection bias [38, 58]. Initial experience in treatment of RSV in transplant recipients utilized aerosolized ribavirin to minimize systemic toxicity. However, this method of administration is costly with recent prohibitively expensive increases in drug costs in recent years. Aerosolized ribavirin also has risks of pulmonary side effects such as bronchospasm and requires special precautions during administration which may interfere with other treatments and patient assessment. This method of administration also raises concerns regarding pregnant healthcare worker exposure and teratogenicity.

Administration of oral ribavirin is an alternative option for treatment of infection with RSV and has been shown to have similar rates of progression to LRTI and similar mortality to treatment with aerosolized ribavirin in HSCT [59]. Similarly, in a small study of lung transplant recipients, treatment with oral ribavirin did not differ from treatment with inhaled ribavirin for the outcomes of mortality or progression of bronchiolitis obliterans syndrome (BOS) although treatment choice was based on clinician preference [60]. It can be dosed in several different ways – as a fixed dose of 600 –800 mg every 8–12 h or as a loading dose of 10 mg/kg and then 20 mg/kg/day divided into three doses [59]. The bioavailability is 64% [61]. The main concern with oral ribavirin is the risk of reversible hemolytic anemia although there are additional concerns of absorption in HSCT patients with GVHD or mucositis. Despite this concern, the risk of hemolytic anemia in recent reports is low, occurring in between 1% and 5% of treated patients [62, 63]. This is rarely a treatment-limiting side effect with the average drop in hemoglobin in one study being 5 g/L. [64]

Intravenous ribavirin is available in Europe but not in North America outside of a limited supply for virologic emergencies such as Lassa fever. There are few studies of this mode of administration. Intravenous delivery overcomes the issue of absorption of the oral formulation and also penetration with the aerosolized formulation when there is significant pulmonary consolidation. A few small case series have shown intravenous ribavirin for the treatment of RSV to be relatively well tolerated with only mild hemolytic anemia reported in one study [65, 66].

Despite multiple studies examining the impact of ribavirin for the treatment of RSV infection, how best to use this treatment in vulnerable patient groups remains unclear. The lack of a prospective, randomized controlled trial definitely demonstrating efficacy and the concerns regarding cost, side effects, and logistics of administration, particularly with the aerosolized formulation, are all important considerations. This has led some authors to propose a risk stratification tool

(immunodeficiency scoring index) to decide which allogeneic HSCT patients may best benefit from ribavirin therapy, with therapy reserved for only those with high-risk scores who are at greatest risk for poor outcome [37]. The immunodeficiency scoring index considers patient characteristics including absolute neutrophil and lymphocyte counts, patient age, conditioning regimen, graft-versus-host disease, and engraftment status to assign a weighted score. Treatment stratification based on risk score demonstrated little treatment effect for those with low or moderate risk scores but a significant reduction in mortality in the high-risk group with a mortality rate of 63% in the untreated group as compared to 8% in the treated group [37]. This scoring system awaits further validation. There is no equivalent scoring system for autologous HSCT recipients and even less data in other hematological malignancies such as acute leukemia that might be at risk of poor outcome. Currently, there is center-to-center variation in practice. Similarly, outside of lung transplant where ribavirin may be used at certain centers, there is almost no data on ribavirin use in other solid organ transplant groups. Duration of therapy is also unknown as there may be differences between clinical resolution of symptoms and negative laboratory studies, particularly as testing becomes more sensitive. Both have been used as end points for therapy. Ideally, as research continues into new treatment options for RSV, these questions about ribavirin therapy may no longer need to be answered.

Immunoglobulins

Palivizumab is a humanized monoclonal antibody to the RSV fusion (F) glycoprotein. It is approved for use as prophylaxis in high-risk children during peak RSV season based on the IMpact-RSV study which demonstrated a 55% relative risk reduction for hospitalization due to RSV in infants who received prophylactic palivizumab compared to infants who did not [67]. Convincing data of the utility of palivizumab for treatment of established RSV infection however is lacking. In the transplant population, data has been limited to two small phase I studies, a few retrospective reviews, and individual case reports [16, 68]. Although it appears safe, the largest review of 40 HSCT patients did not show a difference in outcome [69]. Intravenous immunoglobulin (IVIG) is another therapy that has been proposed for treatment of respiratory viral infection for both direct antiviral activity and immunomodulatory benefits. A dose of 500 mg/kg of IVIG is commonly given by centers with variation in the number of repeats and interval of dosing. At our center, we administer a single dose, with others reporting as many as seven doses administered every other day [57]. A pooled analysis of RSV treatment in HSCT recipient found lower mortality in individuals who received a combination of aerosolized ribavirin and immunoglobulin compared to aerosolized ribavirin alone [36]. Guidelines support the use of IVIG in the management of HSCT recipients with RSV infection and specifically recommend against the use of palivizumab in this patient population [40].

Studies of immunoglobulin therapy for RSV in lung transplant recipients are limited. Similar to the concerns regarding studies of ribavirin therapy, studies of immunoglobulin therapy involve multiple components to the treatment regimen and

lack a control group. For example, 1 study of 23 cases of RSV in single-, double-, or heart-lung transplant recipients treated with aerosolized ribavirin, immunoglobulin, corticosteroids, and palivizumab demonstrated stable forced expiratory volume in 1 s at treatment follow-up. Although this was reported as being an effective therapy, all patients received all four interventions, and there was no control group for comparison [53]. Based on data from HSCT populations, American guideline recommendations are for consideration of ribavirin and IVIG plus/or minus corticosteroids in lung transplant recipients with severe infection due to RSV [50]. Despite the lack of evidence, this recommendation extends to other SOT groups with severe infection.

New Treatments

New treatments for RSV infection are needed. Several candidate therapies are at various stages of investigation. The major targets of drug development include nucleoside analogues and non-nucleoside inhibitors of RNA-dependent RNA-polymerase, inhibition of viral attachment and fusion, and inhibition of messenger RNA (mRNA) signaling (see Fig. 1).

ALN-RSV01 is a small interfering RNA (siRNA) which inhibits mRNA signaling, blocking nucleocapsid production. In a phase 2b study of aerosolized ALN-

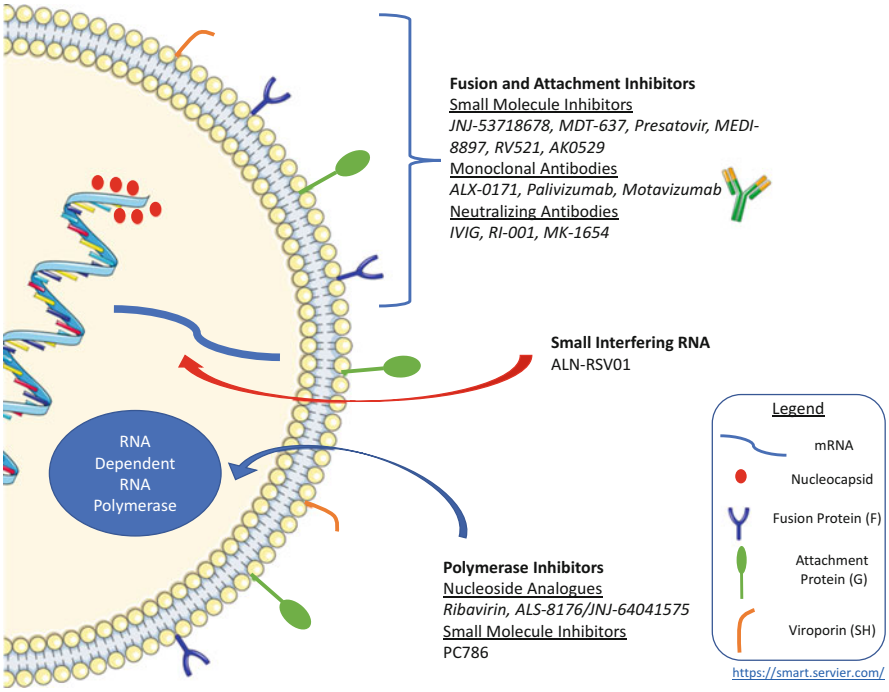


Fig. 1 Current and investigational therapies for RSV

RSV01 compared to placebo in lung transplant recipients, there was a trend toward a decrease in the development of BOS in the treatment arm which was significant in the per protocol analysis [70].

Several candidate fusion inhibitors have been explored including MDT-637, JNJ-53718678, MEDI-8897, ALX-0171, AK0529, RV521, and presatovir (GS-5806). Presatovir showed promising results in viral challenge studies in healthy volunteers. However, two recently completed phase 2b, randomized, double-blind, placebo-controlled trials of presatovir in HSCT recipients with URTI and LRTI, respectively, failed to show a reduction in respiratory failure or mortality [71, 72]. There was a trend in the URTI study for presatovir to decrease progression to LRTI. More data from these trials are coming as reports are currently limited to abstract form. In a healthy volunteer challenge study, JNJ-53718678 demonstrated reduction in RSV viral load and symptom scores compared to placebo [73]. Further clinical trials are required to establish the utility of these treatments.

ALS-8176 and ALS-8112 are nucleoside analogues with activity against RSV in vitro. Data on their treatment efficacy in hospitalized patients with RSV infection is not yet available. PC786 is a small-molecule inhibitor of viral RNA replication which is also pending further study [74].

Monoclonal antibodies against the viral F protein include palivizumab and motavizumab. Both have been studied as prophylaxis to prevent RSV infection in at-risk infants. Neither is routinely recommended for treatment of established RSV infection. Based on the efficacy of targeted monoclonal antibodies for prevention of RSV infection, researchers have investigated the possibility of an inhaled mRNA preparation which would stimulate endogenous production of anti-F antibodies and have shown this to be effective in transfected mouse models [75]. Additional neutralizing antibodies that have been assessed include RI-001 and MK-1654. More data may be coming from these molecules.

Key Points

- RSV is a common respiratory viral infection with significant morbidity and mortality in the HSCT and lung transplant populations.
- Improved diagnostics have led to better understanding of the epidemiology of this infection.
- Antiviral therapy is currently limited to ribavirin and IVIG, with support for their use primarily from observational studies of retrospective cohorts.
- New treatments are needed, and there are several candidates under evaluation.

Conclusion

RSV is an RNA virus infection with significant morbidity and mortality – particularly for HSCT and lung transplant recipients. Despite improvements in detection of this virus in recent years, there is still limited evidence for therapy. Ribavirin remains

an option, particularly for patients with severe infection: however, conclusive evidence of its efficacy is lacking. Immunoglobulin replacement is often used as an adjunctive measure. Ultimately, the best intervention is still prevention, which includes good respiratory hygiene and infection control measures to prevent nosocomial transmission from occurring. Research is still needed to find novel solutions to manage this common pathogen, including vaccination and new targeted therapies of which studies are ongoing.

References

1. Falsey AR, Hennessey P, Formica M, et al. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med. 2005;352:1749–59.
2. Rose EB, Wheatley A, Langley G, et al. Respiratory syncytial virus seasonality – United States, 2014–2017. Morb Mortal Wkly Rep. 2018;67:71–6.
3. Bloom-feshbach K, Alonso WJ, Charu V, et al. Latitudinal variations in seasonal activity of influenza and respiratory syncytial virus (RSV): a global comparative review. PLoS One. 2013;8:e54445.
4. Weber M, Muholland E, Greenwood B. Respiratory syncytial virus infection in tropical and developing countries. Tropical Med Int Health. 1998;3:268–80.
5. Kelly SG, Mph KM, Bolon MK, et al. Respiratory syncytial virus outbreak on an adult stem cell transplant unit. Am J Infect Control. 2016;44:1022–6.
6. Jensen TO, Stelzer-braid S, Willenborg C, et al. Outbreak of respiratory syncytial virus (RSV) infection in immunocompromised adults on a hematology ward. J Med Virol. 2016;88:1827–31.
7. Colosia AD, Yang J, Hillson E, et al. The epidemiology of medically attended respiratory syncytial virus in older adults in the United States: a systematic review. PLoS One. 2017;12: e0182321.
8. Pilie P, Werbel WA, Iv JR, et al. Adult patients with respiratory syncytial virus infection: impact of solid organ and hematopoietic stem cell transplantation on outcomes. Transpl Infect Dis. 2015;17:551–7.
9. RSV Outbreak Investigation Team. Contributing and terminating factors of a large RSV outbreak in an adult hematology and transplant unit. PLos Curr Outbreaks. 2014;1.
10. Manzoni P, Figueras-aloy J, Simo EAF, et al. Defining the incidence and associated morbidity and mortality of severe respiratory syncytial virus infection among children with chronic diseases. Infect Dis Ther. 2017;6:383–411.
11. Martino R, Porras RP, Rabella N, et al. Prospective study of the incidence, clinical features, and outcome of symptomatic upper and lower respiratory tract infections by respiratory viruses in adult recipients of hematopoietic stem cell transplants for hematologic malignancies. Biol Blood Marrow Transplant. 2005;11:781–96.
12. Williams JV, Martino R, Rabella N, et al. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. J Infect Dis. 2005;192:1061–5.
13. Peck AJ, Englund JA, Kuypers J, et al. Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. Blood. 2007;110:1681–8.
14. Schiffer JT, Kirby K, Sandmaier B, et al. Timing and severity of community acquired respiratory virus infections after myeloablative versus non-myeloablative hematopoietic stem cell transplantation. Haematologica. 2009;94:1101–8.

15. Avetisyan G, Mattsson J, Sparrelid E, et al. Respiratory syncytial virus infection in recipients of allogeneic stem-cell transplantation: a retrospective study of the incidence, clinical features, and outcome. Transplantation. 2009;88:1222–6.
16. Nichols WG, Gooley T, Boeckh M. Community-acquired respiratory syncytial virus and parainfluenza virus infections after hematopoietic stem cell transplantation: the Fred Hutchinson Cancer Research Center Experience. Biol Blood Marrow Transplant. 2001;7:11–5.
17. Weinberg A, Lyu DM, Li S, et al. Incidence and morbidity of human metapneumovirus and other community-acquired respiratory viruses in lung transplant recipients. Transpl Infect Dis. 2010;12:330–5.
18. Milstone AP, Brumble LM, Barnes J, et al. A single-season prospective study of respiratory viral infections in lung transplant recipients. Eur Respir J. 2006;28:131–7.
19. Gottlieb J, Schulz TF, Welte T, et al. Community-acquired respiratory viral infections in lung transplant recipients: a single season cohort study. Transplantation. 2009;87:1530–7.
20. Pohl C, Green M, Wald ER, et al. Respiratory syncytial virus infections in pediatric liver transplant recipients. J Infect Dis. 1992;165:166–9.
21. Feldman A, Sundaram S, Beaty B, et al. Hospitalizations for respiratory syncytial virus and vaccine-preventable infections in the first 2 years after pediatric liver transplant. J Pediatr. 2012;182:232–8.
22. Rowan CM, Gertz SJ, Zinter MS, et al. A multicenter investigation of respiratory syncytial viral infection in children with hematopoietic cell transplantation. Transpl Infect Dis. 2018;20: e12882.
23. Hutspardol S, Essa M, Richardson S, et al. Significant transplantation-related mortality from respiratory virus infections within the first one hundred days in children after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015;21:1802–7.
24. Anderson NW, Binnicker MJ, Harris DM, et al. Morbidity and mortality among patients with respiratory syncytial virus infection: a 2-year retrospective review. Diagn Microbiol Infect Dis. 2016;85:367–71.
25. Uckay I, Gasche-Soccal P, Kaiser L, et al. Low incidence of severe respiratory syncytial virus infections in lung transplant recipients despite the absence of specific therapy. J Heart Lung Transplant. 2010;29:299–305.
26. Waghmare A, Campbell AP, Xie H, et al. Respiratory syncytial virus lower respiratory disease in hematopoietic cell transplant recipients: viral RNA detection in blood, antiviral treatment, and clinical outcomes. Clin Infect Dis. 2013;57:1731–41.
27. Fisher CE, Preiksaitis CM, Lease ED, et al. Symptomatic respiratory virus infection and chronic lung allograft dysfunction. Clin Infect Dis. 2016;62:313–9.
28. Griffiths C, Drews SJ, Marchant DJ. Respiratory syncytial virus: infection, detection, and new options for prevention and treatment. Clin Microbiol Rev. 2017;30:277–319.
29. Peck AJ, Corey L, Boeckh M. Pretransplantation respiratory syncytial virus infection: impact of a strategy to delay transplantation. Clin Infect Dis. 2004;39:673–80.
30. Charlton C, Babady E, Ginocchio C, et al. Practical guidance for clinical microbiology laboratories: viruses causing acute respiratory tract infections. Clin Microbiol Rev. 2019;32:1–49.
31. Casiano-colon AE, Hulbert BB, Mayer TK, et al. Lack of sensitivity of rapid antigen tests for the diagnosis of respiratory syncytial virus infection in adults. J Clin Virol. 2003;28:169–74.
32. Hawkinson D, Abhyankar S, Aljitawi O, et al. Delayed RSV diagnosis in a stem cell transplant population due to mutations that result in negative polymerase chain reaction. Diagn Microbiol Infect Dis. 2013;75:426–30.
33. Richardson L, Brite J, Del Castillo M, et al. Comparison of respiratory virus shedding by conventional and molecular testing methods in patients with haematological malignancy. Clin Microbiol Infect. 2016;22:380.e1–7.
34. Ariza-Heredia EJ, Fishman JE, Cleary T, et al. Clinical and radiological features of respiratory syncytial virus in solid organ transplant recipients: a single-center experience. Transpl Infect Dis. 2012;14:64–71.

35. Geis S, Prifert C, Weissbrich B, et al. Molecular characterization of a respiratory syncytial virus outbreak in a hematology unit in Heidelberg, Germany. J Clin Microbiol. 2013;51:155–62.
36. Shah JN, Chemaly RF. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. Blood. 2011;117:2755–64.
37. Shah DP, Ghantoji SS, Ariza-heredia EJ, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. Blood. 2014;123:3263–9.
38. Kim Y, Guthrie KA, Waghmare A, et al. Respiratory syncytial virus in hematopoietic cell transplant recipients: factors determining progression to lower respiratory tract disease. J Infect Dis. 2014;209:1195–204.
39. Siegel JD, Rhinehart E, Cic RNMPH, et al. Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. Atlanta: Center for Disease Control; 2007.
40. Hirsch HH, Martino R, Ward KN, et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfl uenza virus, metapneumovirus, rhinovirus, and coronavirus. Clin Infect Dis. 2013;56:258–66.
41. Lehners N, Schnitzler P, Geis S, et al. Risk factors and containment of respiratory syncytial virus outbreak in a hematology and transplant unit. Bone Marrow Transplant. 2013;48:1548–53.
42. Bolyard EA, Tablan OC, Williams WW, et al. Guideline for infection control in healthcare personnel. Infect Control Hosp Epidemiol. 1998;19:407–63.
43. Sokol KA, De I, Sara VKE, et al. Masks for prevention of respiratory viruses on the BMT unit: results of a quality initiative. Transpl Infect Dis. 2016;18:965–7.
44. Sung AD, Sung JAM, Thomas S, et al. Universal mask usage for reduction of respiratory viral infections after stem cell transplant: a prospective trial. Clin Infect Dis. 2016;63:999–1006.
45. Kassis C, Champlin RE, Hachem RY, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. Biol Blood Marrow Transplant. 2010;16:1265–71.
46. Teusink-cross A, Davies SM, Danziger-isakov L, et al. Restrictive palivizumab use does not lead to increased morbidity and mortality in pediatric hematopoietic stem cell transplantation patients. Biol Blood Marrow Transplant. 2016;22:1904–6.
47. Borchers AT, Chang C, Gershwin ME, et al. Respiratory syncytial virus – a comprehensive review. Clin Rev Allergy Immunol. 2013;45:331–79.
48. Graham BS. ScienceDirect vaccine development for respiratory syncytial virus. Curr Opin Virol. 2017;23:107–12.
49. August A, Glenn GM, Kpamegan E, et al. Trial of aluminum-adjuvanted respiratory syncytial virus F particle vaccine formulations in healthy women of childbearing age. Vaccine. 2017;35:3749–59.
50. Manuel O, Estabrook M. RNA respiratory viral infections in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13511.
51. Beaird O, Freifeld A, Ison M, et al. Current practices for treatment of respiratory syncytial virus and other non-influenza respiratory viruses in high-risk patient populations: a survey of institutions in the Midwestern Respiratory Virus Collaborative. Transpl Infect Dis. 2016;18:210–5.
52. Danziger-isakov L, Arslan D, Sweet S, et al. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the International Pediatric Lung Tranplant Collaborative. Pediatr Transplant. 2012;16:638–44.
53. Liu V, Dhillon GS, Weill D. A multi – drug regimen for respiratory syncytial virus and parain uenza virus infections in adult lung and heart–lung transplant recipients. Transpl Infect Dis. 2010;12:38–44.
54. Boeckh M, Englund J, Li Y, et al. Randomized controlled multicenter trial of aerosolized ribavirin for respiratory syncytial virus upper respiratory tract infection in hematopoietic cell transplant recipients. Clin Infect Dis. 2007;44:245–9.

55. Fuehner T, Dierich M, Duesberg C, et al. Single-centre experience with oral ribavirin in lung transplant recipients with paramyxovirus infections. Antivir Ther. 2011;16:733–40.
56. Chemaly RF, Torres HA, Munsell MF, et al. An adaptive randomized trial of an intermittent dosing schedule of aerosolized ribavirin in patients with cancer and respiratory syncytial virus infection. J Infect Dis. 2012;206:1367–71.
57. Shah DP, Ghantoji SS, Shah JN, et al. Impact of aerosolized ribavirin on mortality in 280 allogeneic haematopoietic stem cell transplant recipients with respiratory syncytial virus infections. J Antimicrob Chemother. 2013;68:1872–80.
58. Wang L, Allen J, Diong C, et al. Respiratory virus infection after allogeneic hematopoietic stem cell transplant in a tropical center: predictive value of the immunodeficiency scoring index. Transpl Infect Dis. 2017;19:e12693.
59. Foolad F, Aitken SL, Shigle TL, et al. Oral versus aerosolized ribavirin for the treatment of respiratory syncytial virus infections in hematopoietic cell transplant recipients. Clin Infect Dis. 2018;68:1–9.
60. Li L, Avery R, Budev M, et al. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. J Heart Lung Transplant. 2012;31:839–44.
61. Wolters Kluwer Health Inc., Riverwoods IL. Ribavirin (systemic): drug information. Lexi-Drugs. Accessed on 2 Jun 2019. http://online.lexi.com
62. Gorcea CM, Tholouli E, Turner A, et al. Effective use of oral ribavirin for respiratory syncytial viral infections in allogeneic haematopoietic stem cell transplant recipients. J Hosp Infect. 2017;95:214–7.
63. Marcelin JR, Wilson JW, Razonable RR. Oral ribavirin therapy for respiratory syncytial virus infections in moderately to severely immunocompromised patients. Transpl Infect Dis. 2014;250:242–50.
64. Burrows FS, Pgdip B, Carlos LM, et al. Oral ribavirin for respiratory syncytial virus infection after lung transplantation: efficacy and cost-efficiency. J Heart Lung Transplant. 2015;34:958–62.
65. Glanville AR, Scott AIR, Morton JM, et al. Intravenous ribavirin is a safe and cost-effective treatment for respiratory syncytial virus infection after lung transplantation. J Heart Lung Transplant. 2005;24:2114–9.
66. Molinos-Quintana A, Perez-de Soto C, Gomez-Rosa M, et al. Intravenous ribavirin for respiratory syncytial viral infections in pediatric hematopoietic SCT recipients. Bone Marrow Transplant. 2013;48:265–8.
67. Group TIm-RS. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics. 1998;1:531–7.
68. Tsitsikas DA, Oakervee H, Cavenagh JD, et al. Treatment of respiratory syncytial virus infection in haemopoietic stem cell transplant recipients with aerosolized ribavirin and the humanized monoclonal antibody palivizumab: a single centre experience. Br J Haematol. 2009;146:574–6.
69. De Fontbrune FS, Robin M, Porcher R, et al. Palivizumab treatment of respiratory syncytial virus infection after allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2007;45:1019–24.
70. Gottlieb J, Zamora MR, Hodges T, et al. ALN-RSV01 for prevention of bronchiolitis obliterans syndrome after respiratory syncytial virus infection in lung transplant recipients. J Heart Lung Transplant. 2016;35:213–21.
71. Marty FM, Chemaly RF, Bergeron A, et al. LBA6: a phase 2b, randomized, double-blind, placebo-controlled trial of presatovir (GS-5806) for the treatment of respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) in hematopoietic-cell transplantation (HCT) recipients. In BMT Tandem Meetings. 2018.
72. Chemaly RF, Dadwal SS, Bergeron A, et al. LBA7: a phase 2b, randomized, double-blind, placebo-controlled trial of presatovir (GS-5806), an oral fusion inhibitor for the treatment of

respiratory syncytial virus (RSV) upper respiratory tract infection (URTI) in hematopoietic- cell transplant. In BMT Tandem Meetings. 2018.
73. Stevens M, Rusch S, Devincenzo J, et al. Antiviral activity of oral JNJ-53718678 in healthy adult volunteers challenged with respiratory syncytial virus: a placebo-controlled study. J Infect Dis. 2018;218:748–56.
74. Coates M, Brookes D, Kim Y, et al. Preclinical characterization of PC786, an inhaled small-molecule respiratory syncytial virus L protein polymerase inhibitor. Antimicrob Agents Chemother. 2017;61:1–18.
75. Tiwari PM, Vanover D, Lindsay KE, et al. Engineered mRNA-expressed antibodies prevent respiratory syncytial virus infection. Nat Commun. 2018;9:3999.

Respiratory Viral Pathogens in Solid Organ and Hematopoietic Stem Cell Transplant Recipients

31

Steven A. Pergam and Michael G. Ison

Contents

Introduction 744
Respiratory Virus Epidemiology Among Transplant Patients 745
Clinical Presentations of Respiratory Viral Pathogens in Transplant Recipients 746
Disease Progression and Complications 747
Diagnostic Overview 748
Common and Uncommon Respiratory Viral Pathogens 750
Influenza 750
  Treatment 751
  Prevention 752
Respiratory Syncytial Virus 754
  Treatment 755
  Prevention 757
  Human Metapneumovirus 757
  Treatment and Prevention 758
Parainfluenza Viruses 758
  Treatment and Prevention 759
Rhinovirus 760
  Treatment and Prevention 760
Coronavirus 760
  Treatment and Prevention 761
Adenovirus 761
  Treatment and Prevention 762

S. A. Pergam
Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center and University of Washington, Seattle, WA, USA
e-mail: spergam@fredhutch.org

M. G. Ison (✉)
Division of Infectious Diseases and Organ Transplantation, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
e-mail: mgison@northwestern.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_32

Bocavirus and Other Uncommon Respiratory Viruses 763
Infection Control Practices to Protect Transplant Patients from Respiratory Viruses 763
Unique Donor and Recipient Issues 765
Conclusions 766
Key Points 766
References 766

Abbreviations

2019-nCoV	2019 novel Coronavirus
AdV	Adenovirus
BAL	Bronchoalveolar lavage
BoV	Bocavirus
CLAD	Chronic lung allograft dysfunction
CoV	Coronavirus
CT	Computed tomography
EV	Enterovirus
GVHD	Graft-versus-host disease
hMPV	Human metapneumovirus
hRhV	Human rhinovirus
HSCT	Hematopoietic stem cell transplant
ICU	Intensive care unit
IV	Intravenous
LRTI	Lower respiratory tract infection
MDR	Multidrug resistance
MERS-CoV	Middle Eastern Respiratory Syndrome Coronavirus
PCR	Polymerase chain reaction
PFT	Pulmonary function tests
PIV	Parainfluenza
RSV	Respiratory syncytial virus
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SOT	Solid organ transplant
URI	Upper respiratory infection

Introduction

Despite significant advances in surgical and medical therapies, infections remain a significant risk for morbidity and mortality among patients who have undergone both solid organ (SOT) and hematopoietic stem cell transplantation (HSCT). Respiratory viruses, including influenza A and B, respiratory syncytial virus (RSV), parainfluenza viruses (PIV), human metapneumovirus (hMPV), human rhinovirus (hRhV), coronaviruses (CoV), adenoviruses (AdV), and bocavirus (BoV), are among the most common causes of posttransplant infections (Fig. 1) [1].

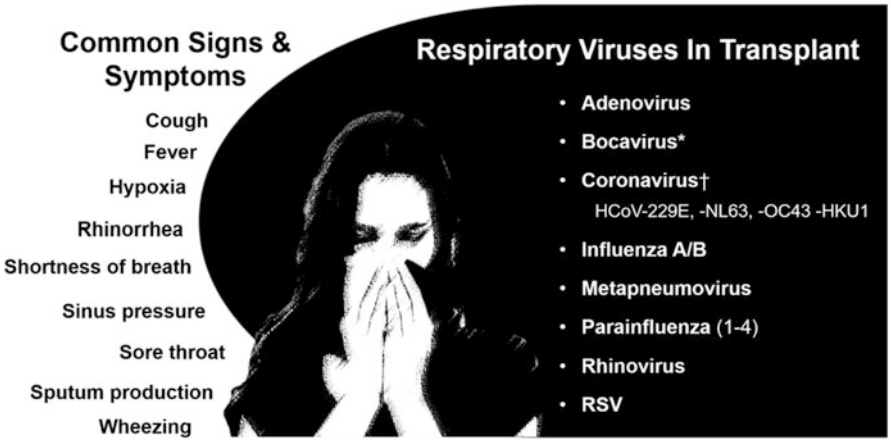


Fig. 1 Common respiratory viruses seen in transplant patients are those seen in normal populations and during seasonal outbreaks. Although most are community acquired pathogens, adenoviruses are often viruses that can reactivate from latent reservoirs in the host or be newly acquired from exposures. *Bocavirus is more often considered a gastrointestinal pathogen, and many multiplex PCR panels may not include this for routine testing. †These four Coronaviruses are human strains see as common community strains, and do not include outbreak strains such as MERS-CoV, SARS-CoV, and 2109-novel CoV. Abbreviations: RSV-respiratory syncytial virus

Clinically, it is challenging to differentiate specific respiratory viruses based on symptoms or presentation. In transplant recipients, a high-index of suspicion is needed as these patients can present with a myriad of infections and inflammatory conditions associated with sinopulmonary symptoms (e.g., idiopathic pneumonia syndrome, cryptogenic organizing pneumonia) [2]. In addition, symptoms may be minimal to absent in many immunosuppressed patients [3]. As a result, it is crucial that the early diagnostic workup include an evaluation for respiratory viruses in order to identify and guide therapy. There are currently approved agents for the prevention and treatment of influenza and RSV. Although emerging experimental therapies [4–6] are becoming more available for other respiratory viruses, alternate antiviral agents which are licensed for other viral infections (e.g., ribavirin) have been used particularly among those with severe disease [7, 8]. In this chapter, we will review the general approach to respiratory viruses and then discuss approaches to the common respiratory viruses with a focus and prevention and treatment.

Respiratory Virus Epidemiology Among Transplant Patients

Multiple contemporary studies of SOT and HSCT patients using molecular diagnostics have helped to define the epidemiology of respiratory viruses in this population. Patients early after their transplant, particularly lung transplant recipients, allogeneic HSCT, and pediatric transplant recipients consistently, have higher rates of clinically significant respiratory viral infections and a higher rate of progression to lower tract

disease. Other risk factors associated with more severe and progressive illness include recent use of lymphocyte depleting agents, lymphopenia <300 cells/μL, use of high-dose glucocorticoids, and severe hypogammaglobulinemia (≤4.5 g/mL) [1]. Seasonal epidemics of respiratory viruses in transplant recipients follow the local seasonal patterns, although disease may be first recognized among immunosuppressed populations [1]. For the northern hemisphere, influenza and RSV are most common November–April; hRhV and CoV are most common in the fall and spring; and there is less seasonal variation with PIV and AdV. HRhV consistently is the most commonly identified pathogen, followed by coronavirus, RSV, PIV viruses, influenza, AdV, human hMPV, and BoV [9]. While cases are more severe among the most immunosuppressed transplant patients, such as those immediately posttransplant or those undergoing treatment of rejection, infections from respiratory viruses can occur at any time after transplant.

Clinical Presentations of Respiratory Viral Pathogens in Transplant Recipients

Transplant patients develop symptoms commonly described with respiratory viral infections, including rhinorrhea, sore throat, cough, and fever; however, presentation is much more varied among these patient populations ranging from asymptomatic shedding to severe lower respiratory tract infections. However, it has been demonstrated that transplant patients are more likely to present with less prominent early signs and symptoms, for example, rhinorrhea, reduced PFTs, or fever may be the sole presenting signs of a respiratory viral infection in lung transplant recipients [1, 9–11]. Similarly, HSCT recipients are more apt to have atypical symptoms, such as fever only, and many patients present with limited symptoms. As a result, clinicians must have a low index of suspicion for testing patients and considering early therapy (Fig. 1, Table 1) [3]. A recent large study of SOT and HSCT recipients with influenza clearly demonstrated the paucity of classic symptoms with only 64% presenting with fever, 35% with a sore throat, and 40% with myalgias [12].

Importantly, immunosuppressed patients are more likely to present with severe life-threatening lower tract infections such as pneumonia [12]. Patients with more severe lower tract disease presentations are more likely to present with shortness of breath, hypoxia, and severe respiratory symptoms that require intubation and ICU level support. Any transplant patient presenting with acute respiratory distress, particularly those with diffuse infiltrates on radiologic assessment, should have common respiratory viral infections included in their early differential diagnosis.

With the development of molecular techniques for detection of respiratory viruses, several studies have also noted that transplant patients can present with asymptomatic infections. These studies which screen patients prospectively demonstrate that patients have documented viral detection (by PCR) but a lack of clinical symptoms [13]; some patients never develop clinical evidence of disease [3]. In part due to atypical symptoms and the potential for asymptomatic shedding, nosocomial transmission in HSCT clinic and hospital settings has been well described. Even well

Table 1 Common respiratory viruses in transplantation

Virus	Isolation recommendations	Prophylactic options	Therapeutic options
Influenza	Contact and droplet	Inactivated influenza vaccine seasonal NAI	NAI
RSV	Contact and droplet	Palivizumab	Ribavirin ± immunoglobulin
hMPV	Contact and droplet	None	Ribavirin ± immunoglobulin
PIV	Contact and droplet	None	Ribavirin ± immunoglobulin
Rhinovirus	Contact and droplet	None	None ([a]DAS181)
Coronavirus	Contact and droplet [a]2019 novel CoV, MERS, or SARS which require contact, droplet and airborne precautions	None	None
Adenovirus	Contact & droplet	None	Cidofovir, ([a]brincidofovir)
Bocavirus	Contact & droplet	None	None

Contact Precautions: gown, gloves; Droplet: Mask and Eye protection
Abbreviation: NAI, Neuraminidase inhibitor (oseltamivir, peramivir, or zanamivir); RSV, Respiratory Syncytial virus; hMPV, Human metapneumonvirus; PIV, Parainfluenzas; CoV, Coronavirus; MERS, Middle East respiratory syndrome-related Coronavirus; SARS, Severe acute respiratory syndrome-related Coronavirus
[a]unapproved agents currently in clinical trials

organized, careful infection prevention and screening programs targeted to these high-risk patients mitigate but cannot eliminate the risk of respiratory viruses and the development of clinical disease [14, 15].

Disease Progression and Complications

Respiratory viral infections can become life threatening as patients are more likely to progress from upper tract infections to lower tract infections. Rates of progression vary between virus, ranging from 5% to 50% depending on the pathogen and the level of immunosuppression in the host [16]. Among HSCT recipients, a number of factors have been identified to be associated with an increased risk of progression from upper to lower respiratory tract involvement. Commonly identified risk factors include neutropenia ($\leq$500 neutrophils/mL), lymphopenia ($\leq$200 lymphocytes/mL), age $\geq$ 40 years, presence of graft-versus-host disease (GVHD), use of glucocorticoids within 2 weeks of infection, myeloablative chemotherapy within 1 year of infection, cumulative antibiotic exposure, and infection early, especially within 1 year, of HSCT [16, 17]. Other factors include a history of smoking, nosocomial acquisition, hypoxia at diagnosis, use of matched unrelated or mismatched donor,

prior autologous HSCT, and cord blood transplant [5, 18, 19]. Two risk scores have been developed to risk stratify HSCT recipients with respiratory viruses and predict response to therapy [7, 18–20].

In transplant populations with respiratory viruses, bacterial infections are a known but infrequent complications linked to these infections, with *Pseudomonas aeruginosa*, *Streptococcus pneumoniae,* and other common oral and aerodigestive tract bacterial species commonly seen as co-pathogens. Multidrug resistant (MDR) infections such as methicillin-resistant *Staphylococcus aureus* and MDR gram negative infections may also be more likely in these patients due to significant prior hospitalization and antibiotic exposures [21–23].

In addition to disease specific complications, emerging reports have linked respiratory viruses with rejection, particularly among lung transplant recipients. While there remains controversy about the role of respiratory viruses in triggering acute rejection, there is a clear association with an increased risk of developing chronic rejection or chronic lung allograft dysfunction (CLAD) and infectious complications, including aspergillosis, among lung transplant recipients [24–27]. Risk of CLAD appears to be greatest with infection with the pneumoviruses (RSV and HPMV) and with lower respiratory tract involvement (HR 3.0, 95% CI 1.5–5.9) [28, 29]. Although data suggests that this association is linked to symptomatic infections, additional studies on the role of asymptomatic respiratory viral infections on lung transplant patients are needed [24]. Lower respiratory tract involvement with the respiratory viruses is associated with late onset airflow obstruction and infectious complications, including invasive aspergillosis, in HSCT recipients as well [30–32]. Transplant patients may develop encephalitis, myocarditis, or other rare complications that have been reported seen in patients with influenza infection.

Diagnostic Overview

As with most respiratory infections, patients with signs and symptoms of pneumonia should undergo radiologic evaluation. Although patients with upper tract symptoms do not routinely need imaging, patients with lower tract disease symptoms (e.g., cough or shortness of breath) should undergo imaging to evaluate the extent of lower tract involvement [33]. Chest x-rays can be a useful test (Fig. 2a), but among immunosuppressed transplant patients, noncontrast CT scans have increased sensitivity for lower tract disease manifestations and are often the preferred method for radiologic evaluation (Fig. 2b). Transplant patients with lower tract respiratory viral infections most commonly present with diffuse or patchy ground glass infiltrates, tree-in-bud findings, and/or consolidation on CT scans (Fig. 3).

It is nearly impossible to clinically distinguish infection due to one respiratory virus from another. Making a diagnosis among SOT and HSCT recipients is critical not only for infection prevention purposes, but also to enable the treating physician to select the optimal therapy for the patient. While a range of diagnostic methods are available, including serology, culture, and antigen detection, nucleic acid testing

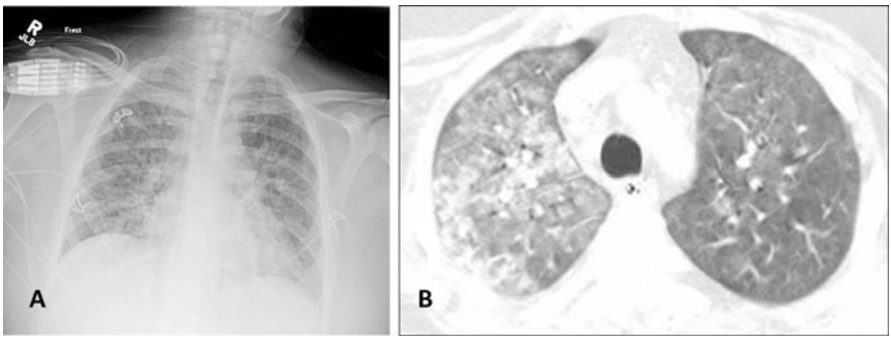
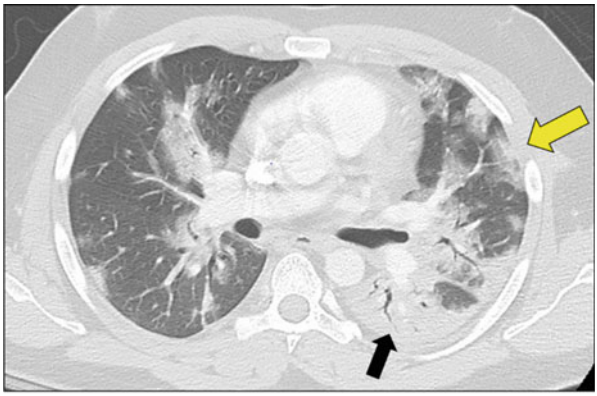


Fig. 2 Allogeneic transplant patient who presented with worsening hypoxia, fever and dry cough, documented to have lower tract human metapneumovirus. Human metapneumovirus documented by multiplex PCR from nasal wash and by bronchoaveolar lavage. A. Posterior anterior chest radiograph demonstrating a diffuse interstitial pattern. B. CT scan of the chest demonstrating diffuse multifocal lung infiltrates

Fig. 3 Transplant patient with multifocal ground glass (yellow arrow) infiltrates and consolidations (black arrow) from documented Influenza A



Transplant patient with multifocal ground glass (yellow arrow) infiltrates and consolidations (black arrow) from documented Influenza A.

(PCR) has become the gold standard for the diagnosis of respiratory viruses. There are a range of molecular diagnostics, including a number multiplex panels that allow for concurrent screening of multiple pathogens from a single sample, and most of the currently available assays provide rapid results. In general, immunocompromised patients with any upper respiratory tract symptoms and/or fever should have upper airway samples (nares or nasopharyngeal) sent for multiplex PCR testing for respiratory viruses. False negative testing from the upper respiratory tract samples can occur from inadequate sample collection or among patients who have progressed to lower respiratory tract involvement. In one recent study, upper respiratory tract samples failed to detect a pathogen present in lower airway samples in 14.8% of

patients [34]. Failure to detect a pathogen using upper respiratory samples, particularly in patients with symptoms of lower tract involvement, should prompt collection of lower airway samples, by deep tracheal aspirate or preferably by bronchoalveolar lavage (BAL); BAL samples should be tested using by similar multiplex PCR methods [5, 35]. Sampling lower tract specimens also helps determine if patients with respiratory viral infections have associated co-infections, which may require additional targeted therapy.

Common and Uncommon Respiratory Viral Pathogens

Respiratory viruses present with similar findings, but outcomes, complications, and treatment for various viruses are unique. In the following section, we describe specific epidemiologic details of individual viruses and describe available methods for treatment.

Influenza

Influenza viruses are *Orthomyxoviridae* enveloped and segmented RNA viruses, and Influenza A and B cause the vast majority of infections in transplant patients. Influenza results in seasonal epidemics typically from November to May in the northern and May to October in the southern hemisphere, respectively. Influenza A typically occurs early in the season, followed by Influenza B, however seasonal variation can shift normal patterns. Seasonal epidemics are triggered by viruses that have antigenically drifted, with minor changes in which hemagglutinin or neuraminidase proteins are altered slightly so that preexisting antibodies present in the population no longer neutralize the virus. Far less frequently, an antigenic shift can occur when a novel hemagglutinin or neuraminidase that has not circulated in the human population for a long period of time emerges (e.g., H1N1 Swine influenza) and causes transmission, often associated with significant morbidity and mortality [36].

Among SOT patients, patients present most frequently with cough (85%) and fever (64%) but other cardinal symptoms, such as sore throat (35%) and myalgias (40%), are less common. Twenty-two percent have radiologic evidence of pneumonia on initial evaluation although proven bacterial superinfections are less frequently reported. While most patients in one large study were hospitalized, the length of stay was short (median 6 days), only 11% required ICU-level care, and even fewer required mechanical ventilation (8%) [12]. Among HSCT recipients, symptom presentations are similar, with cough as the most common symptom in most studies, appearing in 70–90% of patients [12, 18, 37]. Reported rates of fever range from 30% to 70% in these same studies, while myalgias are less frequently reported (5–39%). Importantly, rhinorrhea, sore throat, and other URI symptoms can also be among the only presenting symptoms in HSCT patients with influenza [38]. Additionally, patients who develop influenza in the early post-HSCT period may have

less frequent and or prominent symptoms [3]. Hospitalizations and ICU level admission rates appear to be similar to SOT patient populations [12]. Presentations in transplant patients may vary from mild to severe, and this variation is likely related to host immunity, symptom distribution which may also shift between seasons, and unique characteristics of new seasonal strains [18, 39].

Treatment

Currently, there are three classes of antivirals approved for the treatment of influenza: M2 ion channel inhibitors (amantadine and rimantadine), neuraminidase inhibitors (oseltamivir, peramivir, and zanamivir), and the polymerase inhibitor (baloxavir marboxil). Due to widespread resistance in all circulating strains, the M2 ion channel inhibitors are not recommended for the prevention or treatment of influenza. While the polymerase inhibitor baloxavir marboxil is associated with improved activity against influenza B and is associated with much more rapid reduction in viral replication, clinical studies are only available in immunocompetent patients [40–42]. Resistance among these immunocompetent emerged quickly in ~10% of adults and up to 40% of children treated with baloxavir marboxil. Resistance was seen more commonly in those with poor preinfection immunity and in individuals infected with A/H3N2 viruses. The resistant variants are transmissible and associated with longer durations of clinical symptoms and viral shedding in many of the patients [43–50]. As such, routine use of baloxavir is not recommended for transplant patients. If considered for use, it should always only be used in combination with a neuraminidase inhibitor and likely requires repeat dosing; the pharmacokinetics to inform optimal timing for repeat dosing are currently being studied (ClincalTrials.gov: NCT03684044) [36].

Prospective studies have not been performed in the transplant setting to define the optimal dose, route, or duration of antiviral NAI therapy, or the role of combination therapies. NAI therapy is associated with reduced mortality, reduced progression to pneumonia, reduced duration of shedding, and among lung transplant recipients, reduced risk of development of chronic lung allograft dysfunction [51, 52]. Early therapy, defined as initiation in <48 h after symptom onset, is associated with the best outcomes 53]. Nonetheless, therapy appears to be beneficial in this population beyond 48 h and all symptomatic patients should be treated. Unless diagnostic testing results are readily available, antiviral therapy should be initiated as soon as influenza is suspected in a transplant patient presenting with symptoms during influenza season, even if diagnostics are pending; patients without evidence of lower tract disease with negative testing can have therapy discontinued. Viral replication is prolonged with the average duration of shedding being significantly longer than 5 days in HSCT and SOT patients [12, 20]. As such, most experts recommend at least 10 days of therapy with longer therapy in patients that remain symptomatic and have ongoing shedding beyond 10 days. Higher than usual doses (i.e., oseltamivir 150 mg p.o. BID) may be associated with reduced risk of resistance emergence [54] or considered for

treatment of patients with influenza B infections (ClincalTrials.gov: NCT00545532), but recommendations vary about the utility of routine use of high-dose therapy in transplant patients [55–57].

Patients that have persistent symptoms with sustained or worsening viral replication after 5 days should be considered to have virologic resistance, which is more frequently detected among transplant recipients. Not all contemporary multiplex assays provide surrogates for estimating viral loads (i.e., mean florescence or cycle threshold (CT) values or viral loads), but if available, an increasing viral load among patients on therapy is often evidence of virologic failure. Additionally, resistance assays are available at specific centers, the CDC, and some State Health Departments. If your assay does not provide these surrogate endpoints, it is recommended that repeat testing with assays that provide such data can help discern whether the patient is experiencing virologic failure. At medical centers without such tools, patients with prolonged viral detection and progression of symptoms while on therapy should also be considered to potentially have antiviral resistant infection.

If resistance is considered or documented by molecular techniques, most recommend a switch to an alternative therapy (ideally a combination of two active drugs). Resistance testing of clinical samples is recommended, if not already sent. Since most centers use oseltamivir as primary therapy, a switch to zanamivir is often recommended if possible as some resistant strains maintain susceptibility to zanamavir [58]; however, resistance to oseltamivir is more likely to be associated with cross resistance to peramivir [59]. Caution should be used with zanamivir in patients with reactive airways and among those intubated and receiving respirator support, as the drug is inhaled. Transplant patients with mutations conferring multidrug resistance have been described, so consideration for dual therapy (NA inhibitor and another agent) is often recommended in those failing therapy [60]. The most effective combination therapies have not been studied except in small case series [61]. Most commercially available assays only detect the most common H275Y mutation that is relevant for oseltamivir resistance in influenza A/H1N1 viruses; if other agents or other viruses require testing, samples can be sent to CDC through state or local health departments in the USA or through local public health agencies.

Prevention

Although screening and contact precautions can help prevent healthcare associated transmission, prevention is best accomplished through use of the inactivated influenza vaccine. Current guidelines recommend annual influenza vaccination in all transplant patients, their close contacts, and healthcare workers caring for them. Ideally, vaccination should happen 3–6 months posttransplant, but earlier vaccination should be considered if there is an ongoing local epidemic [5, 35]. A number of different formulations of influenza vaccine are available. Although studies have generally been small, all formulations of inactivated vaccine have been demonstrated to be effective in preventing influenza [62]. In general, live-attenuated influenza

vaccine (Flumist®) should be avoided in transplant patients because of the theoretical risk of developing clinical disease from the vaccine, unless the live attenuated vaccine is the only option for vaccinating the patient during a period where the potential risk outweighs the benefit of vaccination (e.g., center level outbreak). The CDC and others also recommend that family members should not receive the live virus vaccine, but if live vaccine receipt occurs, close contacts should be separated from the transplant recipient for at least one week [63, 64].

In general, transplant patients have less robust responses to influenza vaccine than normal hosts even when considering seasonal variance in vaccine responses [65]. Poor responses have been demonstrated with vaccine within 6 months of HSCT, in patients with active GVHD, in patients who are on ≥2 g of daily mycophenolate mofetil (MMF) and who are ≥65 years [66–69]. All standard dose inactivated vaccines are quadrivalent (currently containing the influenza A/H1N1, A/H3N2, B/Victoria and B/Yamagata strains), including the egg-based, cell culture based, and recombinant HA vaccines. The high dose and MF59 adjuvanted influenza vaccine currently are only approved in trivalent forms (both lack the B/Yamagata strain); a quadrivalent high dose vaccine was recently approved and will become commercially available during the 2020–2021 season. Adjuvanted vaccine did not appear to result in improved responses compared to unadjuvanted vaccine in HSCT and SOT recipients [70–72]. Among HSCT patients, lower seroconversion was associated with use of calcineurin inhibitors ($P < 0.001$) and shorter duration from transplantation, as well as GVHD [68, 72].

Two commonly studied approaches to improve vaccine responses include giving a booster vaccine 4–8 weeks after initial vaccine or the use of high-dose influenza vaccine. Booster dosing has been most commonly utilized in individuals needing vaccination shortly after transplant. This approach is associated with a consistent but modest improvement in seroconversion and seroprotective humoral responses (10–12% increased seroprotection with the second vaccine) [73]. The European Conference of Infections in Leukemia (ECIL) recommend a second dose of inactivated vaccine administered 3–4 weeks after the first dose should be considered in patients with severe GVHD or low lymphocyte counts. Additionally, they recommend early vaccination at 3 months post-HSCT during outbreaks, followed by an additional dose 3–4 weeks later [74]. Current US guidelines recommend single dose inactivated influenza vaccine at 6 months post-HSCT, or a dose at 3 months followed by a second dose 3–4 weeks later [64].

Inactivated high-dose influenza is a trivalent vaccine that contains 4 times the hemagglutinin protein and has also been studied in various transplant populations. In the largest study to date, high-dose vaccine was associated with an improved seroconversion rate in SOT recipients (A/H1N1: 40.7% vs. 20.5%; A/H3N2: 57.1% vs. 32.5%; B: 58.3% vs. 41.6%) although there were no significant difference in seroprotection, likely due to high prevaccine titers [75]. A pilot study in adult HSCT patients demonstrated that high dose vaccine was found to be safe and associated with a higher percentage of individuals with titers ≥1:40 and a higher geometric mean titer (GMT) against the H3N2 strain compared with that of the standard dose group [76]; a randomized clinical trial comparing standard dose or

high-dose followed by boosting at 4 weeks post-HSCT has currently completed enrollment but final study results are pending at the time of this update. Without Advisory Committee of Immunization Practices, support for routine use in immunosuppressed patients access may be limited due to costs and insurance restrictions, so high-dose vaccine is generally only given to patients ≥65 years of age for which it is specifically indicated [77]. None-the-less, current guidelines in SOT recommend the routine use of high-dose vaccine in all SOT recipients; available HSCT guidelines have not supported routine use, other than to those ≥65 years of age, due to the limited data available in this population [35, 78].

For patients who would be predicted to have a poor influenza vaccine response, seasonal antiviral prophylaxis can be considered, particularly during large outbreaks and is supported by a randomized clinical trial. In this trial, patients were given oseltamivir 75 mg once daily or placebo for 12 weeks when influenza was first recognized in the community. Such an approach was associated with reduced incidence of PCR positive (1.7% vs. 8.4%) and culture-confirmed (<1% vs. 3.8%) viral breakthrough infections, yet without evidence of NAI resistance mutations in viruses that failed prophylaxis [79]. While generally well tolerated, clinical experience demonstrates challenges in drug approvals from health insurance carriers and a high incidence of discontinuation because of intolerance.

Postexposure prophylaxis using the lower prophylactic dose (daily vs. twice daily) is not recommended for those with documented household or nosocomial exposures since transmission may have already occurred and the risk of clinical and virologic influenza breakthrough should be clearly avoided, especially in immunocompromised patients where resistance is a major concern [36]. Instead, empiric treatment using the therapeutic dosing (i.e., 10 days of twice daily oseltamivir) should be restricted to high risk transplant recipients (HSCT within 6 months of transplant or unvaccinated lung transplant recipients). Low-risk patients should be monitored closely and started on early full dose treatment if they develop any symptoms. If used, therapy be should be started as soon as the diagnosis is confirmed in the contact but should not be started if there has been >48 h of exposure to a symptomatic contact; in such situations active symptom monitoring with proactive treatment is recommended.

Respiratory Syncytial Virus

RSV was reclassified in 2016 into the family *Pneumoviridae,* genus *Orthopneumoviridae* [80, 81]. RSV is an enveloped, nonsegmented, negative sense, single-stranded RNA virus which encodes 11 proteins: 2 nonstructural and 9 structural [82]. RSV isolates are divided into two major antigenic groups A and B, each of which are further divided into 13 RSV A genotypes and 20 RSV B genotypes [80, 81].

Studies involving SOT recipients have demonstrated significant mortality in lung transplant patients where LRTIs with RSV can be a risk factor for CLAD and for death (mortality rate up to 20%) [83, 84]. Local inflammation and enhanced exposure of the immune system to pulmonary antigens lead to injury in the airway

epithelial cells and sub-epithelial structures, leading to obliteration of the small airways, and subsequently CLAD [85].

Among adult HSCT recipients with RSV infection, progression from URTI to LRTI occurs in 40–60% of cases and LRTI is associated with mortality rates up to 80% [86–89]. Two available risk scores (see Table 2) are helpful in predicting outcomes for RSV.

In the first scoring system, patients with more than 2 SID (Severe Immunodeficiency) factors (VSID, Very Severe Immunodeficiency) were found with RSV-attributed mortality rates of 18% [7]. The immunodeficiency scoring index RSV (ISI-RSV) score demonstrates an increasing risk of progression from upper to lower tract disease (7%, 15% and 48%, respectively) and mortality (0%, 3%, and 29%, respectively) across the 3 risk levels [19]. These scores have been proposed as tools for determining need for hospital admission and antiviral therapy.

Treatment

The standard of care for the management of RSV infection in adults is mainly limited to supportive care with bronchodilators, supplemental oxygen, intravenous fluid, and antipyretics [90]. Ribavirin and immunoglobulin preparations are the therapies most

Table 2 Risk score for HSCT recipients with RVI to predict risk of progression to lower tract disease and death

(a) Severe immunodeficiency score		
Moderate immunodeficiency (MID)	Severe immunodeficiency (SID)	Very severe immunodeficiency (VSID)
≥1 factor present	Only 1 factor present	≥ 2 factors present
	Neutropenia <0.5 10^9/L	Neutropenia <0.5 10^9/L
	Lymphopenia <0.1 10^9/L	Lymphopenia <0.1 10^9/L
	Allo-HSCT <6 months ago	Allo-HSCT <6 months ago
GVHD<2 or limited GVHD	GVHD ≥2 or requiring treatment	GVHD ≥2 or requiring treatment
T-cell depletion >3 months	T-cell depletion <3 months	T-cell depletion <3 months
B-cell depletion >3 months	B-cell depletion <3 months	B-cell depletion <3 months
	Hypo-γ-globulinemia <4.5 g/L	Hypo-γ-globulinemia <4.5 g/L

(b) Immunodeficiency Score Index (ISI)	
Factor	Score
Neutropenia <0.5 10^9/L	3
Lymphopenia <0.2 10^9/L	3
Pre-engraftment or Allo-HSCT <1 months	1
GVHD (acute/chronic)	1
Corticosteroids	1
Myeloablative conditioning	1
Age > 40 years	2
Interpretation: 0–2: Low risk; 3–6: Moderate risk; 7–12: High risk	

commonly utilized and are generally limited to HSCT and lung transplant recipients [82]. Ribavirin is a guanosine analogue that is FDA approved, in its aerosolized formulation, for the treatment of RSV in infants and young children only. Intravenous ribavirin is not commercially available in most parts of the world. Due to challenges in drug delivery and the high cost of aerosolized ribavirin, many centers utilize oral ribavirin off-label for the treatment of immunocompromised patients despite a lack of prospective efficacy data [91–93].

Available studies suggest that if therapy is to be used, it is of greatest benefit when started early to prevent progression to lower tract disease [94–96]. When used for lower tract disease, ribavirin has been shown to be less effective [97]. Furthermore, a comprehensive review of the available literature shows that patients treated with aerosolized ribavirin and an antibody preparation, including standard intravenous immune globulin (IVIG), RSV immunoglobulin, or palivizumab had the lowest risk of progression to lower tract disease and death [16, 89]. If aerosolized ribavirin is used, continuous dosing (6 g over 18 h daily) has been shown to be equally effective in preventing progression to LRTI compared to intermittent dosing (2 g over 3 h every 8 h) [98].

Oral ribavirin is increasingly used among transplant centers globally due to ease and cost considerations [92]. A range of doses have been utilized across centers, although aggressive dosing is likely needed to approach the drug concentration needed in alveolar lung fluid to treat RSV; dosing approaches must be balanced with adverse effects such as hemolytic anemia [93, 99–119]. Most studies in HSCT recipients that have compared oral to inhaled ribavirin have demonstrated similar efficacy, although none have been randomized and none included a placebo arm. Among lung transplant recipients, IV ribavirin has been associated with 100% survival rate with only 5% developing post-RSV BOS, a rate lower than historical data [120]. Several retrospective observational studies of oral ribavirin have consistently shown similar outcomes compared with IV or inhaled ribavirin in lung transplant patients. The majority of studies have not been placebo controlled. However, two recent studies of novel RSV drugs found that when ribavirin was utilized in transplant patients, it was not shown to convincingly improve outcomes compared to patients that did not receive ribavirin [6, 121]. These data, the lack of availability of intravenous ribavirin, the cost of inhaled ribavirin, and the lack of placebo-controlled studies in transplant patients highlight the challenges and ongoing need for safety and efficacy studies of ribavirin in the transplant population.

The current approach used in most centers is the use of aerosolized or oral ribavirin for treatment of RSV in HSCT and lung transplant recipients; antibody preparations are often added to ribavirin in HSCT recipients with more severe disease [5, 35, 82, 122]. Most centers will utilize standard intravenous immunoglobulin (IVIG) for the antibody preparation. Recently, a high titer RSV immunoglobulin (RI-002) was approved by the FDA without a specific RSV indication but in a compassionate use study was associated with a high survival rate in HSCT recipients (73%) [123]. Limited data on treatment in adults suggests reduced progression to lower tract disease with early combination therapy using IV palivizumab and ribavirin [16, 124, 125]. However, use of palivizumab in combination therapy was

not found to decrease mortality in a large retrospective study of patients with RSV pneumonia and is not recommended by most centers [126]. The largest single-center study utilizing palivizumab in younger adults (median age 16 years old) with HSCT also failed to show significant impact on outcome as assessed by progression to LRTI, early mortality rate, and 1-year overall survival rate [124].

Two investigational agents provide further insight into antiviral therapy in this population but did not meet prespecified study endpoints. The first, ALN-RSV01, is a small interfering RNA (siRNA) that was studied to assess its impact on prevention of BOS in lung transplant patients. Two completed studies in lung transplant patients demonstrated a statistically nonsignificant decrease in new or progressive BOS at day 180 (13.6% vs. 30.3%, $p = 0.058$) but no impact on viral parameters or symptom scores. Early therapy was associated with greatest impact [127]. Although this agent is no longer under clinical development, it demonstrated the ability to use CLAD as a potential endpoint in studies in lung transplant recipients. The second, presatovir (oral GS-5806), is an oral small molecule fusion inhibitor that has been studied in lung and HSCT recipients. Unfortunately, despite excellent data in human challenge models and a clear safety profile in clinical studies, presatovir did not achieve the clinical or virologic endpoints in the transplant studies [6, 121, 128]. A small effect was noted in patients with significant lymphopenia with upper respiratory tract illness [121]. Several other experimental therapies (JNJ-53718678: ClinicalTrials.gov Identifier NCT03379675, NCT03656510; RV521: ClinicalTrials.gov Identifier NCT03782662; and MDT-637) are undergoing clinical development, including some studies in the transplant setting [82].

Prevention

Prevention of RSV is limited by the lack of an approved vaccine to protect against the virus. Palivizumab is an RSV-specific monoclonal F antibody that is currently approved by the FDA and European Medicines Agency for the prevention of RSV only in pediatric patients at high risk of RSV disease. In high risk infants, palivizumab is 55% effective at reducing rates of pediatric hospitalization and has been shown to reduce morbidity although not mortality [129]. Given cost constraints, there are limited data on the use of palivizumab as preventative therapy in immunosuppressed patients and the elderly (cost per adult dose of >$10,000 in the United States) [130]. Palivizumab has been successfully utilized to prevent an outbreak of nosocomial RSV transmission in a single adult HSCT unit [131]. Palivizumab is used by some but not all pediatric lung transplant programs based on a recent survey [132].

Human Metapneumovirus

Human metapneumovirus (hMPV) is a negative-sense, single-stranded RNA virus in the Paramyxoviridae family. There are two subtypes with two subgroups of each (A1, A2, B1, and B2). Much like other members of the Paramyxoviruses, hMPV

has a propensity to cause LRTI and has been associated with high mortality. There are limited data in SOT, with the preponderance of data in lung transplant recipients. hMPV is symptomatic in about half of patients and two-thirds go on to develop LRTI [11]. hMPV has also been associated with development of CLAD and additional infectious complications including CMV reactivation in these patients [133–135].

There have been few HSCT patient studies focused on hMPV but those suggest that approximately 25% of patients develop LRTI progression at a median of 7 days after initial symptom onset (range 2–63 days). Progression to LRTI is associated with steroid use $\geq$1 mg/kg prior to URI diagnosis (HR, 5.10; P = 0.004), low lymphocyte count (HR, 3.43; $P = 0.011$), and early onset of HMPV infection after HSCT (before day 30 after HSCT; HR, 3.54; $P = 0.013$) [136]. Interestingly, baseline viral load was not associated with risk of LRTI progression. Mortality rates at 100 days in some studies have been high (reported as high as 43%), and mortality has been associated with steroid therapy, oxygen requirement >2 L, or mechanical ventilation, and bone marrow as stem cell source [137]. Reports also suggest higher attack rates in pediatric and adolescent HSCT populations [138].

Treatment and Prevention

Based on in vitro activity, some centers use inhaled, intravenous, or oral ribavirin with and without immunoglobulin for the treatment of hMPV [139–145]. Since there are no comparative studies, the clinical benefit of this approach cannot be assessed particularly since the majority of patients who receive treatment are those with severe LRTI disease; due to lack of other treatment options, these remain in clinical use [145, 146]. There are few novel drugs in the market that target hMPV, but a novel sialidase inhibitor, DAS-181, has shown some promise in animal models of hMPV infection [147]. There are no currently available vaccines to prevent hMPV and no prophylactic treatments approved that target hMPV.

Parainfluenza Viruses

PIVs are negative sense single stranded RNA viruses in the Paramyxoviridae family. There are four different serotypes (PIV-1, -2, -3, -4) [148]. The envelope is studded with two different glycoproteins; one contains hemagglutinin-neuraminidase (HN protein) activity while the other is involved in the fusion mechanism (F protein) [149]. The HN is responsible for attachment to sialic acid-containing receptors on respiratory epithelial cell surfaces. The four different serotypes of PIV also show remarkably different seasonal patterns, but together lead to a nearly yearly presence of PIV infections. PIV-1 follows a biennial pattern, marked by a dramatic increase in cases in the months of September to December of odd-numbered years [150]. Outbreaks of PIV-3 infections occur yearly, primarily in the months of April to

June. During the absence of endemic PIV-1, PIV-3 shows increased activity, either as a longer spring season or as a second but milder season in the months of November to December [151]. Similar to PIV-3, outbreaks of infection with PIV-2, which are smaller in magnitude, occur on an annual basis [151]. Data on PIV-4 is less clear, as it is less frequently tested for and isolated, making it difficult to draw conclusions on the seasonality of this serotype [150, 151].

Together PIV infections pose a large infectious burden on HSCT and lung transplant recipients [152, 153]. In multiple studies investigating the incidence of PIV infection in adult and pediatric HSCT patients, an overall incidence of 4% (0.2–30%) was identified, with an estimated 3–7% of patients becoming infected with PIV within the first 100 days of transplantation [154]. PIV-associated mortality is highest in patients with LRTI disease, who often progress to multiorgan failure, and in those who develop pulmonary co-pathogens, with an average mortality rate of 27% (0–62%) [152].

Treatment and Prevention

There are currently no approved vaccines for the prevention of PIV [155]. Likewise, there are currently no FDA approved treatments for PIV infection. Ribavirin is active against paramyxoviruses through the depletion of intracellular GTP pools secondary to the inhibition of IMP dehydrogenase [156]. However, ribavirin and IVIG have been studied in transplant patients without convincing efficacy in most retrospective nonrandomized studies. Oral, aerosolized, and intravenous ribavirin have been administered in the management of PIV but studies have not demonstrated significant improvement in viral shedding or mortality (77.3% aerosolized ribavirin vs. 86.3% no treatment) among patients with PIV-associated pneumonia when compared to no treatment [155]. Despite the negative data, ribavirin ± IVIG therapy are recommended for the treatment of severe PIV infection in transplant patients in current guidelines, due to the lack of other available therapies [35, 122].

DAS181 is a novel inhaled host-active antiviral composed of the *Actinomyces viscosus* sialidase catalytic domain linked to the human AR glycosaminoglycan-binding sequence [157]. DAS181 selectively cleaves host cell sialic acids, which are required by influenza and PIV for binding of the virus to the host cell [158]. DAS181 showed promise when used as treatment for patients who received the drug through compassionate use access to the drug, where DAS181 resulted in improved pulmonary function and reduced viral loads in most, but not all of the patients [159–165]. Nonresponders frequently had co-infections and typically died of the infection [165]. A randomized, placebo-controlled study of DAS181 in HSCT recipients with PIV was recently conducted (Clinicaltrials.gov identifier: NCT01644877). DAS181 was well tolerated but the study failed to demonstrate superiority in terms of the primary endpoint (clinical stability survival, defined as being alive with normalization of vital signs and resolution of supplemental oxygen requirements, at day 45 after randomization; 39.2% DAS181 vs. 31.4% placebo, $p = 0.29$). Post hoc analysis demonstrated a trend to higher rates of return to room air

in patients with lower tract PIV infection who did not require mechanical ventilation [166]. Based on this post hoc analysis and being granted Breakthrough Therapy Designation by the US FDA, a phase 3 trial is currently enrolling nationally (Clinicaltrials.gov identifier: NCT03808922).

Rhinovirus

Human rhinoviruses (hRhV) are *Picornaviridae* in the genus *Enterovirus*. They are small enveloped single-stranded positive sense RNA viruses [167]. While there are a large number of recognized hRV types (over 160), they can all be classified into three species (A–C). hRVs are the most commonly isolated viruses among both SOT and HSCT recipients and are associated with prolonged shedding, which can occur for months posttransplant [11, 168–171]. A high baseline viral load has been associated with more prolonged shedding for all 3 species [169]. Among lung transplant recipients, hRV is not convincingly associated with acute or chronic rejection but may be associated with an increased risk of bacterial co-infections [10, 172]. Despite a sense by many treating transplant patients that hRV infections are a nuisance, fatal cases of LRTI hRV infections have been described and reports suggest that mortality rates associated with LRTI are similar to other respiratory viruses [173, 174]. More recently, a risk score that incorporates the risk of low lymphocyte count, low albumin, positive cytomegalovirus serostatus, recipient statin use, and steroid use $\geq$2 mg/kg/day has been developed that predicts progression to LRTI among HSCT patients [175].

Treatment and Prevention

Unfortunately there are no active vaccines for hRhV, and while several drugs have been studied against hRhV, none have been licensed to date; there are no current trials of investigational agents in transplant patients [176].

Coronavirus

CoVs are a broad group of respiratory pathogens that span animal and human strains. The most common circulating human CoVs, HCoV-229E, and -NL63, and HCoV-OC43 and -HKU1, are large, enveloped single-stranded RNA viruses that are common causes of respiratory infection in transplant recipients [171]. Clinically, most patients have mild upper respiratory tract infections often with a prolonged period of asymptomatic shedding [171, 177–180]. Clinical symptoms of LRTI appear to be less frequent than other respiratory viruses among SOT and HSCT patients, but often when present co-infection is noted in patients with severe lower tract signs or symptoms. Data among HSCT recipients with clinical signs and symptoms of LRTI and detection of CoV in the lower tract demonstrate increased

oxygen requirements (60%) at presentation and mortality rates within 90 days (54%) of diagnosis were high [179].

Animal CoV of clinical significance to humans, MERS-CoV, SARS-CoV, and the recently emergent SARS-CoV-2, have resulted in high rates of mortality in regional epidemics and global pandemics [181, 182]. Data on outcomes in transplant patients are uncertain with these pandemic CoVs as few patients have been affected by MERS-CoV or SARS-CoV. When infection has been described with these emerging CoV, patients may have atypical infection or a prolonged period of asymptomatic shedding [183–185]. Data on transplant patients affected by the emergent COVID-19 are still undergoing study at this time but slightly worse outcomes have been described clinically [186–188].

Treatment and Prevention

There are no known vaccine candidates for human or emergent animal CoVs, and there are no currently available therapies which are approved for treatment. Respiratory etiquette and infection prevention strategies remain the foundation of CoV prevention. Importantly, for MERS-CoV, SARS-CoV, and COVID-19, airborne, droplet, contact, and standard precautions are recommended; eye protection is recommended for aerosol-generating procedures [189].

Adenovirus

Human adenoviruses (AdV) are divided into seven species (A–G) with over 80 serotypes [190]. They are double-stranded, nonenveloped DNA viruses. AdV can cause a range of clinical manifestations including infections of the central nervous system, the eye, the gastrointestinal tract, the genitourinary tract, and the respiratory tract; disseminated disease, defined as $\geq$2 sites of infection typically with viremia, is more common among HSCT patients and associated with the highest risk of mortality (Fig. 4). Asymptomatic AdV viremia is common in SOT recipients [191, 192]. Adenoviral hepatitis, typically caused by AdV 1, 2, or 5, is most common among liver transplant recipients and can be diagnosed by detection of viremia, elevated liver function tests, or visualization of viral intranuclear inclusions on liver biopsy [1]. AdV enterocolitis occurs in small bowel transplant recipients and may mimic rejection [193]. Adenoviral pneumonia is associated with graft loss, death, or progression to obliterative bronchiolitis in lung transplant recipients. AdV causes hemorrhagic cystitis with or without interstitial nephritis in kidney transplant recipients. Such patients typically present with fever, hematuria, and new-onset renal dysfunction [194]. AdV DNA in biopsy specimens is associated with worse outcomes among pediatric heart transplant patients [195, 196]. AdV in SOT patients is most common in pediatric transplantation, small bowel recipients, and those who receive lymphocyte depletion [1].

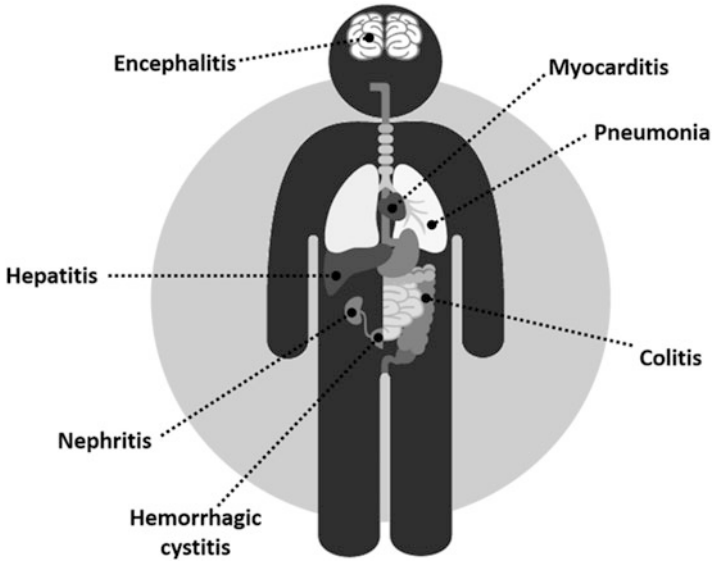


Fig. 4 Locations of adenovirus disease among transplant patients. (Most common locations for AdV disease among transplant patients include the gastro-intestinal tract, genitourinary tract and respiratory tract. This figure does not represent isolated viremia or disseminated disease (with multiple organ sites and associated viremia). Image courtesy of Kyoko Kurosawa)

Among HSCT recipients, AdV is most common among those with an allogeneic transplant, cord blood transplant, cell-depleted graft, receipt of alemtuzumab, and patients with acute graft-versus-host disease. Severe lymphopenia (<300 cells/μL) has been associated with disseminated disease. Although disseminated disease only effects 1–7% of HSCT recipients, it is associated with a significant risk of mortality (8–26%) [1]. Similar to SOT recipients, AdV can present as a number of manifestations, including pneumonia/pneumonitis, CNS disease, colitis, hepatitis, cystitis, and nephritis, and can be seen as isolated viremia in some patients (Fig. 4) [197, 198]. While the majority of AdV cases are associated with reactivation from latent infection in the recipient, patients can also develop community-acquired upper and lower respiratory tract infections, and there are reports of outbreaks of AdV on HSCT units [199]. Since early detection of AdV in stool and blood predicts disease, especially in pediatric HSCT patients, screening is often utilized to identify patients in whom interventions may reduce risk of disease progression [200–204]. A threshold of 5 $\log^{10}$ copies/g feces has been shown to be predictive of developing adenoviremia in one study [200].

Treatment and Prevention

No antiviral agents are currently FDA approved for the treatment of AdV. Ribavirin has not shown clear clinical benefit, despite having activity in vitro

[190, 205]. Cidofovir has demonstrated clinical utility using either 5 mg/kg weekly or 1 mg/kg three times a week in combination with probenecid and hydration, but significant nephrotoxicity and bone marrow toxicity limit its long term treatment of severe infections; toxicity also makes routine use of preemptive Cidofovir challenging for pediatric hematopoietic stem cell transplantation patients with persistent or rising AdV viral loads [1, 190, 205, 206]. Brincidofovir, a lipid conjugate of cidofovir available in tablet and liquid form, appears more potent against AdV than cidofovir with less bone marrow and renal toxicity [207]. The one significant toxicity that is associated with Brincidofovir is GI toxicity which often presents as diarrhea and can mimic GVHD [208, 209]. In clinical studies and case series, brincidofovir appears to be associated with antiviral and clinical benefit among HSCT and SOT patients who have been treated [207, 210–212]. Unfortunately, clinical development of the drug for AdV has been suspended although the drug remains available for compassionate use until current supplies of the drug are expended (ClinicalTrial.gov Identified: NCT02596997).

A newer approach using AdV- and multivirus-specific T cells is also being studied in prospective trials (ClinicalTrial.gov Identified: NCT03425526, NCT03378102, NCT03266627, NCT02532452, NCT02510417, NCT02007356, NCT01945814) [203, 213]. While these agents remain experimental, early studies suggest significant clinical benefit.

Bocavirus and Other Uncommon Respiratory Viruses

Bocaviruses (BoV) are single stranded nonenveloped DNA viruses of the Parvovirinae in the virus family Parvoviridae that are associated with both respiratory and GI infections. Data on prevalence and outcomes in transplant patients are limited, but among those with a documented respiratory pathogen BoV may make approximately 8% of all detected viruses. These same data suggest that BoV patients with active symptoms are more likely to have a co-pathogen when detected [214]. Increased detection among HSCT recipients in stool may be evidence that the GI tract is the more common location for this pathogen [215]. Novel enterovirus (EV), such as EV-D68, are infrequent but are known to lead to clinical illness among transplant patients that varies between mild URI to severe respiratory disease [216].

Infection Control Practices to Protect Transplant Patients from Respiratory Viruses

Transplant centers should develop standard respiratory virus protocols which can be implemented throughout floors, units and clinics where high risk immunosuppressed patients receive care. In order to track such efforts and plan for interventions, Infection Prevention teams should set up surveillance systems among transplant patients, as outbreaks of multiple respiratory virus pathogens have been seen in these

populations [14, 217, 218]. Policies should target high level compliance with hand hygiene, and protocols aimed at early identification of patients with active respiratory symptoms. Enhanced Infection Control strategies, for respiratory virus prevention including recommended contact and droplet precautions for many of these pathogens; most centers isolate patients with active respiratory symptoms (Table 1) [189]. Eye protection should be routinely used (face shield or goggles) and consideration for use of N95-level protection is recommended for aerosol-generating procedures (Fig. 5) [189]. Airborne isolation, droplet, and contact isolation (including placement in negative pressure rooms) are required for patients who are diagnosed with MERS-CoV, SARS-CoV, or 2019-nCoV due to high risk for transmission.

Placing patients with active respiratory symptoms into single rooms, masking symptomatic patients on units while outside of their rooms, shifting efforts to rapid molecular testing and implementing early treatment protocols, particularly for influenza, are targeted efforts that can help limit the spread of respiratory viruses. In addition, actively screening those who enter the unit or clinic for respiratory symptoms on arrival can assure early detection and isolation of patients and prevent transmission from caregivers or hospital staff with symptoms [15]. Some centers also restrict access to high-risk units to young children, to help limit possible exposures.

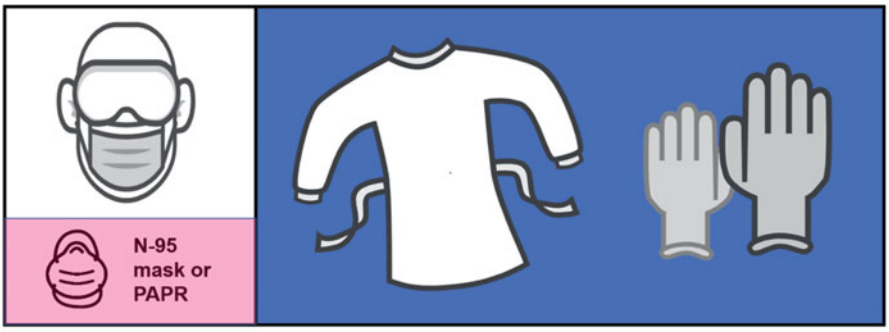


Fig. 5 Personal protective equipment for patients with respiratory viruses. Respiratory virus prevention should include personal protective equipment for contact and droplet precautions with a surgical mask (with goggles or face shield to protect eyes), a gown and gloves. Red: For some high-risk invasive procedures, such as bronchoalveolar lavage, interventionalists may want to consider wearing a fit-tested N-95 mask for infections such as Influenza. It is critical to remember that N-95 or Powered Air Purifying Respirator (PAPR) is required for emerging coronaviruses, such as MERS-CoV, SARS-CoV and 2019-nCoV. (Image courtesy of Kyoko Kurosawa)

Standardized CDC guidelines recommend contact and droplet precautions (masks with face shields or goggles) for staff caring for patients during the duration of clinical illness [189]. Masking of symptomatic patients can help limit production of secretions and help limit spread of respiratory viruses; however, routine use among patients and staff remains controversial. Many centers consider routine masking during high risk respiratory virus season (November–April), while others target all inpatients regardless of season. Older studies gave conflicting data on the utility of wearing gowns and masks to decrease nosocomial transmission, but two recent studies demonstrate a potential correlation with universal mask use, particularly for providers who come in contact with HSCT patients; albeit nonrandomized longitudinal single center comparison studies may be biased by seasonal variations [219–223]. Routine masking can help address those who are contagious prior to development of symptoms (e.g., influenza) or patients with asymptomatic shedding.

It is also critical for centers to assure staff, caregivers, and visitors avoid entering units or clinics while symptomatic. Efforts to avoid presentism can be critical as health care workers can both be affected by and possibly be linked to outbreaks in such units [15, 224]. Since outpatient care for transplant patients is often through transplant clinics, organized outpatient prevention programs are needed in clinic spaces where patients cohort together [15]. As discussed above, efforts to assure high-level influenza vaccine compliance and dedicated vaccination campaigns among healthcare workers and families/caregivers can also provide additional protection for high risk patients [225, 226]. Furthermore, dedicated educational efforts targeted towards patients and their caregivers to promote active hand hygiene, appropriate respiratory hygiene, and awareness of the risks of respiratory viruses are important.

Unique Donor and Recipient Issues

Respiratory viruses raise unique concerns of donor-derived infectious disease transmission to the recipient. In general, viremia is not a significant concern for most respiratory viruses, so risk of disease transmission is generally limited to lung donors. As such, lung donors with proven influenza, RSV, hMPV, PIV, or AdV infections are generally excluded from infection. Although current guidelines do not recommend the use of influenza-infected donors and infection has been demonstrated to be transmitted to recipients, some centers will consider the use of these organs with the concurrent use of oseltamivir in the recipient, especially if the donor has received therapy as well [35, 227–229]. Viremia and disseminated infection can occur with novel influenza viruses, including avian viruses, as well as AdV. As such, donors with these viruses generally should not be used. Donors with other respiratory viruses can generally be considered for nonlung donation without significant risk of disease transmission.

In the setting of HSCT, one controversial issue is how to proceed with transplant in recipients who have symptomatic infections at the time of induction. Symptomatic patients with viruses detected pre-HSCT had increased mortality (unadjusted HR

3.5, 95% CI 1.0–12.1, $p = 0.05$); asymptomatic patients with detected virus had no increased mortality risk [37]. As such, most experts recommend testing patients with symptoms of a URI prior to induction and delaying transplant if virus, even hRhV, is detected if feasible [5].

Conclusions

Respiratory viral pathogens remain common complications among SOT and HSCT recipients. Despite progress in improved diagnostics, transplant immunosuppression, and prevention strategies, respiratory viral pathogens are a still major cause of morbidity and mortality among transplant patients, particularly among the most immunosuppressed. Respiratory viruses often present with fever, cough, and upper respiratory symptoms, but classic symptoms seen in normal hosts may be missing in these patients, and they are more likely to progress to life-threatening pneumonia. Only influenza viruses have approved therapies, but emerging trials may provide future options for treatment for other respiratory viruses. Influenza vaccination for patients and their close contacts and infection prevention practices to prevent person-to-person transmission remain critically important to limit outbreaks and clusters in transplant centers.

Key Points

- Respiratory viruses are common causes of morbidity and mortality among transplant patients.
- Patients with respiratory viruses may present with atypical or less prominent clinical symptoms, despite being at high risk for progression to lower tract disease.
- Awareness and early diagnosis are key to managing and isolating patients with active respiratory viral symptoms.
- All patients with influenza should be treated with antiviral agents, but treating teams should be aware of increased risk for the development of resistance.
- Influenza vaccine should be promoted to all patients, caregivers/family, and staff at transplant centers.
- Aggressive infection prevention policies aimed at early detection, isolation, and treatment of patients is key to prevent outbreaks in transplant populations.

References

1. Ison MG, Hirsch HH. Community-acquired respiratory viruses in transplant patients: diversity, impact, unmet clinical needs. Clin Microbiol Rev. 2019;32(4):e00042–19.
2. Vande Vusse LK, Madtes DK. Early onset noninfectious pulmonary syndromes after hematopoietic cell transplantation. Clin Chest Med. 2017;38(2):233–48.

3. Peck AJ, Englund JA, Kuypers J, Guthrie KA, Corey L, Morrow R, et al. Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. Blood. 2007;110(5):1681–8.
4. Hayden FG. Advances in antivirals for non-influenza respiratory virus infections. Influenza Other Respir Viruses. 2013;7(Suppl 3):36–43.
5. Waghmare A, Englund JA, Boeckh M. How I treat respiratory viral infections in the setting of intensive chemotherapy or hematopoietic cell transplantation. Blood. 2016;127(22):2682–92.
6. Beigel JH, Nam HH, Adams PL, Krafft A, Ince WL, El-Kamary SS, et al. Advances in respiratory virus therapeutics – a meeting report from the 6th isirv antiviral group conference. Antivir Res. 2019;167:45–67.
7. Khanna N, Widmer AF, Decker M, Steffen I, Halter J, Heim D, et al. Respiratory syncytial virus infection in patients with hematological diseases: single-center study and review of the literature. Clin Infect Dis. 2008;46(3):402–12.
8. Haffizulla J, Hartman A, Hoppers M, Resnick H, Samudrala S, Ginocchio C, et al. Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomised, placebo-controlled, phase 2b/3 trial. Lancet Infect Dis. 2014;14(7):609–18.
9. Paulsen GC, Danziger-Isakov L. Respiratory viral infections in solid organ and hematopoietic stem cell transplantation. Clin Chest Med. 2017;38(4):707–26.
10. Vu DL, Bridevaux PO, Aubert JD, Soccal PM, Kaiser L. Respiratory viruses in lung transplant recipients: a critical review and pooled analysis of clinical studies. Am J Transplant. 2011;11(5):1071–8.
11. Peghin M, Hirsch HH, Len O, Codina G, Berastegui C, Saez B, et al. Epidemiology and immediate indirect effects of respiratory viruses in lung transplant recipients: a 5-year prospective study. Am J Transplant. 2017;17(5):1304–12.
12. Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P, et al. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67(9):1322–9.
13. Adams RH. Preemptive treatment of pediatric bone marrow transplant patients with asymptomatic respiratory syncytial virus infection with aerosolized ribavirin. Biol Blood Marrow Transplant. 2001;7(Suppl):16S–8S.
14. Kelly SG, Metzger K, Bolon MK, Silkaitis C, Mielnicki M, Cullen J, et al. Respiratory syncytial virus outbreak on an adult stem cell transplant unit. Am J Infect Control. 2016;44(9):1022–6.
15. Chu HY, Englund JA, Podczervinski S, Kuypers J, Campbell AP, Boeckh M, et al. Nosocomial transmission of respiratory syncytial virus in an outpatient cancer center. Biol Blood Marrow Transplant. 2014;20(6):844–51.
16. Chemaly RF, Shah DP, Boeckh MJ. Management of respiratory viral infections in hematopoietic cell transplant recipients and patients with hematologic malignancies. Clin Infect Dis. 2014;59(Suppl_5):S344–51.
17. Ogimi C, Krantz EM, Golob JL, Waghmare A, Liu C, Leisenring WM, et al. Antibiotic exposure prior to respiratory viral infection is associated with progression to lower respiratory tract disease in allogeneic hematopoietic cell transplant recipients. Biol Blood Marrow Transplant. 2018;24(11):2293–301.
18. Kmeid J, Vanichanan J, Shah DP, El Chaer F, Azzi J, Ariza-Heredia EJ, et al. Outcomes of influenza infections in hematopoietic cell transplant recipients: application of an immunodeficiency scoring index. Biol Blood Marrow Transplant. 2016;22(3):542–8.
19. Shah DP, Ghantoji SS, Ariza-Heredia EJ, Shah JN, El Taoum KK, Shah PK, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. Blood. 2014;123(21):3263–8.
20. Khanna N, Steffen I, Studt JD, Schreiber A, Lehmann T, Weisser M, et al. Outcome of influenza infections in outpatients after allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis. 2009;11(2):100–5.
21. Pinana JL, Gomez MD, Perez A, Madrid S, Balaguer-Rosello A, Gimenez E, et al. Community-acquired respiratory virus lower respiratory tract disease in allogeneic stem cell

transplantation recipient: risk factors and mortality from pulmonary virus-bacterial mixed infections. Transpl Infect Dis. 2018;20(4):e12926.
22. Cordero E, Perez-Romero P, Moreno A, Len O, Montejo M, Vidal E, et al. Pandemic influenza A(H1N1) virus infection in solid organ transplant recipients: impact of viral and non-viral co-infection. Clin Microbiol Infect. 2012;18(1):67–73.
23. Hodson A, Kasliwal M, Streetly M, MacMahon E, Raj K. A parainfluenza-3 outbreak in a SCT unit: sepsis with multi-organ failure and multiple co-pathogens are associated with increased mortality. Bone Marrow Transplant. 2011;46(12):1545–50.
24. Fisher CE, Preiksaitis CM, Lease ED, Edelman J, Kirby KA, Leisenring WM, et al. Symptomatic respiratory virus infection and chronic lung allograft dysfunction. Clin Infect Dis. 2016;62(3):313–9.
25. Garcia-Vidal C, Barba P, Arnan M, Moreno A, Ruiz-Camps I, Gudiol C, et al. Invasive aspergillosis complicating pandemic influenza A (H1N1) infection in severely immunocompromised patients. Clin Infect Dis. 2011;53(6):e16–9.
26. Gregson AL. Infectious triggers of chronic lung allograft dysfunction. Curr Infect Dis Rep. 2016;18(7):21.
27. Martin-Gandul C, Mueller NJ, Pascual M, Manuel O. The impact of infection on chronic allograft dysfunction and allograft survival after solid organ transplantation. Am J Transplant. 2015;15(12):3024–40.
28. Fisher CE, Mohanakumar T, Limaye AP. Respiratory virus infections and chronic lung allograft dysfunction: assessment of virology determinants. J Heart Lung Transplant. 2016;35(7):946–7.
29. Peghin M, Los-Arcos I, Hirsch HH, Codina G, Monforte V, Bravo C, et al. Community-acquired respiratory viruses are a risk factor for chronic lung allograft dysfunction. Clin Infect Dis. 2019;69(7):1192–7.
30. Erard V, Chien JW, Kim HW, Nichols WG, Flowers ME, Martin PJ, et al. Airflow decline after myeloablative allogeneic hematopoietic cell transplantation: the role of community respiratory viruses. J Infect Dis. 2006;193(12):1619–25.
31. Pinana JL, Gomez MD, Montoro J, Lorenzo I, Perez A, Gimenez E, et al. Incidence, risk factors, and outcome of pulmonary invasive fungal disease after respiratory virus infection in allogeneic hematopoietic stem cell transplantation recipients. Transpl Infect Dis. 2019;21(5):e13158.
32. Versluys AB, Rossen JW, van Ewijk B, Schuurman R, Bierings MB, Boelens JJ. Strong association between respiratory viral infection early after hematopoietic stem cell transplantation and the development of life-threatening acute and chronic alloimmune lung syndromes. Biol Blood Marrow Transplant. 2010;16(6):782–91.
33. von Lilienfeld-Toal M, Berger A, Christopeit M, Hentrich M, Heussel CP, Kalkreuth J, et al. Community acquired respiratory virus infections in cancer patients-guideline on diagnosis and management by the Infectious Diseases Working Party of the German Society for haematology and Medical Oncology. Eur J Cancer. 2016;67:200–12.
34. Boonyaratanakornkit J, Vivek M, Xie H, Pergam SA, Cheng GS, Mielcarek M, et al. Predictive value of respiratory viral detection in the upper respiratory tract for infection of the lower respiratory tract with hematopoietic stem cell transplantation. J Infect Dis. 2020;221(3):379–388.
35. Manuel O, Estabrook M, American Society of Transplantation Infectious Diseases Community of Practice. RNA respiratory viral infections in solid organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33(9):e13511.
36. Uyeki TM, Bernstein HH, Bradley JS, Englund JA, File TM, Fry AM, et al. Clinical practice guidelines by the Infectious Diseases Society of America: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenzaa. Clin Infect Dis. 2019;68(6):895–902.
37. Campbell AP, Guthrie KA, Englund JA, Farney RM, Minerich EL, Kuypers J, et al. Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. Clin Infect Dis. 2015;61(2):192–202.

38. Reid G, Huprikar S, Patel G, Razonable RR, Mossad S, Levi M, et al. A multicenter evaluation of pandemic influenza A/H1N1 in hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2013;15(5):487–92.
39. Mohty B, Thomas Y, Vukicevic M, Nagy M, Levrat E, Bernimoulin M, et al. Clinical features and outcome of 2009-influenza A (H1N1) after allogeneic hematopoietic SCT. Bone Marrow Transplant. 2012;47(2):236–42.
40. Hayden FG, Sugaya N, Hirotsu N, Lee N, de Jong MD, Hurt AC, et al. Baloxavir Marboxil for uncomplicated influenza in adults and adolescents. N Engl J Med. 2018;379(10):913–23.
41. Hirotsu N, Sakaguchi H, Sato C, Ishibashi T, Baba K, Omoto S, et al. Baloxavir marboxil in Japanese pediatric patients with influenza: safety and clinical and virologic outcomes. Clin Infect Dis. 2019.
42. Ison MG, Portsmouth S, Yoshida Y, Shishido T, Hayden F, Uehara T. LB16. Phase 3 trial of Baloxavir Marboxil in high-risk influenza patients (CAPSTONE-2 study). Open Forum Infect Dis. 2018;5(Suppl 1):s764–s5.
43. Ikematsu H, Kawai N, Tani N, Chong Y, Iwaki N, Bando T, et al. Duration of fever and PA/I38X-substituted virus emergence in patients treated with baloxavir in the 2018–2019 influenza season. J Infect Chemother. 2019.
44. Imai M, Yamashita M, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Kiso M, Murakami J, et al. Influenza a variants with reduced susceptibility to baloxavir isolated from Japanese patients are fit and transmit through respiratory droplets. Nat Microbiol. 2020;5(1):27–33.
45. Omoto S, Speranzini V, Hashimoto T, Noshi T, Yamaguchi H, Kawai M, et al. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Sci Rep. 2018;8(1):9633.
46. Takashita E, Ichikawa M, Morita H, Ogawa R, Fujisaki S, Shirakura M, et al. Human-to-human transmission of influenza A(H3N2) virus with reduced susceptibility to Baloxavir, Japan, February 2019. Emerg Infect Dis. 2019;25(11):2108–11.
47. Takashita E, Kawakami C, Morita H, Ogawa R, Fujisaki S, Shirakura M, et al. Detection of influenza A(H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan, December 2018. Euro Surveill. 2019;24(3).
48. Takashita E, Kawakami C, Ogawa R, Morita H, Fujisaki S, Shirakura M, et al. Influenza A (H3N2) virus exhibiting reduced susceptibility to baloxavir due to a polymerase acidic subunit I38T substitution detected from a hospitalised child without prior baloxavir treatment, Japan, January 2019. Euro Surveill. 2019;24(12).
49. Takashita E, Morita H, Ogawa R, Nakamura K, Fujisaki S, Shirakura M, et al. Susceptibility of influenza viruses to the novel cap-dependent endonuclease inhibitor Baloxavir Marboxil. Front Microbiol. 2018;9:3026.
50. Uehara T, Hayden FG, Kawaguchi K, Omoto S, Hurt AC, De Jong MD, et al. Treatment-emergent influenza variant viruses with reduced baloxavir susceptibility: impact on clinical and virologic outcomes in uncomplicated influenza. J Infect Dis. 2020;221(3):346–355.
51. Ison MG, Sharma A, Shepard JA, Wain JC, Ginns LC. Outcome of influenza infection managed with oseltamivir in lung transplant recipients. J Heart Lung Transplant. 2008;27(3):282–8.
52. Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P, et al. A five-year prospective multi-center evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67(9):1322–1329.
53. Kumar D, Michaels MG, Morris MI, Green M, Avery RK, Liu C, et al. Outcomes from pandemic influenza a H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. Lancet Infect Dis. 2010;10(8):521–6.
54. Mitha E, Krivan G, Jacobs F, Nagler A, Alrabaa S, Mykietiuk A, et al. Safety, resistance, and efficacy results from a phase IIIb study of conventional- and double-dose oseltamivir regimens for treatment of influenza in immunocompromised patients. Infect Dis Ther. 2019;8(4):613–26.
55. Ison MG, Hirsch HH. Influenza: a recurrent challenge to transplantation. Transpl Infect Dis. 2010;12(2):95–7.

56. Lee N, Hui DS, Zuo Z, Ngai KL, Lui GC, Wo SK, et al. A prospective intervention study on higher-dose oseltamivir treatment in adults hospitalized with influenza a and B infections. Clin Infect Dis. 2013;57(11):1511–9.
57. South East Asia Infectious Disease Clinical Research Network. Effect of double dose oseltamivir on clinical and virological outcomes in children and adults admitted to hospital with severe influenza: double blind randomised controlled trial. BMJ. 2013;346:f3039.
58. Gaur AH, Bagga B, Barman S, Hayden R, Lamptey A, Hoffman JM, et al. Intravenous zanamivir for oseltamivir-resistant 2009 H1N1 influenza. N Engl J Med. 2010;362(1):88–9.
59. Takashita E, Fujisaki S, Shirakura M, Nakamura K, Kishida N, Kuwahara T, et al. Influenza A (H1N1)pdm09 virus exhibiting enhanced cross-resistance to oseltamivir and peramivir due to a dual H275Y/G147R substitution, Japan, March 2016. Euro Surveill. 2016;21(24). https://doi.org/10.2807/1560-7917.ES.2016.21.24.30258.
60. L'Huillier AG, Abed Y, Petty TJ, Cordey S, Thomas Y, Bouhy X, et al. E119D neuraminidase mutation conferring Pan-resistance to neuraminidase inhibitors in an A(H1N1)pdm09 isolate from a stem-cell transplant recipient. J Infect Dis. 2015;212(11):1726–34.
61. Seo S, Englund JA, Nguyen JT, Pukrittayakamee S, Lindegardh N, Tarning J, et al. Combination therapy with amantadine, oseltamivir and ribavirin for influenza A infection: safety and pharmacokinetics. Antivir Ther. 2013;18(3):377–86.
62. Krueger KM, Ison MG, Ghossein C. Practical guide to vaccination in all stages of CKD, including patients treated by dialysis or kidney transplantation. Am J Kidney Dis. 2019.
63. Grohskopf LA, Alyanak E, Broder KR, Walter EB, Fry AM, Jernigan DB. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee On Immunization Practices - United States, 2019-20 influenza season. MMWR Recomm Rep. 2019;68(3):1–21.
64. Carpenter PA, Englund JA. How I vaccinate blood and marrow transplant recipients. Blood. 2016;127(23):2824–32.
65. Bosaeed M, Kumar D. Seasonal influenza vaccine in immunocompromised persons. Hum Vaccin Immunother. 2018;14(6):1311–22.
66. Hirzel C, Kumar D. Influenza vaccine strategies for solid organ transplant recipients. Curr Opin Infect Dis. 2018;31(4):309–15.
67. Chong PP, Avery RK. A comprehensive review of immunization practices in solid organ transplant and hematopoietic stem cell transplant recipients. Clin Ther. 2017;39(8):1581–98.
68. Mohty B, Bel M, Vukicevic M, Nagy M, Levrat E, Meier S, et al. Graft-versus-host disease is the major determinant of humoral responses to the AS03-adjuvanted influenza A/09/H1N1 vaccine in allogeneic hematopoietic stem cell transplant recipients. Haematologica. 2011;96(6):896–904.
69. Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant. 2008;42(10):637–41.
70. Kumar A, McElhaney JE, Walrond L, Cyr TD, Merani S, Kollmann TR, et al. Cellular immune responses of older adults to four influenza vaccines: results of a randomized, controlled comparison. Hum Vaccin Immunother. 2017;13(9):2048–57.
71. Kumar D, Campbell P, Hoschler K, Hidalgo L, Al-Dabbagh M, Wilson L, et al. Randomized controlled trial of adjuvanted versus nonadjuvanted influenza vaccine in kidney transplant recipients. Transplantation. 2016;100(3):662–9.
72. Natori Y, Humar A, Lipton J, Kim DD, Ashton P, Hoschler K, et al. A pilot randomized trial of adjuvanted influenza vaccine in adult allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2017;52(7):1016–21.
73. Cordero E, Roca-Oporto C, Bulnes-Ramos A, Aydillo T, Gavalda J, Moreno A, et al. Two doses of inactivated influenza vaccine improve immune response in solid organ transplant recipients: results of TRANSGRIPE 1-2, a randomized controlled clinical trial. Clin Infect Dis. 2017;64(7):829–38.

74. Cordonnier C, Einarsdottir S, Cesaro S, Di Blasi R, Mikulska M, Rieger C, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European conference on infections in leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(6):e200–e12.
75. Natori Y, Shiotsuka M, Slomovic J, Hoschler K, Ferreira V, Ashton P, et al. A double-blind, randomized trial of high-dose vs standard-dose influenza vaccine in adult solid-organ transplant recipients. Clin Infect Dis. 2018;66(11):1698–704.
76. Halasa NB, Savani BN, Asokan I, Kassim A, Simons R, Summers C, et al. Randomized double-blind study of the safety and immunogenicity of standard-dose trivalent inactivated influenza vaccine versus high-dose trivalent inactivated influenza vaccine in adult hematopoietic stem cell transplantation patients. Biol Blood Marrow Transplant. 2016;22(3):528–35.
77. Kumar D, Blumberg EA, Danziger-Isakov L, Kotton CN, Halasa NB, Ison MG, et al. Influenza vaccination in the organ transplant recipient: review and summary recommendations. Am J Transplant. 2011;11(10):2020–30.
78. Danziger-Isakov L, Kumar D, Practice AICo. Vaccination of solid organ transplant candidates and recipients: Guidelines from the American society of transplantation infectious diseases community of practice. Clin Transplant. 2019;33(9):e13563.
79. Ison MG, Szakaly P, Shapira MY, Krivan G, Nist A, Dutkowski R. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. Antivir Ther. 2012;17(6):955–64.
80. Anderson LJ, Hierholzer JC, Tsou C, Hendry RM, Fernie BF, Stone Y, et al. Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. J Infect Dis. 1985;151(4):626–33.
81. Pangesti KNA, Abd El Ghany M, Walsh MG, Kesson AM, Hill-Cawthorne GA. Molecular epidemiology of respiratory syncytial virus. Rev Med Virol. 2018;28(2). https://doi.org/10.1002/rmv.1968
82. Nam HH, Ison MG. Respiratory syncytial virus infection in adults. BMJ. 2019;366:l5021.
83. Weigt SS, Gregson AL, Deng JC, Lynch JP 3rd, Belperio JA. Respiratory viral infections in hematopoietic stem cell and solid organ transplant recipients. Semin Respir Crit Care Med. 2011;32(4):471–93.
84. Lopez-Medrano F, Aguado JM, Lizasoain M, Folgueira D, Juan RS, Diaz-Pedroche C, et al. Clinical implications of respiratory virus infections in solid organ transplant recipients: a prospective study. Transplantation. 2007;84(7):851–6.
85. Belperio JA, Weigt SS, Fishbein MC, Lynch JP 3rd. Chronic lung allograft rejection: mechanisms and therapy. Proc Am Thorac Soc. 2009;6(1):108–21.
86. Waghmare A, Campbell AP, Xie H, Seo S, Kuypers J, Leisenring W, et al. Respiratory syncytial virus lower respiratory disease in hematopoietic cell transplant recipients: viral RNA detection in blood, antiviral treatment, and clinical outcomes. Clin Infect Dis. 2013;57(12):1731–41.
87. Chemaly RF, Ghosh S, Bodey GP, Rohatgi N, Safdar A, Keating MJ, et al. Respiratory viral infections in adults with hematologic malignancies and human stem cell transplantation recipients: a retrospective study at a major cancer center. Medicine (Baltimore). 2006;85(5):278–87.
88. Ljungman P. Respiratory syncytial virus in hematopoietic cell transplant recipients: factors determining progression to lower respiratory tract disease. J Infect Dis. 2014;209(8):1151–2.
89. Shah JN, Chemaly RF. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. Blood. 2011;117(10):2755–63.
90. Broadbent L, Groves H, Shields MD, Power UF. Respiratory syncytial virus, an ongoing medical dilemma: an expert commentary on respiratory syncytial virus prophylactic and therapeutic pharmaceuticals currently in clinical trials. Influenza Other Respir Viruses. 2015;9(4):169–78.
91. Chemaly RF, Aitken SL, Wolfe CR, Jain R, Boeckh MJ. Aerosolized ribavirin: the most expensive drug for pneumonia. Transpl Infect Dis. 2016;18(4):634–6.

92. Beaird OE, Freifeld A, Ison MG, Lawrence SJ, Theodoropoulos N, Clark NM, et al. Current practices for treatment of respiratory syncytial virus and other non-influenza respiratory viruses in high-risk patient populations: a survey of institutions in the Midwestern respiratory virus collaborative. Transpl Infect Dis. 2016;18(2):210–5.
93. Trang TP, Whalen M, Hilts-Horeczko A, Doernberg SB, Liu C. Comparative effectiveness of aerosolized versus oral ribavirin for the treatment of respiratory syncytial virus infections: a single-center retrospective cohort study and review of the literature. Transpl Infect Dis. 2018;20(2):e12844.
94. Ghosh S, Champlin RE, Englund J, Giralt SA, Rolston K, Raad I, et al. Respiratory syncytial virus upper respiratory tract illnesses in adult blood and marrow transplant recipients: combination therapy with aerosolized ribavirin and intravenous immunoglobulin. Bone Marrow Transplant. 2000;25(7):751–5.
95. McCarthy AJ, Kingman HM, Kelly C, Taylor GS, Caul EO, Grier D, et al. The outcome of 26 patients with respiratory syncytial virus infection following allogeneic stem cell transplantation. Bone Marrow Transplant. 1999;24(12):1315–22.
96. Nichols WG, Gooley T, Boeckh M. Community-acquired respiratory syncytial virus and parainfluenza virus infections after hematopoietic stem cell transplantation: the Fred Hutchinson Cancer Research Center experience. Biol Blood Marrow Transplant. 2001;7 (Suppl):11S–5S.
97. Lewinsohn DM, Bowden RA, Mattson D, Crawford SW. Phase I study of intravenous ribavirin treatment of respiratory syncytial virus pneumonia after marrow transplantation. Antimicrob Agents Chemother. 1996;40(11):2555–7.
98. Chemaly RF, Torres HA, Munsell MF, Shah DP, Rathod DB, Bodey GP, et al. An adaptive randomized trial of an intermittent dosing schedule of aerosolized ribavirin in patients with cancer and respiratory syncytial virus infection. J Infect Dis. 2012;206(9):1367–71.
99. Abe S, Miyamura K, Oba T, Terakura S, Kasai M, Kitaori K, et al. Oral ribavirin for severe adenovirus infection after allogeneic marrow transplantation. Bone Marrow Transplant. 2003;32(11):1107–8.
100. Burrows FS, Carlos LM, Benzimra M, Marriott DJ, Havryk AP, Plit ML, et al. Oral ribavirin for respiratory syncytial virus infection after lung transplantation: efficacy and cost-efficiency. J Heart Lung Transplant. 2015;34(7):958–62.
101. Casey J, Morris K, Narayana M, Nakagaki M, Kennedy GA. Oral ribavirin for treatment of respiratory syncitial virus and parainfluenza 3 virus infections post allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant. 2013;48(12):1558–61.
102. Chakrabarti S, Collingham KE, Holder K, Fegan CD, Osman H, Milligan DW. Pre-emptive oral ribavirin therapy of paramyxovirus infections after haematopoietic stem cell transplantation: a pilot study. Bone Marrow Transplant. 2001;28(8):759–63.
103. Dai D, Chen H, Tang J, Tang Y. Inhibition of mTOR/eIF4E by anti-viral drug ribavirin effectively enhances the effects of paclitaxel in oral tongue squamous cell carcinoma. Biochem Biophys Res Commun. 2017;482(4):1259–64.
104. de Zwart AES, Riezebos-Brilman A, Kerstjens HAM, Verschuuren EAM, Alffenaar JC. Repurposed oral ribavirin for respiratory virus infections requires pharmacokinetic-pharmacodynamics for dose optimization. Clin Infect Dis. 2020;70(6):1258.
105. Dreyzin A, Michaels MG, Vander Lugt MT, Szabolcs P. Oral ribavirin for paramyxovirus infection after alemtuzumab-containing reduced-intensity conditioning HCT regimen. Pediatr Transplant. 2019;23(2):e13358.
106. Foolad F, Aitken SL, Shigle TL, Prayag A, Ghantoji S, Ariza-Heredia E, et al. Oral versus aerosolized ribavirin for the treatment of respiratory syncytial virus infections in hematopoietic cell transplant recipients. Clin Infect Dis. 2019;68(10):1641–9.
107. Fuehner T, Dierich M, Duesberg C, DeWall C, Welte T, Haverich A, et al. Single-centre experience with oral ribavirin in lung transplant recipients with paramyxovirus infections. Antivir Ther. 2011;16(5):733–40.

108. Garcia B, Sharma N, Johnson K, Salgado J, Wille K. Clinical outcomes of paramyxovirus infections in lung transplant recipients treated with oral ribavirin: a two-center case series. Exp Clin Transplant. 2019;17(3):393–7.
109. Gorcea CM, Tholouli E, Turner A, Saif M, Davies E, Battersby E, et al. Effective use of oral ribavirin for respiratory syncytial viral infections in allogeneic haematopoietic stem cell transplant recipients. J Hosp Infect. 2017;95(2):214–7.
110. Jain SR, Phoompoung P, Husain S. Is Oral ribavirin for the treatment of respiratory syncytial virus in high-risk hematopoietic stem cell transplant recipients really safe? Clin Infect Dis. 2019;69(12):2234–5.
111. Keck M, Mindru C, Kalil AC, Mercer DF, Florescu DF. Respiratory syncytial virus lower respiratory tract infection in a pediatric liver transplant recipient treated with oral ribavirin. Pediatr Transplant. 2012;16(8):E348–51.
112. Li L, Avery R, Budev M, Mossad S, Danziger-Isakov L. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. J Heart Lung Transplant. 2012;31(8):839–44.
113. Marcelin JR, Wilson JW, Razonable RR, Mayo Clinic HO. Transplant infectious diseases S. Oral ribavirin therapy for respiratory syncytial virus infections in moderately to severely immunocompromised patients. Transpl Infect Dis. 2014;16(2):242–50.
114. Mori T, Nakamura Y, Kato J, Yamane A, Aisa Y, Takeshita K, et al. Oral ribavirin therapy for lower respiratory tract infection of respiratory syncytial virus complicating bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2011;93(1):132–4.
115. Pelaez A, Lyon GM, Force SD, Ramirez AM, Neujahr DC, Foster M, et al. Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. J Heart Lung Transplant. 2009;28(1):67–71.
116. Permpalung N, Thaniyavarn T, Saullo JL, Arif S, Miller RA, Reynolds JM, et al. Oral and inhaled ribavirin treatment for respiratory syncytial virus infection in lung transplant recipients. Transplantation. 2020;104(6):1280–1286.
117. Pinana JL, Hernandez-Boluda JC, Calabuig M, Ballester I, Marin M, Madrid S, et al. A risk-adapted approach to treating respiratory syncytial virus and human parainfluenza virus in allogeneic stem cell transplantation recipients with oral ribavirin therapy: a pilot study. Transpl Infect Dis. 2017;19(4). https://doi.org/10.1111/tid.12729.
118. Shima T, Yoshimoto G, Nonami A, Yoshida S, Kamezaki K, Iwasaki H, et al. Successful treatment of parainfluenza virus 3 pneumonia with oral ribavirin and methylprednisolone in a bone marrow transplant recipient. Int J Hematol. 2008;88(3):336–40.
119. Yoon BW, Song YG, Lee SH. Severe community-acquired adenovirus pneumonia treated with oral ribavirin: a case report. BMC Res Notes. 2017;10(1):47.
120. Glanville AR, Scott AI, Morton JM, Aboyoun CL, Plit ML, Carter IW, et al. Intravenous ribavirin is a safe and cost-effective treatment for respiratory syncytial virus infection after lung transplantation. J Heart Lung Transplant. 2005;24(12):2114–9.
121. Chemaly RF, Dadwal SS, Bergeron A, Ljungman P, Kim YJ, Cheng GS, et al. A phase 2, randomized, double-blind, placebo-controlled trial of presatovir for the treatment of respiratory syncytial virus upper respiratory tract infection in hematopoietic-cell transplant recipients. Clin Infect Dis. 2019.
122. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European conference on infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. Clin Infect Dis. 2013;56(2):258–66.
123. Falsey AR, Koval C, DeVincenzo JP, Walsh EE. Compassionate use experience with high-titer respiratory syncytical virus (RSV) immunoglobulin in RSV-infected immunocompromised persons. Transpl Infect Dis. 2017;19(2). https://doi.org/10.1111/tid.12657.

124. de Fontbrune FS, Robin M, Porcher R, Scieux C, de Latour RP, Ferry C, et al. Palivizumab treatment of respiratory syncytial virus infection after allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2007;45(8):1019–24.
125. Liu V, Dhillon GS, Weill D. A multi-drug regimen for respiratory syncytial virus and parainfluenza virus infections in adult lung and heart-lung transplant recipients. Transpl Infect Dis. 2010;12(1):38–44.
126. Seo S, Campbell AP, Xie H, Chien JW, Leisenring WM, Englund JA, et al. Outcome of respiratory syncytial virus lower respiratory tract disease in hematopoietic cell transplant recipients receiving aerosolized ribavirin: significance of stem cell source and oxygen requirement. Biol Blood Marrow Transplant. 2013;19(4):589–96.
127. Simon A, Karsten V, Cehelsky J, Shah S, Gollob J, Meyers R, et al. Results of a phase 2b multi-center trial of ALN-RSV01 in respiratory syncytial virus (RSV)-infected lung transplant patients. Eur Respir J 2012;40(Suppl 56):P1476.
128. DeVincenzo JP, Whitley RJ, Mackman RL, Scaglioni-Weinlich C, Harrison L, Farrell E, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. N Engl J Med. 2014;371(8):711–22.
129. American Academy of Pediatrics Committee on Infectious Diseases, American Academy of Pediatrics Bronchiolitis Guidelines Committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. Pediatrics. 2014;134(2):415–20.
130. Shadman KA, Wald ER. A review of palivizumab and emerging therapies for respiratory syncytial virus. Expert Opin Biol Ther. 2011;11(11):1455–67.
131. Kassis C, Champlin RE, Hachem RY, Hosing C, Tarrand JJ, Perego CA, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. Biol Blood Marrow Transplant. 2010;16(9):1265–71.
132. Danziger-Isakov LA, Arslan D, Sweet S, Benden C, Goldfarb S, Wong J. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the international pediatric lung transplant collaborative. Pediatr Transplant. 2012;16 (6):638–44.
133. Dosanjh A. Respiratory metapneumoviral infection without co-infection in association with acute and chronic lung allograft dysfunction. J Inflamm Res. 2015;8:79–82.
134. Peghin M, Los-Arcos I, Hirsch HH, Len O, Codina G, Monforte V, et al. Respiratory viruses are a risk factor for chronic lung allograft dysfunction. Clin Infect Dis. 2019;69(7):1192–1197.
135. Peghin M, Los-Arcos I, Hirsch HH, Codina G, Monforte V, Bravo C, et al. Community-acquired respiratory viruses are a risk factor for chronic lung allograft dysfunction Clin Infect Dis. 2018.
136. Seo S, Gooley TA, Kuypers JM, Stednick Z, Jerome KR, Englund JA, et al. Human Metapneumovirus infections following hematopoietic cell transplantation: factors associated with disease progression. Clin Infect Dis. 2016;63(2):178–85.
137. Renaud C, Xie H, Seo S, Kuypers J, Cent A, Corey L, et al. Mortality rates of human metapneumovirus and respiratory syncytial virus lower respiratory tract infections in hematopoietic cell transplantation recipients. Biol Blood Marrow Transplant. 2013;19(8):1220–6.
138. Martinez-Rodriguez C, Banos-Lara MDR. HMPV in immunocompromised patients: frequency and severity in pediatric oncology patients. Pathogens. 2020;9(1):51.
139. Kitanovski L, Kopriva S, Pokorn M, Dolnicar MB, Rajic V, Stefanovic M, et al. Treatment of severe human metapneumovirus (hMPV) pneumonia in an immunocompromised child with oral ribavirin and IVIG. J Pediatr Hematol Oncol. 2013;35(7):e311–3.
140. Park SY, Baek S, Lee SO, Choi SH, Kim YS, Woo JH, et al. Efficacy of oral ribavirin in hematologic disease patients with paramyxovirus infection: analytic strategy using propensity scores. Antimicrob Agents Chemother. 2013;57(2):983–9.
141. Raza K, Ismailjee SB, Crespo M, Studer SM, Sanghavi S, Paterson DL, et al. Successful outcome of human metapneumovirus (hMPV) pneumonia in a lung transplant recipient treated with intravenous ribavirin. J Heart Lung Transplant. 2007;26(8):862–4.
142. Shachor-Meyouhas Y, Ben-Barak A, Kassis I. Treatment with oral ribavirin and IVIG of severe human metapneumovirus pneumonia (HMPV) in immune compromised child. Pediatr Blood Cancer. 2011;57(2):350–1.

143. Weinberg A, Lyu DM, Li S, Marquesen J, Zamora MR. Incidence and morbidity of human metapneumovirus and other community-acquired respiratory viruses in lung transplant recipients. Transpl Infect Dis. 2010;12(4):330–5.
144. Wyde PR, Chetty SN, Jewell AM, Boivin G, Piedra PA. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin in vitro. Antivir Res. 2003;60(1):51–9.
145. Shah DP, Shah PK, Azzi JM, El Chaer F, Chemaly RF. Human metapneumovirus infections in hematopoietic cell transplant recipients and hematologic malignancy patients: a systematic review. Cancer Lett. 2016;379(1):100–6.
146. Spahr Y, Tschudin-Sutter S, Baettig V, Compagno F, Tamm M, Halter J, et al. Community-acquired respiratory paramyxovirus infection after allogeneic hematopoietic cell transplantation: a single-center experience. Open Forum Infect Dis. 2018;5(5):ofy077.
147. Thammawat S, Sadlon TA, Adamson P, Gordon DL. Effect of sialidase fusion protein (DAS 181) on human metapneumovirus infection of Hep-2 cells. Antivir Chem Chemother. 2015;24(5–6):161–5.
148. Henrickson KJ. Parainfluenza viruses. Clin Microbiol Rev. 2003;16(2):242–64.
149. White JM. Viral and cellular membrane fusion proteins. Annu Rev Physiol. 1990;52:675–97.
150. Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med. 2001;344(25):1917–28.
151. Fry AM, Curns AT, Harbour K, Hutwagner L, Holman RC, Anderson LJ. Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. Clin Infect Dis. 2006;43(8):1016–22.
152. Shah DP, Shah PK, Azzi JM, Chemaly RF. Parainfluenza virus infections in hematopoietic cell transplant recipients and hematologic malignancy patients: a systematic review. Cancer Lett. 2016;370(2):358–64.
153. Chemaly RF, Hanmod SS, Rathod DB, Ghantoji SS, Jiang Y, Doshi A, et al. The characteristics and outcomes of parainfluenza virus infections in 200 patients with leukemia or recipients of hematopoietic stem cell transplantation. Blood. 2012;119(12):2738–45.. quiz 969
154. Ustun C, Slaby J, Shanley RM, Vydra J, Smith AR, Wagner JE, et al. Human parainfluenza virus infection after hematopoietic stem cell transplantation: risk factors, management, mortality, and changes over time. Biol Blood Marrow Transplant. 2012;18(10):1580–8.
155. Russell E, Ison MG. Parainfluenza virus in the hospitalized adult. Clin Infect Dis. 2017;65 (9):1570–6.
156. Leyssen P, Balzarini J, De Clercq E, Neyts J. The predominant mechanism by which ribavirin exerts its antiviral activity in vitro against flaviviruses and paramyxoviruses is mediated by inhibition of IMP dehydrogenase. J Virol. 2005;79(3):1943–7.
157. Moss RB, Davey RT, Steigbigel RT, Fang F. Targeting pandemic influenza: a primer on influenza antivirals and drug resistance. J Antimicrob Chemother. 2010;65(6):1086–93.
158. Branche AR, Falsey AR. Parainfluenza virus infection. Semin Respir Crit Care Med. 2016;37(4):538–54.
159. Chalkias S, Mackenzie MR, Gay C, Dooley C, Marty FM, Moss RB, et al. DAS181 treatment of hematopoietic stem cell transplant patients with parainfluenza virus lung disease requiring mechanical ventilation. Transpl Infect Dis. 2014;16(1):141–4.
160. Waghmare A, Wagner T, Andrews R, Smith S, Kuypers J, Boeckh M, et al. Successful treatment of parainfluenza virus respiratory tract infection with DAS181 in 4 immunocompromised children. J Pediatric Infect Dis Soc. 2015;4(2):114–8.
161. Chen YB, Driscoll JP, McAfee SL, Spitzer TR, Rosenberg ES, Sanders R, et al. Treatment of parainfluenza 3 infection with DAS181 in a patient after allogeneic stem cell transplantation. Clin Infect Dis. 2011;53(7):e77–80.
162. Guzman-Suarez BB, Buckley MW, Gilmore ET, Vocca E, Moss R, Marty FM, et al. Clinical potential of DAS181 for treatment of parainfluenza-3 infections in transplant recipients. Transpl Infect Dis. 2012;14(4):427–33.
163. Dhakal B, D'Souza A, Pasquini M, Saber W, Fenske TS, Moss RB, et al. DAS181 treatment of severe parainfluenza virus 3 pneumonia in allogeneic hematopoietic stem cell transplant recipients requiring mechanical ventilation. Case Rep Med. 2016;2016:8503275.

164. Drozd DR, Limaye AP, Moss RB, Sanders RL, Hansen C, Edelman JD, et al. DAS181 treatment of severe parainfluenza type 3 pneumonia in a lung transplant recipient. Transpl Infect Dis. 2013;15(1):E28–32.
165. Salvatore M, Satlin MJ, Jacobs SE, Jenkins SG, Schuetz AN, Moss RB, et al. DAS181 for the treatment of parainfluenza virus infections in 16 hematopoietic stem cell transplant recipients at a single center. Biol Blood Marrow Transplant. 2016.
166. Chemaly RF, Moss R, Marty FM, Wolfe CR, Lawrence SJ, Dadwal S, et al. A randomized, double-blind, placebo-controlled multicenter phase 2 trial to examine the effects of DAS181 in immunocompromised (IC) patients with parainfluenza virus (PIV) lower respiratory tract infection (LRTI) on supplemental oxygen (SO). IDWeek 2018; San Francisco, CA. 2018.
167. Bioinformatics SSIo. ViralZone:www.expasy.org/viralzone. 2019. [ViralZone is a SIB Swiss Institute of Bioinformatics web-resource for all viral genus and families, providing general molecular and epidemiological information, along with virion and genome figures. Each virus or family page gives an easy access to UniProtKB/Swiss-Prot viral protein entries].
168. Ambrosioni J, Bridevaux PO, Aubert JD, Soccal P, Wagner G, Kaiser L. Role of rhinovirus load in the upper respiratory tract and severity of symptoms in lung transplant recipients. J Clin Virol. 2015;64:1–5.
169. Ogimi C, Xie H, Leisenring WM, Kuypers JM, Jerome KR, Campbell AP, et al. Initial high viral load is associated with prolonged shedding of human rhinovirus in allogeneic hematopoietic cell transplant recipients. Biol Blood Marrow Transplant. 2018;24(10):2160–3.
170. Kaiser L, Aubert JD, Pache JC, Deffernez C, Rochat T, Garbino J, et al. Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med. 2006;174(12):1392–9.
171. Milano F, Campbell AP, Guthrie KA, Kuypers J, Englund JA, Corey L, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. Blood. 2010;115(10):2088–94.
172. Costa C, Bergallo M, Astegiano S, Sidoti F, Terlizzi ME, Gambarino S, et al. Detection of human rhinoviruses in the lower respiratory tract of lung transplant recipients. Arch Virol. 2011;156(8):1439–43.
173. Seo S, Waghmare A, Scott EM, Xie H, Kuypers JM, Hackman RC, et al. Human rhinovirus detection in the lower respiratory tract of hematopoietic cell transplant recipients: association with mortality. Haematologica. 2017;102(6):1120–30.
174. Ison MG, Hayden FG, Kaiser L, Corey L, Boeckh M. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. Clin Infect Dis. 2003;36(9):1139–43.
175. Waghmare A, Xie H, Kuypers J, Sorror ML, Jerome KR, Englund JA, et al. Human rhinovirus infections in hematopoietic cell transplant recipients: risk score for progression to lower respiratory tract infection. Biol Blood Marrow Transplant. 2019;25(5):1011–21.
176. Casanova V, Sousa FH, Stevens C, Barlow PG. Antiviral therapeutic approaches for human rhinovirus infections. Futur Virol. 2018;13(7):505–18.
177. Garbino J, Crespo S, Aubert JD, Rochat T, Ninet B, Deffernez C, et al. A prospective hospital-based study of the clinical impact of non-severe acute respiratory syndrome (non-SARS)-related human coronavirus infection. Clin Infect Dis. 2006;43(8):1009–15.
178. Wolfromm A, Porcher R, Legoff J, Peffault de Latour R, Xhaard A, de Fontbrune FS, et al. Viral respiratory infections diagnosed by multiplex PCR after allogeneic hematopoietic stem cell transplantation: long-term incidence and outcome. Biol Blood Marrow Transplant. 2014;20(8):1238–41.
179. Ogimi C, Waghmare AA, Kuypers JM, Xie H, Yeung CC, Leisenring WM, et al. Clinical significance of human coronavirus in bronchoalveolar lavage samples from hematopoietic cell transplant recipients and patients with hematologic malignancies. Clin Infect Dis. 2017;64(11):1532–9.
180. Ogimi C, Greninger AL, Waghmare AA, Kuypers JM, Shean RC, Xie H, et al. Prolonged shedding of human coronavirus in hematopoietic cell transplant recipients: risk factors and viral genome evolution. J Infect Dis. 2017;216(2):203–9.

181. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016;14(8):523–34.
182. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497.
183. AlGhamdi M, Mushtaq F, Awn N, Shalhoub S. MERS CoV infection in two renal transplant recipients: case report. Am J Transplant. 2015;15(4):1101–4.
184. Kim SH, Ko JH, Park GE, Cho SY, Ha YE, Kang JM, et al. Atypical presentations of MERS-CoV infection in immunocompromised hosts. J Infect Chemother. 2017;23(11):769–73.
185. Kumar D, Tellier R, Draker R, Levy G, Humar A. Severe acute respiratory syndrome (SARS) in a liver transplant recipient and guidelines for donor SARS screening. Am J Transplant. 2003;3(8):977–81.
186. Pereira MR, Mohan S, Cohen DJ, Husain SA, Dube GK, Ratner LE, et al. COVID-19 in solid organ transplant recipients: initial report from the US epicenter. Am J Transplant. 2020.
187. Fernandez-Ruiz M, Andres A, Loinaz C, Delgado JF, Lopez-Medrano F, San Juan R, et al. COVID-19 in solid organ transplant recipients: a single-center case series from Spain. Am J Transplant. 2020.
188. Kumar D, Manuel O, Natori Y, Egawa H, Grossi P, Han SH, et al. COVID-19: a global transplant perspective on successfully navigating a pandemic. Am J Transplant. 2020.
189. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Committee HICPA. Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings 2007. 6/22/19. 2007. https://www.cdc.gov/infectioncontrol/pdf/guidelines/isolation-guidelines-H.pdf
190. Ison MG, Hayden RT. Adenovirus. Microbiol Spectr. 2016;4(4).
191. Humar A, Kumar D, Mazzulli T, Razonable RR, Moussa G, Paya CV, et al. A surveillance study of adenovirus infection in adult solid organ transplant recipients. Am J Transplant. 2005;5(10):2555–9.
192. Sedlacek P, Petterson T, Robin M, Sivaprakasam P, Vainorius E, Brundage T, et al. Incidence of adenovirus infection in hematopoietic stem cell transplantation recipients: findings from the AdVance Study. Biol Blood Marrow Transplant. 2019;25(4):810–8.
193. Florescu DF, Islam MK, Mercer DF, Grant W, Langnas AN, Freifeld AG, et al. Adenovirus infections in pediatric small bowel transplant recipients. Transplantation. 2010;90(2):198–204.
194. Florescu MC, Miles CD, Florescu DF. What do we know about adenovirus in renal transplantation? Nephrol Dial Transplant. 2013;28(8):2003–10.
195. Florescu DF, Kwon JY, Dumitru I. Adenovirus infections in heart transplantation. Cardiol Rev. 2013;21(4):203–6.
196. Shirali GS, Ni J, Chinnock RE, Johnston JK, Rosenthal GL, Bowles NE, et al. Association of viral genome with graft loss in children after cardiac transplantation. N Engl J Med. 2001;344(20):1498–503.
197. Hubmann M, Fritsch S, Zoellner AK, Prevalsek D, Engel N, Bucklein V, et al. Occurrence, risk factors and outcome of adenovirus infection in adult recipients of allogeneic hematopoietic stem cell transplantation. J Clin Virol. 2016;82:33–40.
198. Matthes-Martin S, Feuchtinger T, Shaw PJ, Engelhard D, Hirsch HH, Cordonnier C, et al. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). Transpl Infect Dis. 2012;14(6):555–63.
199. Mattner F, Sykora KW, Meissner B, Heim A. An adenovirus type F41 outbreak in a pediatric bone marrow transplant unit: analysis of clinical impact and preventive strategies. Pediatr Infect Dis J. 2008;27(5):419–24.
200. Hum RM, Deambrosis D, Lum SH, Davies E, Bonney D, Guiver M, et al. Molecular monitoring of adenovirus reactivation in faeces after haematopoietic stem-cell transplantation to predict systemic infection: a retrospective cohort study. Lancet Haematol. 2018;5(9):e422–e9.
201. Erard V, Huang ML, Ferrenberg J, Nguy L, Stevens-Ayers TL, Hackman RC, et al. Quantitative real-time polymerase chain reaction for detection of adenovirus after T cell-replete

hematopoietic cell transplantation: viral load as a marker for invasive disease. Clin Infect Dis. 2007;45(8):958–65.
202. Hill JA, Mayer BT, Xie H, Leisenring WM, Huang ML, Stevens-Ayers T, et al. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. Blood. 2017;129(16):2316–25.
203. Hiwarkar P, Kosulin K, Cesaro S, Mikulska M, Styczynski J, Wynn R, et al. Management of adenovirus infection in patients after haematopoietic stem cell transplantation: state-of-the-art and real-life current approach: a position statement on behalf of the infectious diseases Working Party of the European Society of Blood and Marrow Transplantation. Rev Med Virol. 2018;28(3):e1980.
204. Kosulin K, Berkowitsch B, Matthes S, Pichler H, Lawitschka A, Potschger U, et al. Intestinal adenovirus shedding before allogeneic stem cell transplantation is a risk factor for invasive infection post-transplant. EBioMedicine. 2018;28:114–9.
205. Ison MG. Antiviral treatments. Clin Chest Med. 2017;38(1):139–53.
206. Vora SB, Brothers AW, Englund JA. Renal toxicity in pediatric patients receiving Cidofovir for the treatment of adenovirus infection. J Pediatr Infect Dis Soc. 2017;6(4):399–402.
207. Ramsay ID, Attwood C, Irish D, Griffiths PD, Kyriakou C, Lowe DM. Disseminated adenovirus infection after allogeneic stem cell transplant and the potential role of brincidofovir – case series and 10 year experience of management in an adult transplant cohort. J Clin Virol. 2017;96:73–9.
208. Marty FM, Winston DJ, Chemaly RF, Mullane KM, Shore TB, Papanicolaou GA, et al. A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for cytomegalovirus prophylaxis in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2019;25(2):369–81.
209. Detweiler CJ, Mueller SB, Sung AD, Saullo JL, Prasad VK, Cardona DM. Brincidofovir (CMX001) toxicity associated with epithelial apoptosis and crypt drop out in a hematopoietic cell transplant patient: challenges in distinguishing drug toxicity from GVHD. J Pediatr Hematol Oncol. 2018;40(6):e364–e8.
210. Grimley MS, Chemaly RF, Englund JA, Kurtzberg J, Chittick G, Brundage TM, et al. Brincidofovir for asymptomatic adenovirus viremia in pediatric and adult allogeneic hematopoietic cell transplant recipients: a randomized placebo-controlled phase II trial. Biol Blood Marrow Transplant. 2017;23(3):512–21.
211. Hiwarkar P, Amrolia P, Sivaprakasam P, Lum SH, Doss H, O'Rafferty C, et al. Brincidofovir is highly efficacious in controlling adenoviremia in pediatric recipients of hematopoietic cell transplant. Blood. 2017;129(14):2033–7.
212. Sulejmani N, Nagai S, Safwan M, Rizzari MD, Raoufi M, Abouljoud MS, et al. Brincidofovir as salvage therapy for adenovirus disease in intestinal transplant recipients. Pharmacotherapy. 2018;38(4):470–5.
213. Roemhild A, Reinke P. Virus-specific T-cell therapy in solid organ transplantation. Transpl Int. 2016;29(5):515–26.
214. Pinana JL, Madrid S, Perez A, Hernandez-Boluda JC, Gimenez E, Terol MJ, et al. Epidemiologic and clinical characteristics of coronavirus and bocavirus respiratory infections after allogeneic stem cell transplantation: a prospective single-center study. Biol Blood Marrow Transplant. 2018;24(3):563–70.
215. Costa BCL, Dabilla NAS, Almeida TN, Fiaccadori FS, de Souza TT, das Dores de Paula Cardoso D, et al. Human bocavirus detection and quantification in fecal and serum specimens from recipients of allogeneic hematopoietic stem cell transplantation: a longitudinal study. J Med Virol. 2019. https://doi.org/10.1002/jmv.25486.
216. Waghmare A, Pergam SA, Jerome KR, Englund JA, Boeckh M, Kuypers J. Clinical disease due to enterovirus D68 in adult hematologic malignancy patients and hematopoietic cell transplant recipients. Blood. 2015;125(11):1724–9.

217. Mendes ET, Ramos J, Peixoto D, Dulley F, Alves T, Vilas Boas LS, et al. An outbreak of respiratory syncytial virus infection in hematopoietic stem cell transplantation outpatients: good outcome without specific antiviral treatment. Transpl Infect Dis. 2013;15(1):42–8.
218. Zhu Y, Zembower TR, Metzger KE, Lei Z, Green SJ, Qi C. Investigation of respiratory syncytial virus outbreak on an adult stem cell transplant unit by use of whole-genome sequencing. J Clin Microbiol. 2017;55(10):2956–63.
219. Hall CB, Douglas RG Jr. Nosocomial respiratory syncytial viral infections: should gowns and masks be used? JAMA Pediatr. 1981;135(6):512–5.
220. Murphy D, Todd JK, Chao RK, Orr I, McIntosh K. The use of gowns and masks to control respiratory illness in pediatric hospital personnel. J Pediatr. 1981;99(5):746–50.
221. Leclair JM, Freeman J, Sullivan BF, Crowley CM, Goldmann DA. Prevention of nosocomial respiratory syncytial virus infections through compliance with glove and gown isolation precautions. N Engl J Med. 1987;317(6):329–34.
222. Sokol KA, De la Vega-Diaz I, Edmondson-Martin K, Kim S, Tindle S, Wallach F, et al. Masks for prevention of respiratory viruses on the BMT unit: results of a quality initiative. Transpl Infect Dis. 2016;18(6):965–7.
223. Sung AD, Sung JAM, Thomas S, Hyslop T, Gasparetto C, Long G, et al. Universal mask usage for reduction of respiratory viral infections after stem cell transplant: a prospective trial. Clin Infect Dis. 2016;63(8):999–1006.
224. Nichols WG, Erdman DD, Han A, Zukerman C, Corey L, Boeckh M. Prolonged outbreak of human parainfluenza virus 3 infection in a stem cell transplant outpatient department: insights from molecular epidemiologic analysis. Biol Blood Marrow Transplant. 2004;10 (1):58–64.
225. Podczervinski S, Stednick Z, Helbert L, Davies J, Jagels B, Gooley T, et al. Employee influenza vaccination in a large cancer center with high baseline compliance rates: comparison of carrot versus stick approaches. Am J Infect Control. 2015;43(3):228–33.
226. Price SA, Podczervinski S, MacLeod K, Helbert L, Pergam SA. Understanding influenza vaccination rates and reasons for refusal in caregivers and household contacts of cancer patients. Am J Infect Control. 2019;47(4):468–70.
227. Kumar D, Morris MI, Kotton CN, Fischer SA, Michaels MG, Allen U, et al. Guidance on novel influenza a/H1N1 in solid organ transplant recipients. Am J Transplant. 2010;10(1):18–25.
228. Meylan PR, Aubert JD, Kaiser L. Influenza transmission to recipient through lung transplantation. Transpl Infect Dis. 2007;9(1):55–7.
229. Le Page AK, Kainer G, Glanville AR, Tu E, Bhonagiri D, Rawlinson WD. Influenza B virus transmission in recipients of kidney and lung transplants from an infected donor. Transplantation. 2010;90(1):99–102.

Transplant Adenovirus Infection: Clinical Syndromes, Diagnosis, and Management

32

Maricar Malinis and Marwan M. Azar

Contents

Introduction 781
Virology 782
Epidemiology 782
Mode of Transmission 782
Epidemiology in Transplant Populations 784
Clinical Presentation 785
Hematopoietic Stem Cell Transplant 785
Solid Organ Transplant 786
Diagnosis 789
Treatment 794
Prevention 796
Conclusion 796
Key Points 797
References 797

Introduction

Human adenoviruses (HAdV) can cause self-limiting infections, involving the respiratory tract, intestines, and conjunctiva in immunocompetent hosts. In solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients, HAdV can present as asymptomatic viremia, localized infection, or disseminated disease with significant impact on morbidity and mortality [1, 2]. In addition, HAdV infection can

M. Malinis (✉)
Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA
e-mail: maricar.malinis@yale.edu

M. M. Azar
Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT, USA
e-mail: marwan.azar@yale.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_33

negatively impact the graft survival of SOT recipients [3]. Management of HAdV can be challenging since therapies have limited efficacy and significant toxicities. As with CMV, a preventative strategy using screening for viremia by polymerase chain reaction (PCR) and preemptive antiviral therapy is advocated by experts in at-risk HSCT recipients. This chapter will discuss the virology, epidemiology, clinical presentation, diagnostics, and treatment strategies for HAdV in HSCT and SOT recipients.

Virology

HAdVs are non-enveloped double-stranded DNA viruses of the family *Adenoviridae*. At present, more than 67 HAdV types have been characterized. These are grouped using distinct but intersecting classification schemes which include serotypes, genotypes, and species subgroups. Historically, serotyping of SN and H1 HAdV epitopes leads to the identification of 51 HAdV serotypes, whereas subsequent types, also known as genotypes, were identified using analysis of genomic sequences. HAdVs are further categorized into seven species subgroups (A to G) based on hemagglutination properties, genetic homology, oncogenicity, and cellular tropism (Table 1) [4]. Subgroups B, C, and E typically target respiratory tract and, together with subgroup D, the eye, whereas subgroups A and F preferentially infect the gastrointestinal tract. The ability of certain HAdV subgroups and types to infect specific host cells is reflective of a propensity toward certain disease manifestations, although there can be clinical overlap, especially in severe cases where disseminated disease occurs. For example, HAdV F (types 40 and 41) causes gastroenteritis in children, whereas HAdV B (type 11) is associated with hemorrhagic cystitis post-hematopoietic stem cell transplantation (HSCT). Given their ability to target a wide variety of host cells, HAdV has been utilized as vectors for gene delivery in gene-based therapy [5]. Certain HAdV types, especially within subgroup A, have demonstrated oncogenic potential in animal models, but whether they cause neoplastic alterations in human cells remains unclear [6]. After acute infection, the virus can establish lifelong latent infection in lymphoepithelial tissue including intestinal T cells, lung epithelium, and renal epithelium and can reactivate if HAdV-specific T-cell immunity is impaired as in the posttransplant setting [7–9].

Epidemiology

Mode of Transmission

Most HAdV infections occur in young children, below 4 years of age. Other susceptible populations include persons living in close quarters such as college students and military recruits and immunocompromised patients, particularly those with impaired T-cell immunity [10]. Viral transmission occurs most commonly via droplet inhalation and direct inoculation into the oral mucosa or conjunctiva often from fecal contamination; virions are highly resistant to gastric acid and shed in high quantities in the stool [11]. Acquisition via infected blood and other body fluids and

Table 1 Human adenovirus disease manifestations per subgroup and type

Disease	Frequently associated HAdV subgroups and types	Predilection for immunocompromised hosts
Ocular manifestations		
Conjunctivitis	B: 3, 7 C: 1, 2 E: 4	No
Epidemic keratoconjunctivitis	D: 8, 9, 37	No
Hemorrhagic conjunctivitis	B: 11	No
Pharyngoconjunctival fever	B: 3, 7 E: 4	No
Respiratory manifestations		
Upper respiratory infection	B: 3, 7 C: 1, 2, 5	No
Pneumonia	B: 3, 7, 21 E: 4	Yes
Pertussis-like syndrome	C: 5	No
Acute respiratory distress syndrome	B: 7 E: 4	No (military recruits)
Gastrointestinal manifestations		
Gastroenteritis	F: 40, 41	No
Hepatitis	B: 3, 7 C: 1, 2, 5	Yes
Other manifestations		
Disseminated infection	B: 11, 34, 35 C: 1, 2, 5	Yes
Encephalitis	B: 7	Yes
Hemorrhagic cystitis	B: 11	Yes
Myocarditis	B: 7, 21	No
Reactivation disease	A: 12, 31 B: 3, 11, 16, 34, 35 C: 1, 2, 5 (Predominant)	Yes
Sexually transmitted infection	C: 2 D: 37	No

donor-derived transmission during organ donation have also been reported [12]. The incubation period ranges from 2 to 14 days after acquisition, depending on the mode of transmission and the viral inoculum. After initial infection, HAdV can establish persistent asymptomatic infection in lymphoid tissue of infected hosts and lead to prolonged shedding over months or longer. In one study of immunocompetent adults with respiratory infections, a regression model determined that shedding of HAdV type 55 occurred for 55 days [13]. HAdV infections occur perennially though outbreaks are more common in the winter and spring months. While there is some geographic variation in HAdV strains, global strain circulation occurs [4]. In the environment, HAdVs remain stable for weeks, and their lack of a glycoprotein and lipoprotein-rich envelop makes them much less susceptible to disinfectants than

enveloped viruses. Application of 85–95% alcohol solutions for 2 min or sodium hypochlorite for 10 min is effective at neutralizing the virus from environmental surfaces [14]. In immunocompetent hosts, HAdV infection mostly causes self-limited upper respiratory, conjunctival, or gastrointestinal disease.

Epidemiology in Transplant Populations

Primary infection with HAdV during childhood leads to lifelong latency in lymphoepithelial tissue and development of anti-HAdV immunity, including cellular and humoral responses. By targeting conserved antigenic elements within the HAdV hexon protein, a T-cell-dominant anti-HAdV response offers broad protection across all HAdV types. Immunity develops after exposure to HAdV, preventing reinfection with the same type and partial but not complete protection against other types. A humoral antibody response consisting of neutralizing antibodies against HAdV also forms. Loss of HAdV-specific T cells, as in the post-SOT and HSCT settings, is associated with an increased risk for viral reactivation from a latent state. HAdV infections in adult transplant patients arise chiefly from reactivation of latent viruses (most commonly subgroup C), whereas most infections in pediatric patients represent de novo infection, except for subgroup C serotype infections [15]. In allogeneic HSCT patients, HAdV strains isolated from clinical specimens prior to transplantation are usually identical to those detected after transplantation, and high titers of anti-HAdV dneutralizing antibody titers predict type-specific reactivation in the post-transplant period [16].

Epidemiology in Hematopoietic Stem Cell Transplant

Reactivation disease in transplant patients generally occurs within 100 days of HSCT and usually presents as asymptomatic viremia [17]. In adult HSCT patients, the incidence of HAdV viremia is 3 to 15% post-allogeneic HSCT. In comparison, the incidence of HAdV viremia in pediatric patients is significantly higher up to 47% for allogeneic HSCT, likely as a result of a higher incidence of primary HAdV infection [2]. In adult allogeneic HSCT patients, the progression from viremia to invasive disease occurs in 10–20% of cases but is uncommon in autologous HSCT [18, 19]. Invasive disease is more common in specific transplant populations with profound losses of HAdV-specific cellular immunity either via T-cell-depleted grafts or a requirement for increased immunosuppression. The highest-risk categories for the invasive adenoviral disease are allogeneic HSCT with T-cell depletion, unrelated HSCT, cord blood transplantation, GVHD grades III and IV, and receipt of alemtuzumab (CD52 antibody targeting both cellular and humoral immune branches) or antithymocyte globulin. In adult allogeneic HSCT patients, mortality for overall HAdV infection is low (<1%) but is much higher in symptomatic or disseminated disease (26%) [20], in those with risk factors such as unrelated grafts or receipt of alemtuzumab (32–49%) [21, 22], and in the presence of high-level HAdV viremia (60–80%) [23, 24].

Epidemiology in Solid Organ Transplant Recipients

In SOT, HAdV viremia usually develops within 6 months of transplantation. The incidence of HAdV viremia is less than 10% in adult kidney, heart, and liver SOT [25, 26] except for lung SOT where the incidence is higher (22%) [27], correlating with higher requirements for immunosuppression in the latter group and likely direct symptomatic infection of the graft. As with HSCT, the incidence of HAdV viremia is higher in pediatric patients (up to 38% liver SOT, up to 50% lung and heart SOT, and up to 57% with multi-visceral SOT) [2]. The rate of progression from asymptomatic viremia to invasive disease is not well characterized but is likely lower than HSCT populations except for lung and multi-visceral SOT. Risk factors associated with invasive disease in SOT also include receipt of thymoglobulin or alemtuzumab, prolonged lymphopenia, and donor-positive, recipient-negative HAdV transplants and allograft types with greater lymphoid tissue contents (such as multi-visceral or intestinal transplantation) [26]. Mortality is low overall except for lung transplantation, where courses are often rapidly progressive and early mortality can be seen [26]. In lung SOT, HAdV infection is also implicated in late mortality and graft failure through bronchiolitis obliterans and chronic rejection [28].

Clinical Presentation

HAdV infections in transplant recipients can range from asymptomatic to severe disseminated disease. Establishing the diagnosis of adenoviral disease versus asymptomatic infection can be a challenge as a transplant recipient can shed virus for months in the absence of symptoms. Clinical presentation may vary widely between transplant recipients. There is no consensus on definitions of adenovirus infections and disease; however, for discussion, the definitions proposed by the American Society of Transplantation Infectious Disease Community of Practice guidelines are used for this chapter [29] (Table 2). Several papers cited in this chapter have further defined adenoviral disease into (i) **proven disease**, which requires histopathological confirmation at the appropriate site of infection, and (ii) **probable disease**, which is adenoviral disease without histologic evidence [4, 30, 31].

Hematopoietic Stem Cell Transplant

HAdV infection is most commonly asymptomatic in HSCT recipients. Frequently reported early symptoms and laboratory evidence of adenoviral disease include fever, diarrhea, elevated liver enzymes, and cytopenias [32]. As noted previously, there is variation between HAdV subgroups. The spectrum of adenoviral disease can range from mild gastrointestinal or respiratory symptoms to severe pneumonitis, enteritis, hepatitis, tubulointerstitial nephritis, hemorrhagic cystitis, and meningoencephalitis [2, 23]. Unusual presentations of HAdV include cutaneous involvement

Table 2 Definitions of adenoviral infection[a]

	Definition
Asymptomatic infection	1. No clinical signs and/or symptoms of illness 2. Presence of adenovirus in any biologic sample (i.e., stool, blood, urine, respiratory tract) detected by viral culture, antigen tests, or PCR
Adenoviral disease	1. Signs and /or symptoms of illness 2. Detection adenovirus in one of the following: (a) Biopsy specimen by immunohistochemical stain[b] (b) Cerebrospinal fluid or bronchoalveolar lavage detected by culture, antigen detection, or PCR 3. Absence of other diagnosis
Disseminated adenoviral disease	1. Meets definition of adenoviral disease with 2. Involvement of two or more organs, excluding viremia

[a]Based on Ref. [29]
[b]Detection of adenovirus by PCR from tissue or fluid and negative immunohistochemical stain is **not consistent** with invasive disease

[33] and hemophagocytic lymphohistiocytosis [34, 35]. Disseminated disease occurs in up to 10–20% of cases [18, 36–38]. Retrospective studies have reported the onset of adenoviral disease predominantly within the first 100 days of HSCT, both because this is likely associated with the greatest degree of functional lymphopenia and also the most intensive monitoring [23, 39, 40]. With the implementation of HAdV screening, viral detection in blood, stool, or urine was shown to occur very early in the course of HSCT with a median time of 17–21 days from time of HSCT, almost always preceding clinical manifestation of the disease [2].

Detection of HAdV at multiple sites has been correlated with increased risk for invasive disease in pediatric HSCT [18, 39, 41]. Also, a rapidly rising or a sustained elevated HAdV viral load in blood or plasma has been associated with severe disease in both adult and pediatric HSCT [22, 41, 42]. The mortality of disseminated disease is up to 50% in this population [24, 40, 42].

Solid Organ Transplant

The clinical presentation of HAdV infection in solid organ transplant depends on the site of infection and the type of transplanted organ. Often, infection involves the allograft, representing either a primary infection or reactivation of latent infection in the recipient (recipient-derived) or donor organ (donor-derived). The infection may manifest differently depending on the transplant; bronchiolitis or pneumonitis is common in the lung allograft, hepatitis in the liver allograft, hemorrhagic cystitis in the renal allograft, or enteritis in the intestinal allograft.

Liver Transplant

In liver transplant recipients, HAdV can affect the liver, lungs, kidneys, and gastrointestinal tract. Clinical symptoms may include any of the following: fever, upper respiratory tract symptoms (reactive airway, rhinorrhea), diarrhea, hematuria, or

elevated liver enzymes [43, 44]. Frank hepatitis is a common presentation in pediatric liver transplant recipients. In a retrospective study of 49 pediatric liver transplant recipients who developed HAdV infection, 20 developed invasive disease that manifested as hepatitis ($n = 12$), hepatitis with pneumonia ($n = 2$), and pneumonia ($n = 5$) [43]. The most common serotypes recovered were 1, 2, and 5; type 5 was associated with hepatitis. There was an attributable mortality of 18% due to invasive disease. Eight of the 14 hepatitis cases survived, but among them, 4 patients developed graft loss requiring re-transplantation. In this cohort, the median time from transplant to isolation of the HAdV was 26 days. The timing of infection suggested either reactivation, donor-derived transmission, or hospital nosocomial acquisition. In a separate study at a single center, adenoviral hepatitis was proven by biopsy in seven pediatric liver transplant recipients with an associated mortality of 57% [45]. Focal hepatic lesions with or without associated tissue necrosis, a rare manifestation of adenoviral infections, were reported in two pediatric liver transplant recipients [46, 47].

In a cohort of adult liver transplant recipients, HAdV was isolated in 11 of 191 cases [30]. Of the 11 cases, 4 had an asymptomatic infection, and 7 had tissue-invasive disease (2 definite, 1 probable, and 4 possible). Clinical presentation included urinary tract infection, pneumonia, fulminant hepatitis, and disseminated multi-visceral disease. The mean time from transplant to HAdV isolation by culture was 66 days, while the mean time from transplant to symptomatic disease was 55 days (range 3–180 days). In a prospective cohort study by Humar et al., 263 CMV D+/R- adult SOT recipients were assessed for adenovirus infection using molecular surveillance technique [26]. Eight percent (10/121) of liver transplant recipients had detectable HAdV viremia during the first 12 months of transplantation. Of the 19 SOT recipients with detectable HAdV by PCR, 11 were asymptomatic (58%), and only a few presented with gastrointestinal (2/19, 10.5%) or respiratory (2/19, 10.5%) symptoms. A recent study of Majorant et al. evaluated adult and pediatric SOT recipients with HAdV-positive testing [48]. Four of 17 liver (31%) and 16 of 58 (27%) combined liver/intestine/pancreas recipients with detectable HAdV developed adenoviral disease. In this cohort, there was an increased rejection rate and higher mortality among those with the disease compared to those with asymptomatic infection, although only mortality was found to be significantly different.

Renal Transplant

In the study of Humar et al. that was previously mentioned, HAdV viremia was detected in 6.5% of 92 adult renal transplant recipients prospectively monitored by PCR, and most were asymptomatic [26]. A 2-year prospective cohort study of 75 pediatric renal transplant patients found that 14.5% ($n = 11$) developed HAdV viremia at a median onset of 173 days (interquartile range 109–310 days) and 6 (8%) were asymptomatic [49]. Renal transplant recipients frequently present with hemorrhagic cystitis due to HAdV characterized by fever, dysuria, urgency, frequency, and hematuria [12, 25, 34, 50–54]. In a 3-year, single-center experience [25], 16 of 17 adult kidney transplant recipients developed HAdV hemorrhagic

cystitis, and most (75%) presented during the first 3 months of transplant. Hemorrhagic cystitis in pediatric renal transplant recipients is infrequently reported compared to adult populations. In the study of Engen et al., only 3% had HAdV hemorrhagic cystitis. HAdV infection may occasionally involve the renal parenchyma causing necrotizing granulomatous nephritis that can occur with or without hemorrhagic cystitis [55–60]. The findings of necrosis, granulomas, and hemorrhage on renal biopsy specimens favor HAdV over polyomavirus nephritis, although immunohistochemistry can usually differentiate the two also [61]. Adenoviral nephritis can be confused with acute rejection. Graft dysfunction can often be seen with hemorrhagic cystitis; however, it is once again more frequently reported in adults than in their pediatric counterpart [25, 50, 62, 63]. Hofland et al. [50] and Watcharananan et al. [25] observed graft dysfunction among those with HAdV hemorrhagic cystitis in 35 of 37 patients (95%) and 11 of 17 patients (65%), respectively. The graft dysfunction resolved in the majority of patients after the resolution of adenoviral disease [25].

In addition to hemorrhagic cystitis, adenoviral HAdV disease in renal transplant recipients may present outside the graft, with enteritis, pneumonitis, hepatitis, and orchitis [25, 64, 65]. However, few cases of interstitial pneumonitis [64–66], colitis [67], and hepatitis [68] have progressed to disseminated disease and subsequent death, perhaps consistent with lower degrees of immunosuppression in this solid organ transplant population.

Lung Transplant

Detectable HAdV in plasma was observed in 22.5% of adult lung transplant with a median time of detection from transplant of 134 days (range 1–370 days) [69]. HAdV involving the allograft in lung transplant recipients can have deleterious consequences, including graft dysfunction, acute graft rejection, bronchiolitis obliterans, and death [28, 70, 71].

Mortality due to adenoviral pneumonia is significant in this population. In a study conducted by Ohori et al., all lung transplant recipients (4/4) with adenoviral pneumonia died within 45 days posttransplant despite no evidence of disseminated disease [70]. In a prospective study of 16 pediatric lung and lung-heart transplant recipients for viral pneumonia, HAdV was the most commonly detected virus, accounting for 50% of recipients with a median time from transplant to HAdV detection of 46 days (range 1–458 days) [28]. Of the eight patients, only two survived. Two deaths occurred early in the posttransplant course due to fulminant respiratory failure, one of which was secondary to donor-derived infection. More importantly, this study demonstrated the association of adenoviral infection with bronchiolitis obliterans and graft loss as a late phenomenon sometimes many months after initial infection.

Heart Transplant

As with other SOT recipients, heart transplant recipients are most commonly diagnosed with HAdV infection within the first year of transplant, often by performing HAdV PCR on endomyocardial biopsy specimens [72, 73]. Detection of HAdV by

PCR does not necessarily equate to disease. However, Shirali et al. found that it was predictive of adverse cardiac events, including coronary vasculopathy, acute rejection, or chronic graft failure within 3 months post-biopsy result [72]. Both Shirali et al. [72] and Moulik et al. [73] demonstrated an association between detectable HAdV genome in the myocardium of pediatric transplant patients and reduced graft survival. Pediatric heart transplant recipients with detectable HAdV nucleic acid in allograft specimens diagnosed during rejection surveillance by endomyocardial biopsy were found to have histologic evidence of multifocal moderate to severe rejection [74]. This finding alluded to the possible role of HAdV in rejection, akin to late onset bronchiectasis in lung transplants, but a causal relationship remains to be proven. In addition to cardiac complications from HAdV, extra-cardiac adenoviral disease in heart transplant recipients has been reported, similar to other organs, including conjunctivitis with disseminated disease [75], hepatitis [76], and pneumonia [77, 78].

Intestinal Transplant

Most studies on HAdV infections in intestinal transplant involve pediatric patients [79–81]. Cases may be diagnosed incidentally on routine graft biopsies, not necessarily prompted by symptoms. Ziring et al. performed a retrospective review of 33 patients (30 children, 3 adults) who underwent intestinal transplantation between 1991 and 2003 [80]. Four of the 20 identified cases of enteritis were due to HAdV. One of the four HAdV cases suffered from graft loss, but all survived. Pinchoff et al. [79] reported 14 intestinal transplant recipients (6 intestinal transplant only, 8 liver/intestinal transplant) with routine graft biopsies that were positive for HAdV viral cultures. Of these, only eight were confirmed as adenoviral enteritis by histopathology. The most common symptom in this study was increased ostomy output. Three of the eight patients died; two deaths were attributed to HAdV. In another study, 23.5% of 98 pediatric intestinal transplant recipients developed adenoviral disease, and 15% were asymptomatic shedders that did not progress to invasive disease [81]. Nineteen of 23 cases (82.6%) involved the allograft and occurred within the first year of transplant. The remaining four cases had HAdV pneumonia, three of which developed disseminated disease and died. In both studies, younger age at the time of transplantation was a risk factor for infection [80, 81].

Diagnosis

Several methods are available for the diagnosis of HAdV infections. These include historical methods now rarely used in clinical practice, such as viral culture, shell vial centrifugation, serology, and antigen detection, as well as newer techniques including histopathology with immunohistochemical staining, electron microscopy, PCR, and metagenomic sequencing. Some of the tests, particularly culture immunohistochemical staining, electron microscopy, and metagenomics, which require significant laboratory expertise and hands-on skill are more likely available in resource-replete settings and may not be readily available in smaller medical centers

or rural areas. The specimen types submitted for testing should be guided by the clinical syndrome and include blood, plasma, stool, urine, ocular fluid, cerebrospinal fluid, bronchoalveolar lavage fluid, and nasopharyngeal swabs, among others.

HAdV can be identified using conventional cell culture techniques by detecting a characteristic cytopathic effect (CPE) in a monolayer of host cells. Classic HAdV CPE consists of grape-like aggregates of refractile cells with a lacy configuration [82]. Serotypes 40 and 41 do not produce distinctive CPE and so cannot readily be identified using viral culture. Using this technique, most HAdV can be identified within 2–5 days, but CPE may take up to 4 weeks to develop with specific serotypes or a low viral burden. The sensitivity of culture for HAdV is low, limiting its utility as a routine diagnostic method. Moreover, HAdV can be asymptomatically shed, so positive results must be taken into consideration of the clinical context, with a few exceptions such as ocular fluid in which HAdV detection is considered diagnostic. At the present time, cell culture is limited to public health and epidemiological investigations, given the time and resource consuming nature of the work.

Detection of HAdV can be achieved using rapid culture methods in a shorter time frame. Shell vial cell centrifugation culture techniques employ immunofluorescence to detect early viral antigens prior to the development of detectable CPE. Such methods are particularly useful for viruses that require prolonged incubation periods. After the addition of clinical specimen, shell vial culture tubes are centrifuged to inoculate a cell monolayer. After incubation for up to 4 days, monolayers are stained with virus-specific immunofluorescent antibodies and examined for the presence of intracellular viral antigens. Though more rapid, shell vial centrifugation is less sensitive than culture and may fail to detect low concentrations of virus or certain HAdV types [83].

In the setting of infection, HAdV CPE can be detected in clinical tissue specimens submitted for histopathological evaluation. Histopathologic evidence of HAdV is still considered by many to be the gold standard for the diagnosis of invasive adenoviral disease [29]. Cells infected with HAdV develop large nuclei with prominent basophilic nuclear inclusions and a narrow cytoplasm, giving the appearance of "smudge cells." Formation of granulomas has been described in some case of renal allograft infection. Cytopathic changes are often subtle and easily overlooked in hematoxylin and eosin-stained specimens. Moreover, in SOT, the pathologic findings associated with HAdV graft infection, especially nephritis, can be challenging to discern from acute organ rejection [84]. Therefore, the use of immunohistochemical or immunoperoxidase stains is advisable when HAdV infection is suspected (Fig. 1) [85]. If HAdV is suspected, discussion with pathology colleagues can ensure adequate staining is performed. Electron microscopy, if available, can demonstrate adenoviral particles within infected cells, but this method is mostly reserved for research purposes.

Several commercially available rapid antigen detection assays for HAdV based on enzyme immunoassay, immunochromatography, or latex agglutination are applicable to ocular and stool specimens. In comparison with PCR, the sensitivity of these assays is poor in older and immunocompromised patients [86], but the performance on ocular fluid appears to be better [87]. Direct and indirect

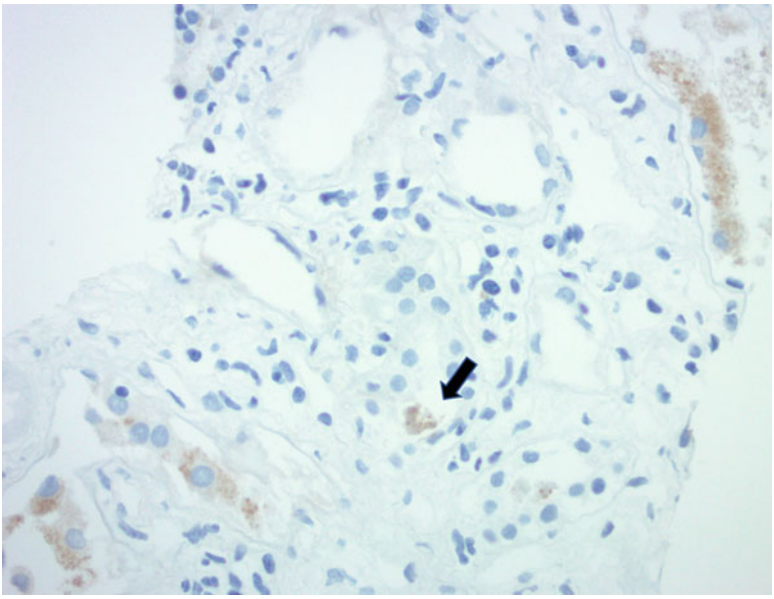


Fig. 1 Immunohistochemical staining of kidney tissue demonstrating presence of adenovirus antigen (see arrow)

immunofluorescence assays are usually performed on respiratory specimens (Fig. 2). The advantage of this method is the ability to evaluate specimen quality by assessing for the presence of exfoliated ciliated columnar epithelial cells from the posterior nasopharynx or mid-turbinate, which is indicative of a well-collected specimen and squamous epithelial cells, indicative of an unsatisfactory specimen. Whereas the use of chromophore-bound monoclonal antibodies allows for high specificity, immunofluorescence techniques are, in fact, less sensitive even than viral culture, which is not the case for other respiratory viruses [88]. Serology for HAdV is not useful diagnostically, especially in immunocompromised patients such as transplant recipients who may be unable to mount a humoral response. However, assessment of neutralizing antibodies to specific serotypes is useful for immunoepidemiologic studies of HAdV infection [16].

By virtue of significantly greater sensitivity and specificity and much shorter processing times, amplification of adenoviral DNA followed by detection using PCR has superseded all other methods to become the test of choice for the diagnosis of HAdV infections, at least in North America [89]. PCR assays capture the vast majority of HAdV types by targeting well-conserved regions including the transactivating region of E1A gene and N-terminal region of hexon gene, though other targets are sometimes included to identify rare HAdV types or to capture emerging types [90]. Both single-target PCR assays and multiplex panels targeting multiple pathogens at once are available. Many academic centers rely on laboratory-developed tests for HAdV detection, especially on blood or plasma.

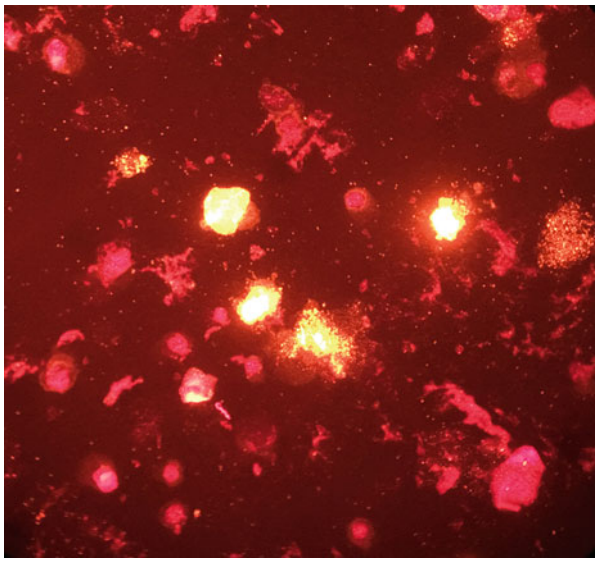


Fig. 2 Direct immunofluorescence assay of a nasopharyngeal swab specimen demonstrating yellow-gold fluorescence of nasopharyngeal cells, indicating the presence of adenovirus antigen

These often employ a real-time PCR approach, which is more rapid, provides quantitative information, and is less prone to contamination [91]. Quantitative PCR assays on blood are especially useful for screening and diagnosis in severely immunocompromised patients, for whom HAdV viremia is predictive of disseminated disease [41]. Analytical sensitivity of 10^2 virus copies/mL is required to ensure early detection of the virus in peripheral blood and to prevent false-negative results. However, due to significant assay heterogeneity and a wide variety of patient populations tested, results of PCR assays cannot readily be interpreted across assays and laboratories. Efforts at developing a universal calibration standard for HAdV are currently underway [92], but there is presently no accepted threshold value of HAdV viremia beyond which antiviral treatment is recommended. Though a well-defined cut-off value to distinguish reactivation from disease is not yet available, a rapid rise in viral load may be associated with the development of invasive or disseminated infection [41]. They can also be helpful to sequentially follow HAdv in the blood, so as to assess virologic improvement. The high sensitivity of PCR assays may result in detection of low-level HAdV from various sites. In asymptomatic subjects, low-level viremia with stable or decreasing kinetics often represents viral shedding rather than disease.

Several commercial FDA-approved multiplex PCR assays that include HAdV targets have become available in recent years. Among these, there are assays applicable to respiratory specimens and stool (Table 3). Respiratory panels generally perform well in comparison to monoplexed real-time PCR methods. However, initial versions of the BioFire RP (v1.6) assay were significantly less sensitive (67%) than comparator PCR assays for the detection of HAdV. An

Table 3 Commercial monoplex and multiplex PCR assays, available in the United States, for HAdV detection

Commercial PCR assays and manufacturer	Monoplex or multiplex	Validated specimen	HAdV subgroups covered	Turnaround time
FilmArray Respiratory Panel (RP) and RP 2 plus, BioFire Diagnostics	Multiplex	Respiratory (NPS)	A, B, C, D, E, F, G	RP: 65 min RP 2 plus: 45 min
FilmArray Respiratory Panel EZ, BioFire Diagnostics	Multiplex	Respiratory (NPS)	A, B, C, D, E, F, G	60 min
FilmArray Respiratory Pneumonia Panel, BioFire Diagnostics	Multiplex	Respiratory (BAL or mini-BAL)	A, B, C, D, E, F, G	60 min
eSensor RVP, GenMark Diagnostics	Multiplex	Respiratory (NPS)	B, C, E	6 h
xTAG RVP, Luminex Corporation	Multiplex	Respiratory (NPS)	A, B, C, D, E, F, G Adenovirus species C and serotypes 7a and 41 not detected	8 h
xTAG RVP Fast v2, Luminex Corporation	Multiplex	Respiratory (NPS)	A, B, C, D, E, F, G	4 h
Panther Fusion AdV/hMPV/RV assay, Hologic	Multiplex	Respiratory (NPS)	A, B, C, D, E, F, G	2 h
Prodesse ProAdeno +assay, Hologic	Monoplex	Respiratory (NPS)	A, B, C, D, E, F, G	3 h
Lyra Adenovirus, Quidel Corporation	Monoplex	Respiratory (NS and NPS)	A, B, C, D, E, F, G	75 min
FilmArray GI panel, BioFire Diagnostics	Multiplex	Stool	F40/41	1 h
xTAG GPP, Luminex Corporation	Multiplex	Stool	F40/41	24 h
Adenovirus R-gene, Biomerieux	Monoplex	Detection and amplification of HAdV DNA on any specimen	A, B, C, D, E, F, G	2 h (post-extraction)

Abbreviation: *NPS*, nasopharyngeal swab; *NS*, nasal swab; *NW*, nasal wash

updated version (v1.7) reported significantly improved sensitivities (91%) mainly due to improved coverage of HAdV types [93], but a subsequent study among a large pediatric cohort found that the BioFire RP v1.7 had lower sensitivity for species A, D, and F [94]. In this study, approximately 5% of specimens that were negative by BioFire RP v1.7 were positive using a laboratory-developed PCR. Several of these cases occurred among immunocompromised patients prompting the authors to caution against the use of the RP v1.7 as the stand-alone test to rule out HAdV in this population.

Unlike respiratory PCR panels that target the majority of HAdV types, most gastrointestinal panels only cover serotypes F40 and F41. Whereas these serotypes are the most common causes of gastroenteritis in immunocompetent children, HAdV species C are more common in the setting of transplantation because they are associated with reactivation. Therefore, these panels should not be used to rule out HAdV infection in high-risk transplant populations [95]. High viral loads of 10^6 virus copies/ml in the stool are associated with a greater risk for future viremia in HSCT patients [17].

Metagenomic next-generation sequencing (mNGS) is an emerging technique that allows for rapid and comprehensive pathogen identification by sequencing the entire nucleic acid present in a clinical specimen. Though less sensitive than directed PCR assays for known targets, mNGS may identify novel, emerging, or genomic mutated HAdV strains not captured by PCR. Several cases of HAdV infections determined by mNGS assays and missed by PCR have been recently reported [96, 97]. As such, mNGS may have a role in the diagnosis of transplant patients with clinical syndromes consistent with HAdV but with negative PCR testing. Testing using mNGS assays may be performed using (1) assays developed within academic medical centers (laboratory-developed assays) and as such may be performed in-house with shorter turnaround times or (2) commercially available assays which require specimen transport/shipping with processing times of up to 10 days. Commercial testing is often very costly, limiting use to difficult or illusive clinical scenarios.

Treatment

Antivirals currently used for the treatment of HAdV infection are not supported by randomized clinical trial data nor FDA-approved for the treatment of HAdV infections. Cidofovir, ganciclovir, and ribavirin have in vitro efficacy against different HAdV serotypes [98, 99] and have been reported in case reports and case series as therapeutic interventions for HAdV infection.

Ganciclovir is a nucleoside analogue of guanosine that has in vitro activity against HAdV. Only one study to date has demonstrated a protective effect against the development of HAdV infection in HSCT [20]. Published reports of its use for the treatment of adenoviral disease in HSCT and SOT are limited to case reports [100–102].

Ribavirin is a guanosine analogue that has in vitro activity against most HAdV isolates within subgroups A, B, and D and all isolates from subgroup C [98]. Its mechanism of action against HAdV is not well established. The limited therapeutic benefit was observed in treated immunocompromised patients, attributed mainly to the failure to decrease viral titers [103, 104]. Ribavirin was not recommended as a treatment of choice for adenoviral disease by the European Conference on Infections in Leukemia [32].

Cidofovir is a cytosine analogue of cytosine that is converted by cellular kinase to an active intracellular metabolite, cidofovir diphosphonate; this form acts as a

competitive inhibitor and alternative substrate for 2-deoxycytidine 5′ triphosphate of the DNA polymerase and subsequently inhibits viral replication [105]. Cidofovir has in vitro activity against different HAdV serotypes [98], and evidence for efficacy against HAdV is the most robust [106–108]. It is currently available in an intravenous formulation which is limited by dose-dependent nephrotoxicity [109] and ocular toxicity [110–112]. To reduce nephrotoxicity, hydration and co-administration with probenecid are recommended [113]. Two regimens can be used for the treatment of adenoviral disease: (1) 1 mg/kg three times a week or (2) 5 mg/kg /week for 2 weeks followed by 5 mg/kg every 2 weeks dosing until clinical resolution of HAdV infection [29, 32]. These two strategies have not been compared with regard to efficacy; however, the 1 mg/kg three times a week is associated with less nephrotoxicity [114, 115]. Virologic response to cidofovir, monitored by plasma HAdV viral PCR, correlates with clinical response [116, 117]. Failure to achieve ≥ 1 $\log_{10}$ reduction in viral load in the first 2 weeks of cidofovir therapy is associated with disease progression and death secondary to symptomatic disease [116].

Brincidofovir (CMX001, Chimerix Inc.), a lipid conjugate of cidofovir, is currently in development. Brincidofovir has good oral availability and achieves better intracellular levels of the active drug compared to cidofovir [118]. Compared to cidofovir, brincidofovir is 5- to $\geq$2500-fold more potent against HAdV [119]. Importantly, brincidofovir is not nephrotoxic; however, dose-limiting gastrointestinal toxicities (such as nausea, vomiting, diarrhea) have been reported more commonly in HSCT recipients [120]. A study involving 13 immunocompromised patients (11 HSCT, 1 intestinal transplant, 1 severe combined immunodeficiency) with life-threatening adenoviral disease refractory or intolerant to cidofovir were treated with brincidofovir [121]. Eight patients (61.5%) had a >1 $\log_{10}$ drop in viral load after the first week of therapy, and 9 patients (69.2%) achieved virologic response by 8 weeks of therapy. Patients with virologic response had more prolonged survival in contrast to those without virologic response (median 196 days vs. 54.5 days, $p = 0.04$). A phase II study evaluated two different doses of brincidofovir in preemptive therapy of HSCT recipients with asymptomatic viremia [122]. Patients were randomized to brincidofovir 100 mg twice weekly, brincidofovir 200 mg weekly, or placebo for 6 to 12 weeks. After 1 week of therapy, 8 of 12 (67%) patients with twice weekly dose had undetectable HAdV viral load compared to 4 of 14 patients (28%) in once weekly dose and 5 of 15 (33%) of placebo. A phase III, open-labeled study on the safety and efficacy of brincidofovir for HAdV infection has been completed; results are not yet published (ClinicalTrials.gov Identifier: NCT02087306). Ongoing availability of brincidofovir is limited, with compassionate access also restricted.

Reduction in immunosuppression remains the cornerstone of treatment. Immune reconstitution is essential in the resolution of HAdV infection. In a study of Feuchtinger et al., individuals who cleared HAdV infection had more HAdV-specific T cells compared to those who failed to control infection [123]. The decline in viremia and survival of HSCT recipients with HAdV infection is associated with increases in lymphocyte count, presence of HAdV-specific CD4+ T-cell responses, and increases in neutralizing antibody titers [40, 124, 125]. Currently, there is no

particular consensus on how to approach to immunosuppression reduction and when to restart immunosuppression after HAdV infection [29]. Due to the role of lymphocyte reconstitution in the clearance of infection, several small studies and phase I/II clinical trials have explored the utility of donor-derived HAdV specific T cells in the treatment of HAdV infection in HSCT [126–131]. Results were encouraging without an increased risk for GVHD associated with the reduction of immunosuppression. To date, there are no available data in SOT recipients. Use of intravenous immunoglobulin has been reported in case reports and case series of severe HAdV disease in combination with antiviral therapy [78, 132–134]. No specific conclusion can be made based on these limited data. However, patients with severe hypogammaglobulinemia (IgG $<$ 400 mg/dl), who are generally at risk for opportunistic infections, may benefit from intravenous immunoglobulin infusions [29, 135].

Prevention

Preemptive treatment strategies have been advocated for certain HSCT with increased risk for HAdV (see the section on "Epidemiology"). Because progression from viremia to disease is uncommon in SOT patients, screening strategies are not recommended in this group. The process involves HAdV monitoring in stool or peripheral blood by PCR and reduction in immunosuppression if HAdV is detected [17, 32, 136]. The rationale of this approach is to initiate therapy before clinical symptoms occur. It was previously observed that a period of asymptomatic viremia precedes disseminated adenoviral disease [41]. In addition to the reduction of immunosuppression, the administration of HAdV-specific T cells as part of the preemptive strategy has been described [17, 136]. Preemptive treatment strategy with cidofovir has been reported [114, 136, 137]; however, nephrotoxicity limits its use. More studies are needed to evaluate the role of brincidofovir in preemptive treatment [122].

Conclusion

HAdV infection most commonly presents as asymptomatic viremia in transplant patients, but progression to tissue-invasive or disseminated disease can occur. The incidence of severe disease is highest in HSCT and lung transplant patients and is associated with very high mortality. Diagnostic testing of clinical specimens should be guided by the presenting clinical syndrome as HAdV types preferentially infect specific host tissues. PCR-based assays are now the tests of choice, but results must be carefully interpreted to distinguish asymptomatic shedding from disease and to rule out the possibility of false-negative results due to serotype noncoverage or novel mutations. Reduction of immunosuppression remains the cornerstone of HAdV treatment as antivirals are often toxic and suboptimally effective. The advent of brincidofovir and HAdV-specific T cells represents new and potentially valuable tools that may revolutionize the treatment of HAdV infections.

Key Points

- Human adenoviruses (HAdV) are non-enveloped double-stranded DNA viruses of the family *Adenoviridae*.
- After acute infection, the virus can establish lifelong latent infection in lymphoepithelial tissue and can reactivate if HAdV-specific T-cell immunity is impaired as in the posttransplant setting.
- Reactivation disease in transplant patients generally occurs within 100 days of HSCT and usually presents as asymptomatic viremia.
- The incidence of HAdV viremia is 3–15% post-allogeneic HSCT in adults, and progression from viremia to invasive disease occurs in 10–20% of cases.
- The incidence of HAdV viremia is less than 10% in adult kidney, heart, and liver SOT except for lung SOT, where the incidence is higher (22%).
- The clinical presentation of HAdV infection can range from asymptomatic infection to end-organ disease, including pneumonitis, enteritis, hepatitis, tubulointerstitial nephritis, hemorrhagic cystitis, and meningoencephalitis in SOT and HSCT.
- In SOT, HAdV infection may involve the allograft, either as a primary infection or a reactivation of latent infection in the recipient or donor organ.
- Graft dysfunction in adenoviral infection has been observed in SOT recipient occasionally resulting to graft loss; however, no causal relationship has been established between HAdV and graft rejection.
- Methods for the diagnosis of HAdV infection include viral culture, shell vial centrifugation, histopathology, immunohistochemical staining, electron microscopy, immunofluorescence assays, antigen detection, serology, polymerase chain reaction (PCR), and metagenomic sequencing.
- By virtue of greater sensitivity and specific and shorter processing times, the test of choice for diagnosis of HAdV infection is PCR.
- Though a well-defined cutoff value to distinguish reactivation from disease is not yet available, a rapid rise in viral load may be associated with the development of invasive or disseminated infection.
- Reduction of immunosuppression is the cornerstone of management of HAdV infection.
- There is no current FDA-approved drug for the treatment of HAdV infection; however, among current antivirals, cidofovir has the most evidence for efficacy against HAdV.
- Immunotherapy may have a role in the treatment of HAdV in cases which poorly responded to reduced immunosuppression and antiviral therapy.
- A preemptive treatment strategy may have a role in the prevention of adenoviral disease progression in at-risk patients with asymptomatic viremia.

References

1. Echavarria M. Adenoviruses in immunocompromised hosts. Clin Microbiol Rev. 2008;21(4):704–15.
2. Ison MG. Adenovirus infections in transplant recipients. Clin Infect Dis. 2006;43(3):331–9.

3. Hoffman JA. Adenovirus infections in solid organ transplant recipients. Curr Opin Organ Transplant. 2009;14(6):625–33.
4. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. 2014;27(3):441–62.
5. Dicks MD, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. PLoS One. 2012;7(7):e40385.
6. Sanchez-Prieto R, de Alava E, Palomino T, Guinea J, Fernandez V, Cebrian S, et al. An association between viral genes and human oncogenic alterations: the adenovirus E1A induces the Ewing tumor fusion transcript EWS-FLI1. Nat Med. 1999;5(9):1076–9.
7. Kosulin K, Geiger E, Vecsei A, Huber WD, Rauch M, Brenner E, et al. Persistence and reactivation of human adenoviruses in the gastrointestinal tract. Clin Microbiol Infect. 2016;22(4):381 e1–8.
8. Roy S, Calcedo R, Medina-Jaszek A, Keough M, Peng H, Wilson JM. Adenoviruses in lymphocytes of the human gastro-intestinal tract. PLoS One. 2011;6(9):e24859.
9. Garnett CT, Talekar G, Mahr JA, Huang W, Zhang Y, Ornelles DA, et al. Latent species C adenoviruses in human tonsil tissues. J Virol. 2009;83(6):2417–28.
10. Lynch JP 3rd, Kajon AE. Adenovirus: epidemiology, global spread of novel serotypes, and advances in treatment and prevention. Semin Respir Crit Care Med. 2016;37(4):586–602.
11. Matthes-Martin S, Boztug H, Lion T. Diagnosis and treatment of adenovirus infection in immunocompromised patients. Expert Rev Anti-Infect Ther. 2013;11(10):1017–28.
12. Pettengill MA, Babu TM, Prasad P, Chuang S, Drage MG, Menegus M, et al. Probable donor-derived human adenovirus type 34 infection in 2 kidney transplant recipients from the same donor. Open Forum Infect Dis. 2019;6(3):ofy354.
13. Huh K, Kim I, Jung J, Lee JE, Jhun BW, Gu SH, et al. Prolonged shedding of type 55 human adenovirus in immunocompetent adults with adenoviral respiratory infections. Eur J Clin Microbiol Infect Dis. 2019;38(4):793–800.
14. Rutala WA, Peacock JE, Gergen MF, Sobsey MD, Weber DJ. Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities. Antimicrob Agents Chemother. 2006;50(4):1419–24.
15. Venard V, Carret A, Corsaro D, Bordigoni P, Le Faou A. Genotyping of adenoviruses isolated in an outbreak in a bone marrow transplant unit shows that diverse strains are involved. J Hosp Infect. 2000;44(1):71–4.
16. Veltrop-Duits LA, van Vreeswijk T, Heemskerk B, Thijssen JC, El Seady R, Jol-van der Zijde EM, et al. High titers of pre-existing adenovirus serotype-specific neutralizing antibodies in the host predict viral reactivation after allogeneic stem cell transplantation in children. Clin Infect Dis. 2011;52(12):1405–13.
17. Lion T, Kosulin K, Landlinger C, Rauch M, Preuner S, Jugovic D, et al. Monitoring of adenovirus load in stool by real-time PCR permits early detection of impending invasive infection in patients after allogeneic stem cell transplantation. Leukemia. 2010; 24(4):706–14.
18. Baldwin A, Kingman H, Darville M, Foot AB, Grier D, Cornish JM, et al. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. Bone Marrow Transplant. 2000;26(12):1333–8.
19. Runde V, Ross S, Trenschel R, Lagemann E, Basu O, Renzing-Kohler K, et al. Adenoviral infection after allogeneic stem cell transplantation (SCT): report on 130 patients from a single SCT unit involved in a prospective multi center surveillance study. Bone Marrow Transplant. 2001;28(1):51–7.
20. Bruno B, Gooley T, Hackman RC, Davis C, Corey L, Boeckh M. Adenovirus infection in hematopoietic stem cell transplantation: effect of ganciclovir and impact on survival. Biol Blood Marrow Transplant. 2003;9(5):341–52.
21. Avivi I, Chakrabarti S, Milligan DW, Waldmann H, Hale G, Osman H, et al. Incidence and outcome of adenovirus disease in transplant recipients after reduced-intensity conditioning with alemtuzumab. Biol Blood Marrow Transplant. 2004;10(3):186–94.

22. Gustafson I, Lindblom A, Yun Z, Omar H, Engstrom L, Lewensohn-Fuchs I, et al. Quantification of adenovirus DNA in unrelated donor hematopoietic stem cell transplant recipients. J Clin Virol. 2008;43(1):79–85.
23. La Rosa AM, Champlin RE, Mirza N, Gajewski J, Giralt S, Rolston KV, et al. Adenovirus infections in adult recipients of blood and marrow transplants. Clin Infect Dis. 2001;32(6):871–6.
24. Ganzenmueller T, Buchholz S, Harste G, Dammann E, Trenschel R, Heim A. High lethality of human adenovirus disease in adult allogeneic stem cell transplant recipients with high adenoviral blood load. J Clin Virol. 2011;52(1):55–9.
25. Watcharananan SP, Avery R, Ingsathit A, Malathum K, Chantratita W, Mavichak V, et al. Adenovirus disease after kidney transplantation: course of infection and outcome in relation to blood viral load and immune recovery. Am J Transplant. 2011;11(6):1308–14.
26. Humar A, Kumar D, Mazzulli T, Razonable RR, Moussa G, Paya CV, et al. A surveillance study of adenovirus infection in adult solid organ transplant recipients. Am J Transplant. 2005;5(10):2555–9.
27. Humar A. Reactivation of viruses in solid organ transplant patients receiving cytomegalovirus prophylaxis. Transplantation. 2006;82(2 Suppl):S9–S14.
28. Bridges ND, Spray TL, Collins MH, Bowles NE, Towbin JA. Adenovirus infection in the lung results in graft failure after lung transplantation. J Thorac Cardiovasc Surg. 1998;116(4):617–23.
29. Florescu DF, Schaenman JM, Practice ASTIDCo. Adenovirus in solid organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13527.
30. McGrath D, Falagas ME, Freeman R, Rohrer R, Fairchild R, Colbach C, et al. Adenovirus infection in adult orthotopic liver transplant recipients: incidence and clinical significance. J Infect Dis. 1998;177(2):459–62.
31. Flomenberg P, Babbitt J, Drobyski WR, Ash RC, Carrigan DR, Sedmak GV, et al. Increasing incidence of adenovirus disease in bone marrow transplant recipients. J Infect Dis. 1994;169(4):775–81.
32. Matthes-Martin S, Feuchtinger T, Shaw PJ, Engelhard D, Hirsch HH, Cordonnier C, et al. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). Transpl Infect Dis. 2012;14(6):555–63.
33. Keyes A, Mathias M, Boulad F, Lee YJ, Marchetti MA, Scaradavou A, et al. Cutaneous involvement of disseminated adenovirus infection in an allogeneic stem cell transplant recipient. Br J Dermatol. 2016;174(4):885–8.
34. Iyama S, Matsunaga T, Fujimi A, Murase K, Kuribayasi K, Sato T, et al. Successful treatment with oral ribavirin of adenovirus-associated hemophagocytic syndrome in a stem cell transplantation recipient. Rinsho Ketsueki. 2005;46(5):363–7.
35. Levy J, Wodell RA, August CS, Bayever E. Adenovirus-related hemophagocytic syndrome after bone marrow transplantation. Bone Marrow Transplant. 1990;6(5):349–52.
36. Yilmaz M, Chemaly RF, Han XY, Thall PF, Fox PS, Tarrand JJ, et al. Adenoviral infections in adult allogeneic hematopoietic SCT recipients: a single center experience. Bone Marrow Transplant. 2013;48(9):1218–23.
37. Ramsay ID, Attwood C, Irish D, Griffiths PD, Kyriakou C, Lowe DM. Disseminated adenovirus infection after allogeneic stem cell transplant and the potential role of brincidofovir – case series and 10 year experience of management in an adult transplant cohort. J Clin Virol. 2017;96:73–9.
38. Hubmann M, Fritsch S, Zoellner AK, Prevalsek D, Engel N, Bucklein V, et al. Occurrence, risk factors and outcome of adenovirus infection in adult recipients of allogeneic hematopoietic stem cell transplantation. J Clin Virol. 2016;82:33–40.
39. Howard DS, Phillips IG, Reece DE, Munn RK, Henslee-Downey J, Pittard M, et al. Adenovirus infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 1999; 29(6):1494–501.

40. Chakrabarti S, Mautner V, Osman H, Collingham KE, Fegan CD, Klapper PE, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. Blood. 2002; 100(5):1619–27.
41. Lion T, Baumgartinger R, Watzinger F, Matthes-Martin S, Suda M, Preuner S, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. Blood. 2003;102(3):1114–20.
42. Omar H, Yun Z, Lewensohn-Fuchs I, Perez-Bercoff L, Orvell C, Engstrom L, et al. Poor outcome of adenovirus infections in adult hematopoietic stem cell transplant patients with sustained adenovirus viremia. Transpl Infect Dis. 2010;12(5):465–9.
43. Michaels MG, Green M, Wald ER, Starzl TE. Adenovirus infection in pediatric liver transplant recipients. J Infect Dis. 1992;165(1):170–4.
44. McLaughlin GE, Delis S, Kashimawo L, Cantwell GP, Mittal N, Cirocco RE, et al. Adenovirus infection in pediatric liver and intestinal transplant recipients: utility of DNA detection by PCR. Am J Transplant. 2003;3(2):224–8.
45. Schaberg KB, Kambham N, Sibley RK, Higgins JPT. Adenovirus hepatitis: clinicopathologic analysis of 12 consecutive cases from a single institution. Am J Surg Pathol. 2017;41(6):810–9.
46. Haas K, Longacre T, Castillo RO. Adenovirus hepatic abscess: a novel source of fever of unknown origin in a pediatric liver transplant recipient. Dig Dis Sci. 2017;62(4):871–3.
47. Mitchell SL, Kajon AE, Kaplan SL, Kim J, Cardenas AM. An unusual case of disseminated adenovirus infection in a cystic fibrosis, liver transplant patient. J Clin Virol. 2016;81:64–7.
48. Majorant D, Qiu F, Kalil AC, Wilson N, Florescu DF. Adenovirus-A deadly disease in the solid organ transplant population: risk factors and outcomes. Transplant Proc. 2018;50(10):3769–74.
49. Engen RM, Huang ML, Park GE, Smith JM, Limaye AP. Prospective assessment of adenovirus infection in pediatric kidney transplant recipients. Transplantation. 2018;102(7):1165–71.
50. Hofland CA, Eron LJ, Washecka RM. Hemorrhagic adenovirus cystitis after renal transplantation. Transplant Proc. 2004;36(10):3025–7.
51. Buchanan W, Bowman JS, Jaffers G. Adenoviral acute hemorrhagic cystitis following renal transplantation. Am J Nephrol. 1990;10(4):350–1.
52. Satoh S, Tada H, Murakami M, Tsuchiya N, Inoue T, Togashi H, et al. The influence of mycophenolate mofetil versus azathioprine and mycophenolic acid pharmacokinetics on the incidence of acute rejection and infectious complications after renal transplantation. Transplant Proc. 2005;37(4):1751–3.
53. Shindo K, Kitayama T, Ura T, Matsuya F, Kusaba Y, Kanetake H, et al. Acute hemorrhagic cystitis caused by adenovirus type 11 after renal transplantation. Urol Int. 1986;41(2):152–5.
54. Shiramizu T, Satoh T, Jinushi K, Oka N, Inokuchi K. Renal allograft dysfunction with acute hemorrhagic cystitis caused by adenovirus in a recipient of a transplanted kidney. Tokai J Exp Clin Med. 1986;11(5):371–5.
55. Asim M, Chong-Lopez A, Nickeleit V. Adenovirus infection of a renal allograft. Am J Kidney Dis. 2003;41(3):696–701.
56. Lim AK, Parsons S, Ierino F. Adenovirus tubulointerstitial nephritis presenting as a renal allograft space occupying lesion. Am J Transplant. 2005;5(8):2062–6.
57. Alsaad KO, Tobar A, Belanger E, Ahmad M, Cattran DC, Herzenberg AM. Late-onset acute haemorrhagic necrotizing granulomatous adenovirus tubulointerstitial nephritis in a renal allograft. Nephrol Dial Transplant. 2007;22(4):1257–60.
58. Hensley JL, Sifri CD, Cathro HP, Lobo P, Sawyer RG, Brayman KL, et al. Adenoviral graft-nephritis: case report and review of the literature. Transpl Int. 2009;22(6):672–7.
59. Kolankiewicz LM, Pullman J, Raffeld M, Kopp JB, Glicklich D. Adenovirus nephritis and obstructive uropathy in a renal transplant recipient: case report and literature review. NDT Plus. 2010;3(4):388–92.

60. Veer M, Abdulmassih R, Como J, Min Z, Bhanot N. Adenoviral nephritis in a renal transplant recipient: case report and literature review. Transpl Infect Dis. 2017;19:e12716.
61. Gaspert A, Luthi B, Mueller NJ, Bossart W, Heim A, Wuthrich RP, et al. Subacute allograft failure with dysuria and hematuria in a kidney transplant recipient. Am J Kidney Dis. 2009;54(1):154–8.
62. Yagisawa T, Takahashi K, Yamaguchi Y, Teraoka S, Horita S, Toma H, et al. Adenovirus induced nephropathy in kidney transplant recipients. Transplant Proc. 1989;21(1 Pt 2):2097–9.
63. Keswani M, Moudgil A. Adenovirus-associated hemorrhagic cystitis in a pediatric renal transplant recipient. Pediatr Transplant. 2007;11(5):568–71.
64. Keller EW, Rubin RH, Black PH, Hirsch MS, Hierholzer JC. Isolation of adenovirus type 34 from a renal transplant recipient with interstitial pneumonia. Transplantation. 1977;23(2):188–91.
65. Stalder H, Hierholzer JC, Oxman MN. New human adenovirus (candidate adenovirus type 35) causing fatal disseminated infection in a renal transplant recipient. J Clin Microbiol. 1977;6(3):257–65.
66. Hierholzer JC, Atuk NO, Gwaltney JM Jr. New human adenovirus isolated from a renal transplant recipient: description and characterization of candidate adenovirus type 34. J Clin Microbiol. 1975;1(4):366–76.
67. Rosario RF, Kimbrough RC, Van Buren DH, Laski ME. Fatal adenovirus serotype-5 in a deceased-donor renal transplant recipient. Transpl Infect Dis. 2006;8(1):54–7.
68. Norris SH, Butler TC, Glass N, Tran R. Fatal hepatic necrosis caused by disseminated type 5 adenovirus infection in a renal transplant recipient. Am J Nephrol. 1989;9(2):101–5.
69. Humar A, Doucette K, Kumar D, Pang XL, Lien D, Jackson K, et al. Assessment of adenovirus infection in adult lung transplant recipients using molecular surveillance. J Heart Lung Transplant. 2006;25(12):1441–6.
70. Ohori NP, Michaels MG, Jaffe R, Williams P, Yousem SA. Adenovirus pneumonia in lung transplant recipients. Hum Pathol. 1995;26(10):1073–9.
71. Simsir A, Greenebaum E, Nuovo G, Schulman LL. Late fatal adenovirus pneumonitis in a lung transplant recipient. Transplantation. 1998;65(4):592–4.
72. Shirali GS, Ni J, Chinnock RE, Johnston JK, Rosenthal GL, Bowles NE, et al. Association of viral genome with graft loss in children after cardiac transplantation. N Engl J Med. 2001;344(20):1498–503.
73. Moulik M, Breinholt JP, Dreyer WJ, Kearney DL, Price JF, Clunie SK, et al. Viral endomyocardial infection is an independent predictor and potentially treatable risk factor for graft loss and coronary vasculopathy in pediatric cardiac transplant recipients. J Am Coll Cardiol. 2010;56(7):582–92.
74. Schowengerdt KO, Ni J, Denfield SW, Gajarski RJ, Radovancevic B, Frazier HO, et al. Diagnosis, surveillance, and epidemiologic evaluation of viral infections in pediatric cardiac transplant recipients with the use of the polymerase chain reaction. J Heart Lung Transplant. 1996;15(2):111–23.
75. Bruminhent J, Athas DM, Hess BD, Flomenberg P. Disseminated adenovirus disease in heart transplant recipient presenting with conjunctivitis. Transpl Infect Dis. 2015;17(1):125–8.
76. Gupta A, Philip A, Ranga K, Cappa J, Dougherty J, Lawlor M. An interesting case of adenoviral hepatitis in a cardiac transplant recipient. Transpl Infect Dis. 2010;12(1):84–6.
77. Refaat M, McNamara D, Teuteberg J, Kormos R, McCurry K, Shullo M, et al. Successful cidofovir treatment in an adult heart transplant recipient with severe adenovirus pneumonia. J Heart Lung Transplant. 2008;27(6):699–700.
78. Permpalung N, Mahoney MV, Alonso CD. Adjunctive use of cidofovir and intravenous immunoglobulin to treat invasive adenoviral disease in solid organ transplant recipients. Pharmacotherapy. 2018;38(12):1260–6.
79. Pinchoff RJ, Kaufman SS, Magid MS, Erdman DD, Gondolesi GE, Mendelson MH, et al. Adenovirus infection in pediatric small bowel transplantation recipients. Transplantation. 2003;76(1):183–9.

80. Ziring D, Tran R, Edelstein S, McDiarmid SV, Gajjar N, Cortina G, et al. Infectious enteritis after intestinal transplantation: incidence, timing, and outcome. Transplantation. 2005;79(6):702–9.
81. Florescu DF, Islam MK, Mercer DF, Grant W, Langnas AN, Freifeld AG, et al. Adenovirus infections in pediatric small bowel transplant recipients. Transplantation. 2010;90(2):198–204.
82. Lipson SM, Poshni IA, Ashley RL, Grady LJ, Ciamician Z, Teichberg S. Presumptive identification of common adenovirus serotypes by the development of differential cytopathic effects in the human lung carcinoma (A549) cell culture. FEMS Microbiol Lett. 1993;113(2):175–82.
83. Van Doornum GJ, De Jong JC. Rapid shell vial culture technique for detection of enteroviruses and adenoviruses in fecal specimens: comparison with conventional virus isolation method. J Clin Microbiol. 1998;36(10):2865–8.
84. Florescu MC, Miles CD, Florescu DF. What do we know about adenovirus in renal transplantation? Nephrol Dial Transplant. 2013;28(8):2003–10.
85. Weidner AS, Panarelli NC, Rennert H, Jessurun J, Yantiss RK. Immunohistochemistry improves the detection of adenovirus in gastrointestinal biopsy specimens from hematopoietic stem cell transplant recipients. Am J Clin Pathol. 2016;146(5):627–31.
86. Levent F, Greer JM, Snider M, Demmler-Harrison GJ. Performance of a new immunochromatographic assay for detection of adenoviruses in children. J Clin Virol. 2009;44(2):173–5.
87. Sambursky R, Trattler W, Tauber S, Starr C, Friedberg M, Boland T, et al. Sensitivity and specificity of the AdenoPlus test for diagnosing adenoviral conjunctivitis. JAMA Ophthalmol. 2013;131(1):17–22.
88. Landry ML, Ferguson D. SimulFluor respiratory screen for rapid detection of multiple respiratory viruses in clinical specimens by immunofluorescence staining. J Clin Microbiol. 2000;38(2):708–11.
89. Kuypers J, Campbell AP, Cent A, Corey L, Boeckh M. Comparison of conventional and molecular detection of respiratory viruses in hematopoietic cell transplant recipients. Transpl Infect Dis. 2009;11(4):298–303.
90. Ebner K, Suda M, Watzinger F, Lion T. Molecular detection and quantitative analysis of the entire spectrum of human adenoviruses by a two-reaction real-time PCR assay. J Clin Microbiol. 2005;43(7):3049–53.
91. Mackay IM, Arden KE, Nitsche A. Real-time PCR in virology. Nucleic Acids Res. 2002;30(6):1292–305.
92. Fryer JF, Hockley JG, Govind S, Morris CL and the Collaborative Study Group. Collaborative study to evaluate the proposed 1st WHO International Standard for Human Adenovirus (HAdV) DNA for nucleic acid amplification techniques (NAT). WHO ECBS Report 2018; WHO/BS/2018.2346.
93. Doern CD, Lacey D, Huang R, Haag C. Evaluation and implementation of FilmArray version 1.7 for improved detection of adenovirus respiratory tract infection. J Clin Microbiol. 2013;51(12):4036–9.
94. Song E, Wang H, Salamon D, Jaggi P, Leber A. Performance characteristics of FilmArray Respiratory Panel v1.7 for detection of adenovirus in a large cohort of pediatric nasopharyngeal samples: one test may not fit all. J Clin Microbiol. 2016;54(6):1479–86.
95. McMillen T, Lee YJ, Kamboj M, Babady NE. Limited diagnostic value of a multiplexed gastrointestinal pathogen panel for the detection of adenovirus infection in an oncology patient population. J Clin Virol. 2017;94:37–41.
96. Zou X, Fan Y, Zhang W, Lu B, Liu Y, Cao B, et al. Metagenomics facilitated complete genome sequencing of adenovirus untyped by real-time PCR. J Infect. 2018;77(2):158–64.
97. Haddad-Boubaker S, Joffret ML, Perot P, Bessaud M, Meddeb Z, Touzi H, et al. Metagenomic analysis identifies human adenovirus 31 in children with acute flaccid paralysis in Tunisia. Arch Virol. 2019;164(3):747–55.

98. Morfin F, Dupuis-Girod S, Mundweiler S, Falcon D, Carrington D, Sedlacek P, et al. In vitro susceptibility of adenovirus to antiviral drugs is species-dependent. Antivir Ther. 2005;10(2):225–9.
99. Naesens L, Lenaerts L, Andrei G, Snoeck R, Van Beers D, Holy A, et al. Antiadenovirus activities of several classes of nucleoside and nucleotide analogues. Antimicrob Agents Chemother. 2005;49(3):1010–6.
100. Chen FE, Liang RH, Lo JY, Yuen KY, Chan TK, Peiris M. Treatment of adenovirus-associated haemorrhagic cystitis with ganciclovir. Bone Marrow Transplant. 1997;20(11):997–9.
101. Nakazawa Y, Suzuki T, Fukuyama T, Katsuyama Y, Tanaka M, Yanagisawa R, et al. Urinary excretion of ganciclovir contributes to improvement of adenovirus-associated hemorrhagic cystitis after allogeneic bone marrow transplantation. Pediatr Transplant. 2009;13(5):632–5.
102. Duggan JM, Farrehi J, Duderstadt S, Turner NJ, Fekety R. Treatment with ganciclovir of adenovirus pneumonia in a cardiac transplant patient. Am J Med. 1997;103(5):439–40.
103. Gavin PJ, Katz BZ. Intravenous ribavirin treatment for severe adenovirus disease in immunocompromised children. Pediatrics. 2002;110(1 Pt 1):e9.
104. Lankester AC, Heemskerk B, Claas EC, Schilham MW, Beersma MF, Bredius RG, et al. Effect of ribavirin on the plasma viral DNA load in patients with disseminating adenovirus infection. Clin Infect Dis. 2004;38(11):1521–5.
105. Lea AP, Bryson HM. Cidofovir. Drugs. 1996;52(2):225–30; discussion 31.
106. Ljungman P, Ribaud P, Eyrich M, Matthes-Martin S, Einsele H, Bleakley M, et al. Cidofovir for adenovirus infections after allogeneic hematopoietic stem cell transplantation: a survey by the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 2003;31(6):481–6.
107. Yusuf U, Hale GA, Carr J, Gu Z, Benaim E, Woodard P, et al. Cidofovir for the treatment of adenoviral infection in pediatric hematopoietic stem cell transplant patients. Transplantation. 2006;81(10):1398–404.
108. Muller WJ, Levin MJ, Shin YK, Robinson C, Quinones R, Malcolm J, et al. Clinical and in vitro evaluation of cidofovir for treatment of adenovirus infection in pediatric hematopoietic stem cell transplant recipients. Clin Infect Dis. 2005;41(12):1812–6.
109. Vittecoq D, Dumitrescu L, Beaufils H, Deray G. Fanconi syndrome associated with cidofovir therapy. Antimicrob Agents Chemother. 1997;41(8):1846.
110. Neau D, Renaud-Rougier MB, Viallard JE, Dutronc H, Cazorla C, Ragnaud JM, et al. Intravenous cidofovir-induced iritis. Clin Infect Dis. 1999;28(1):156–7.
111. Akler ME, Johnson DW, Burman WJ, Johnson SC. Anterior uveitis and hypotony after intravenous cidofovir for the treatment of cytomegalovirus retinitis. Ophthalmology. 1998;105(4):651–7.
112. Scott RA, Pavesio C. Ocular side-effects from systemic HPMPC (Cidofovir) for a non-ocular cytomegalovirus infection. Am J Ophthalmol. 2000;130(1):126–7.
113. Lacy SA, Hitchcock MJ, Lee WA, Tellier P, Cundy KC. Effect of oral probenecid coadministration on the chronic toxicity and pharmacokinetics of intravenous cidofovir in cynomolgus monkeys. Toxicol Sci. 1998;44(2):97–106.
114. Hoffman JA, Shah AJ, Ross LA, Kapoor N. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2001;7(7):388–94.
115. Bhadri VA, Lee-Horn L, Shaw PJ. Safety and tolerability of cidofovir in high-risk pediatric patients. Transpl Infect Dis. 2009;11(4):373–9.
116. Leruez-Ville M, Minard V, Lacaille F, Buzyn A, Abachin E, Blanche S, et al. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. Clin Infect Dis. 2004;38(1):45–52.
117. Seidemann K, Heim A, Pfister ED, Koditz H, Beilken A, Sander A, et al. Monitoring of adenovirus infection in pediatric transplant recipients by quantitative PCR: report of six cases and review of the literature. Am J Transplant. 2004;4(12):2102–8.

118. Painter W, Robertson A, Trost LC, Godkin S, Lampert B, Painter G. First pharmacokinetic and safety study in humans of the novel lipid antiviral conjugate CMX001, a broad-spectrum oral drug active against double-stranded DNA viruses. Antimicrob Agents Chemother. 2012;56(5):2726–34.
119. Toth K, Spencer JF, Dhar D, Sagartz JE, Buller RM, Painter GR, et al. Hexadecyloxypropyl-cidofovir, CMX001, prevents adenovirus-induced mortality in a permissive, immunosuppressed animal model. Proc Natl Acad Sci U S A. 2008;105(20):7293–7.
120. Florescu DF, Keck MA. Development of CMX001 (Brincidofovir) for the treatment of serious diseases or conditions caused by dsDNA viruses. Expert Rev Anti-Infect Ther. 2014;12(10):1171–8.
121. Florescu DF, Pergam SA, Neely MN, Qiu F, Johnston C, Way S, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. Biol Blood Marrow Transplant. 2012;18(5):731–8.
122. Grimley MS, Chemaly RF, Englund JA, Kurtzberg J, Chittick G, Brundage TM, et al. Brincidofovir for asymptomatic adenovirus viremia in pediatric and adult allogeneic hematopoietic cell transplant recipients: a randomized placebo-controlled phase II trial. Biol Blood Marrow Transplant. 2017;23(3):512–21.
123. Feuchtinger T, Lucke J, Hamprecht K, Richard C, Handgretinger R, Schumm M, et al. Detection of adenovirus-specific T cells in children with adenovirus infection after allogeneic stem cell transplantation. Br J Haematol. 2005;128(4):503–9.
124. Heemskerk B, Lankester AC, van Vreeswijk T, Beersma MF, Claas EC, Veltrop-Duits LA, et al. Immune reconstitution and clearance of human adenovirus viremia in pediatric stem-cell recipients. J Infect Dis. 2005;191(4):520–30.
125. van Tol MJ, Claas EC, Heemskerk B, Veltrop-Duits LA, de Brouwer CS, van Vreeswijk T, et al. Adenovirus infection in children after allogeneic stem cell transplantation: diagnosis, treatment and immunity. Bone Marrow Transplant. 2005;35(Suppl 1):S73–6.
126. Feuchtinger T, Matthes-Martin S, Richard C, Lion T, Fuhrer M, Hamprecht K, et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. Br J Haematol. 2006;134(1):64–76.
127. Leen AM, Christin A, Myers GD, Liu H, Cruz CR, Hanley PJ, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. Blood. 2009;114(19):4283–92.
128. Amrolia PJ, Muccioli-Casadei G, Huls H, Adams S, Durett A, Gee A, et al. Adoptive immunotherapy with allodepleted donor T-cells improves immune reconstitution after haploidentical stem cell transplantation. Blood. 2006;108(6):1797–808.
129. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121(26):5113–23.
130. Gerdemann U, Katari UL, Papadopoulou A, Keirnan JM, Craddock JA, Liu H, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. Mol Ther. 2013;21(11):2113–21.
131. Sili U, Leen AM, Vera JF, Gee AP, Huls H, Heslop HE, et al. Production of good manufacturing practice-grade cytotoxic T lymphocytes specific for Epstein-Barr virus, cytomegalovirus and adenovirus to prevent or treat viral infections post-allogeneic hematopoietic stem cell transplant. Cytotherapy. 2012;14(1):7–11.
132. Saquib R, Melton LB, Chandrakantan A, Rice KM, Spak CW, Saad RD, et al. Disseminated adenovirus infection in renal transplant recipients: the role of cidofovir and intravenous immunoglobulin. Transpl Infect Dis. 2010;12(1):77–83.
133. Emovon OE, Lin A, Howell DN, Afzal F, Baillie M, Rogers J, et al. Refractory adenovirus infection after simultaneous kidney-pancreas transplantation: successful treatment with

intravenous ribavirin and pooled human intravenous immunoglobulin. Nephrol Dial Transplant. 2003;18(11):2436–8.
134. Park UJ, Hyun SK, Kim HT, Cho WH, Han SY. Successful treatment of disseminated adenovirus infection with ribavirin and intravenous immunoglobulin in an adult renal transplant recipient: a case report. Transplant Proc. 2015;47(3):791–3.
135. Mawhorter S, Yamani MH. Hypogammaglobulinemia and infection risk in solid organ transplant recipients. Curr Opin Organ Transplant. 2008;13(6):581–5.
136. Lindemans CA, Leen AM, Boelens JJ. How I treat adenovirus in hematopoietic stem cell transplant recipients. Blood. 2010;116(25):5476–85.
137. Anderson EJ, Guzman-Cottrill JA, Kletzel M, Thormann K, Sullivan C, Zheng X, et al. High-risk adenovirus-infected pediatric allogeneic hematopoietic progenitor cell transplant recipients and preemptive cidofovir therapy. Pediatr Transplant. 2008;12(2):219–27.

BK Polyomavirus After Solid Organ and Hematopoietic Cell Transplantation: One Virus – Three Diseases

33

Fabrice E. Graf and Hans H. Hirsch

Contents

Introduction 808
Polyomavirus-Associated Nephropathy: PyVAN 808
Pathology and Clinical Impact 808
Clinical Evaluation and Diagnostics 813
Disease Epidemiology and Risk Factors 815
Management 815
Polyomavirus-Associated Hemorrhagic Cystitis: PyVHC 817
Pathology and Clinical Impact 817
Clinical Evaluation and Diagnostics 820
Disease Epidemiology and Risk Factors 820
Management 821
PyV-Associated Urothelial Cancer: PyVUC 823
Conclusion 824
Key Points 825
References 826

F. E. Graf
Transplantation and Clinical Virology, Department Biomedicine, University of Basel, Basel, Switzerland
e-mail: fabrice.graf@unibas.ch

H. H. Hirsch (✉)
Transplantation and Clinical Virology, Department Biomedicine, University of Basel, Basel, Switzerland

Clinical Virology, Laboratory Medicine, University Hospital Basel, Basel, Switzerland

Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland
e-mail: hans.hirsch@unibas.ch

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_29

Introduction

A frequent complication in kidney transplantation (KT) and allogeneic hematopoietic stem cell transplantation (HSCT) results from uncontrolled replication of BK polyomavirus (BKPyV). BKPyV is the causative agent of polyomavirus-associated nephropathy (PyVAN) seen in 1–15% of KT patients and polyomavirus-associated hemorrhagic cystitis (PyVHC) occurring in 5–20% of HSCT recipients. In addition, PyV-associated urothelial cancer (PyVUC) has been recognized as an emerging entity [52, 71]. Immunocompetent individuals are not affected by these diseases despite three striking facts: First, more than 90% of the general human population has been exposed to BKPyV according seroprevalence studies around the world [20, 51]. Second, the virus is not cleared from the human host, but persists in the kidneys despite the presence of potent neutralizing antibodies and virus-specific T cells [75, 84]. Third, at least 10% of BKPyV-IgG-seropositive immunocompetent healthy blood donors shed the virus asymptomatically in the urine indicating viral immune escape [20]. These virologic characteristics contribute to rendering BKPyV one of the most successful human-specific viruses, whereby the absence of known health impact argues for a long-standing coevolutionary adaptation with the human host. Conversely, this carefully established virus-host balance is likely to be significantly perturbed in patients' developing disease. Moreover, the differences in pathology and between the two key populations at risk for disease argue that specific aspects of relevance to the pathogenesis must differ. If so, research on the relevant viral and host factors and the specific perturbing factors should allow understanding of specific risk profiles as well as targeted prevention and therapy approaches. Indeed, PyVHC is rarely observed after KT, while PyVAN is rarely diagnosed after allogeneic HSCT, and both are very infrequent in non-kidney solid organ transplants (SOT) [29, 85]. Here, we review the key manifestations of BKPyV pathology, the diagnostic tools, and the epidemiological markers of risk and treatment response (Fig. 1).

Polyomavirus-Associated Nephropathy: PyVAN

Pathology and Clinical Impact

Proven PyVAN is defined by the detection of BKPyV proteins in cytopathically altered renal tubular epithelial cells. The most commonly used marker of BKPyVAN is the immunohistochemical detection of the large T antigen (LTag) using the cross-reacting monoclonal antibody raised against the homologous protein of the monkey SV40 polyomavirus [6, 16, 76]. An estimated 8% (95% CI 1–15%) of kidney transplant (KT) recipients are at risk of proven PyVAN [38]. In the first case series from the USA and Europe, increased immunosuppression involving steroid pulses in response to acute rejection episodes and switching to high-dose tacrolimus-containing "rescue" immunosuppression have been consistent hallmarks [34], a combination that also emerged from prospective studies [38, 42].

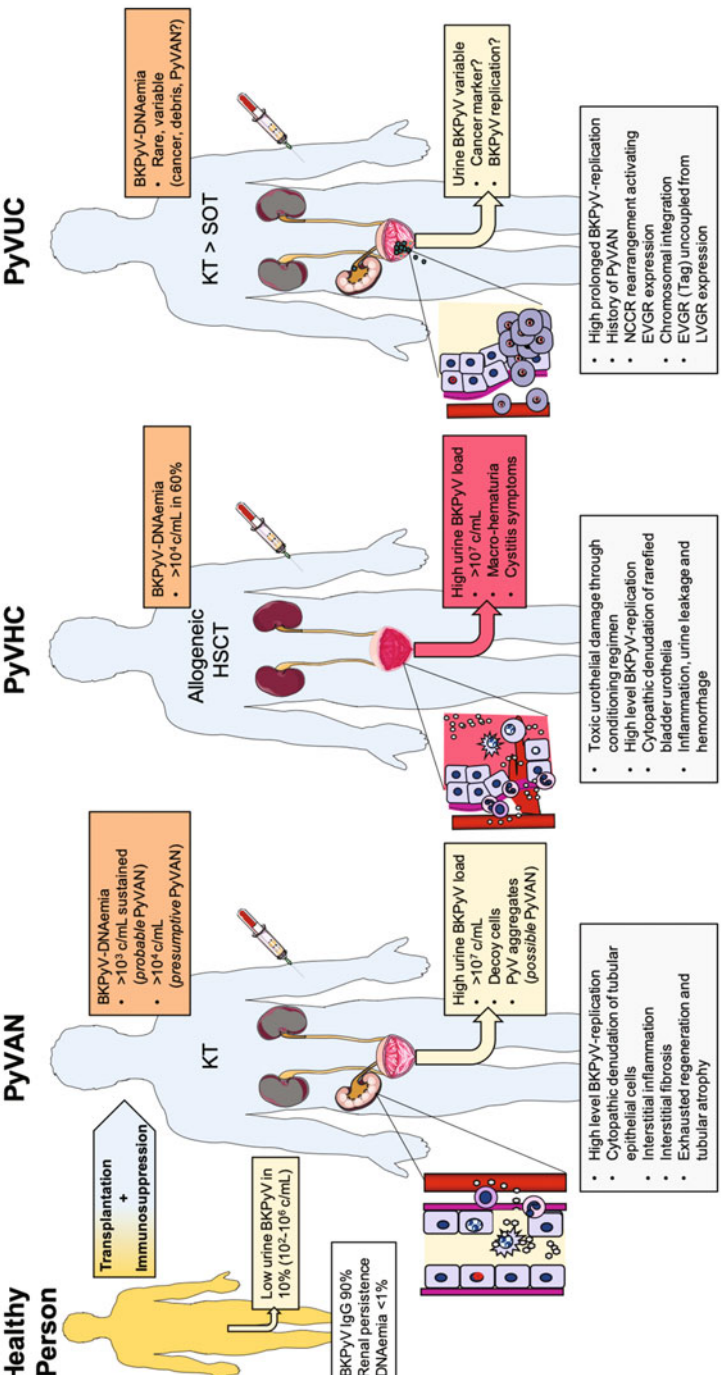


Fig. 1 BK polyomavirus infection, replication, and disease in transplantation. For details see illustrations and text. Abbreviations: *EVGR* early viral gene region, *HSCT* hematopoietic stem cell transplant, *KT* kidney transplant, *LVGR* late viral gene region, *NCCR* noncoding control region, *PyVAN* polyomavirus-associated nephropathy, *PyVHC* polyomavirus-associated hemorrhagic cystitis, *PyVUC* polyomavirus-associated urothelial cancer, *SOT* solid organ transplant, *Tag* tumor antigen. Some illustration were adapted from: https://smart.servier.com (licensed under a Creative Commons Attribution 3.0 Unported License).

The clinical impact of uncontrolled BKPyV replication in KT can be projected when considering that, following induction with depleting antilymphocyte or non-depleting interleukin-2 antibodies, the combination of tacrolimus, mycophenolate, and steroids is the current standard maintenance immunosuppression for approximately 90% of KT recipients around the world. According to a large international multicenter open-label study having BKPyV-DNAemia post-transplant up to month 6 post-transplant as secondary endpoint, BKPyV-DNAemia was detected in 16.3% of the patients in the tacrolimus arm (probable PyVAN), of which 9.4% had plasma BKPyV loads of more than 10,000 copies/mL (presumptive PyVAN) [42]. At the follow-up visit at month 12, BKPyV-DNAemia was still seen in 12% and significantly associated with a reduced estimated glomerular filtration rate (eGFR) of 60.4 mL/min compared to 65.7 mL/min in non-BKPyV-DNAemic patients [42]. In a simplified estimate, approximately 200,000 patients with terminal renal failure are listed for KT in North America and Europe, 50,000 are transplanted each year, and 10,000 patients decease while wait-listed. Taking BKPyV-DNAemia rates of 15% and 10% at month 6 and 12 post-transplant, 7500 and 5000 KT patients annually must be considered at risk for BKPyV-associated premature renal allograft failure in North America and Europe, hence not reaching the average kidney graft survival time of 13–15 years. Given the complex interactions of too high and too low immunosuppression and acute and chronic rejection pathologies, the direct attribution of premature allograft failure to persisting BKPyV replication and PyVAN is difficult. This is not because the ability and interest in modern diagnostic work-up is fading, but because so far, inflammatory changes in the renal allograft biopsy cannot be readily attributed to either viral damage or antiviral or anti-donor immunity. Importantly, these entities should not be regarded as mutually exclusive as long as validated markers are lacking to assess their potential coexistence, contribution, and dynamics. This applies also to subsequent unspecific histological changes including interstitial fibrosis/tubular atrophy ("chronic allograft failure"), which may be partly caused by difficult overlapping management issues for acute cellular rejection and/or antibody-mediated rejection. Importantly, the perspective of returning to waiting lists and retransplantation may be dire because of the higher immunological risk, the prolonged waiting times, and death while waiting as well as the increased risk of recurrent PyVAN. The impact on patient and relatives as well as the associated health-care costs of this condition should not be underestimated.

The first large systematic description of histological features of renal biopsies of PyVAN proposed five histological stages that were increasingly associated with graft loss as the most severe outcome. Cytopathic effects of viral replication, the extent of inflammation, as well as tubular atrophy and fibrosis were considered (Fig. 2). Immunohistochemical detection of LTag using SV40 antibodies showed BKPyV-replicating cells typically confined to nephrons next to unaffected tissue emphasizing the focal nature of the disease (Fig. 2). This (multi)focal distribution increases the likelihood of a PyVAN-negative biopsy especially early in the disease. Indeed, biopsies from the same patients obtained simultaneously had discordant negative and positive biopsies in 10–30% of cases, mostly without inflammatory infiltrates [17].

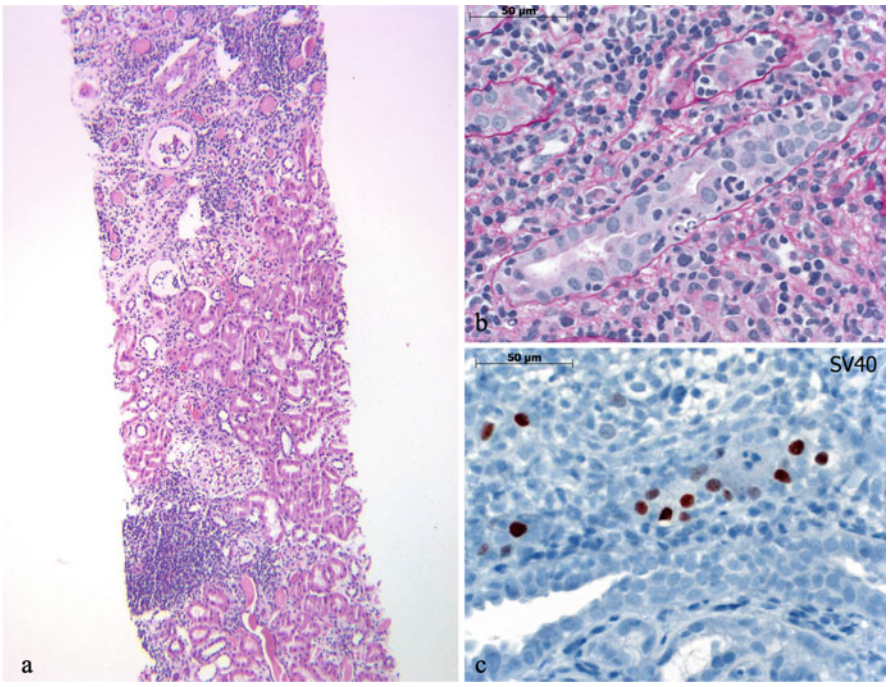


Fig. 2 (**a**) Renal biopsy showing viral cytopathic changes and interstitial inflammation. NB, not all areas are affected. (**b**) Tubulus with viral inclusions (enlarged nuclei) in renal proximal tubular epithelial cells. Viral replication leads to denudation and tubular atrophy. (**c**) Immunohistochemistry with SV40 LTag cross-reacting antibodies (dark spots) demonstrates viral presence. Histology images courtesy of Professor Helmut Hopfer (Pathology, University Hospital Basel).

Based on this initial study, PyVAN is classified into three categories: PyVAN-A (mild viral cytopathic changes), PyVAN-B (cytopathic changes and significant interstitial inflammatory infiltrates of increasing intensity), and PyVAN-C (less cytopathic changes, less interstitial inflammation but extensive tubular atrophy and interstitial fibrosis). PyVAN-B is further subdivided in three subgroups according to the extent of interstitial inflammation in the biopsy core (B1 of 11–25%, B2 26–50%, or B3 >50%, respectively) [17, 40]. All PyVAN patients showed significant viruria (mean of 2.5×10^8 c/mL), decoy cells (cells with enlarged nuclei and viral particle inclusions) in urine, and BKPyV-DNAemia (mean of 2.32×10^7 c/mL) [17, 24] (Table 1).

Recently, the Banff Working Group of Pathologists have proposed scores from 1 to 3 according to the intrarenal PyV load (PyVL) and the interstitial fibrosis ci score from 0 to 3 (Table 1). Analyzing 192 patients with proven PyVAN, PyVL and ci score were significantly correlated with renal function at time of index biopsy, renal function at 2 years post-transplant, and eventual graft failure. Since inflammation may have an independent role in affecting the renal allograft function, proven PyVAN is currently recommended to be scored by either classification until independent prospective re-evaluation is possible in the future [44].

Table 1 Clinical and laboratory observations in patients with BKPyV-associated disease

PyVAN	PyVHC
Clinical diagnosis: • Possible: high-level BKPyV-viruria >7 $\log_{10}$ c/mL and/or decoy cells • Probable: BKPyV-DNAemia >3 $\log_{10}$ c/mL sustained for >2 weeks • Presumptive (laboratory-confirmed): increasing BKPyV-DNAemia of >4 $\log_{10}$ c/mL • Proven: renal biopsy showing cytopathic changes plus viral protein expression demonstrated by immunohistochemistry *Evaluation of biopsy*: PyVAN-A: mild viral cytopathic changes, little or no inflammation, little or no fibrosis or tubular atrophy PyVAN-B: cytopathic changes and significant inflammation (extent of inflammation: B1 = 11–25%, B2 = 26–50%, B3 = >50%). PyVAN-C: little viral cytopathic changes, little interstitial inflammation, extensive (>50%) tubular atrophy and interstitial fibrosis *Classification of PyVAN (Banff working group)* PyVAN class-1: PyVL=1 Banff Ci score = 0-1 PyVAN class-2: 1 2-3 2 0-3 3 0-1 PyVAN class-3:3 2-3 (PyVL 1 ≤ 1%, PyVL 2 > 1% – ≤10%, PyVL 3 >10% of all tubules/ducts SV40+LTag; Ci score: extent of interstitial fibrosis of the cortical area of 0 ≤ 5%, 1 = 6–25%, 2 = 26–50%, 3 >50%)	*Clinical and laboratory triad*: 1. Clinical symptoms of cystitis: dysuria, urge, frequency, lower abdominal pain 2. Grade 2 or higher hematuria 3. BKPyV-viruria of >7 $\log_{10}$ c/mL *and* Exclusion of other relevant causes of HC: other infectious agents, bleeding disorders, metastatic neoplasia, trauma including stents or catheters *Grading of hematuria*: Grade 1: microscopic Grade 2: macroscopic Grade 3: macroscopic with clots Grade 4: macroscopic with clots and post-renal failure *Adjunct diagnostic considerations*: BKPyV-DNAemia >4 $\log_{10}$ c/mL (seen in approximately 70% of cases with triad)

Clearance of PyVAN was recently evaluated by Menter and colleagues in patients treated with reducing immunosuppression [63]. They observed a significant increase in interstitial inflammation and in intraepithelial lymphocytes, often with disappearance of the SV40-LTag signal, but a rise in serum creatinine levels with resolving PyVAN. While these observations support the independent role of inflammation affecting renal allograft function, the self-limiting nature without explicit anti-inflammatory or antirejection treatment in that series argues that regaining BKPyV-specific immunity may transiently affect renal allograft function akin to immune reconstitution inflammatory syndrome (IRIS) prominently described in HIV/AIDS [39] and to multiple sclerosis patients discontinuing natalizumab for progressive multifocal leukoencephalopathy, another PyV pathology caused by JC polyomavirus in the brain [41]. However, acute interstitial cellular rejection remains an important differential diagnosis in this setting, which currently cannot be distinguished molecularly or morphologically.

Nevertheless, plasma BKPyV-DNAemia dynamics can be correlated with the histopathology, whereby inflammation and early decline in BKPyV loads in SV40-cleared renal biopsies favor the diagnosis of antiviral immune reconstitution over acute cellular rejection [63].

The low sensitivity of allograft biopsy compared to plasma BKPyV-DNAemia is supported by another study of proven PyVAN, reporting faster clearance of SV40-LTag in the biopsy than BKPyV-DNAemia [48]. A 50% clearance rate of SV40-LTag detection by immunohistochemistry was seen after approximately 4 months (total 18/22 (81.8%) patients at 6.2 +/− 7.5 months) compared to approximately 8 months for BKPyV-DNAemia (total 17/22; 77.3%) following tacrolimus to cyclosporine A conversion or administration of intravenous immunoglobulin (IVIG) preparations [48]. Finally, allograft nephrectomy in KT patients with terminal PyVAN was followed by a drop to undetectable levels of BKPyV-DNAemia [23]. Since immediate clearance of BKPyV-DNAemia was also observed without reducing immunosuppression as in kidney-pancreas transplantation, it can be concluded that plasma BKPyV loads reflect BKPyV replication in the renal allograft.

The role of inflammation also emerged from two recent studies having similar major findings [19, 70]. SV40-LTag was not detected in approximately 23% of biopsies having minor interstitial inflammation which predated a subsequent diagnosis of proven PyVAN. However, tubular injury attributed to viral replication was associated with inflammation in about three quarters of cases, and interstitial inflammation was stable over repeated biopsies. Viral clearance (resolving PyVAN) concurred with increasing inflammation as reported [63]. Currently, it is not possible to distinguish if inflammation is caused by innate responses to viral cytopathic damage, adaptive immunity to BKPyV antigens or to allo-antigens defining rejection, or their relative contributions in case of coexistence. More than half of the PyVAN cases were diagnosed with acute rejection after reducing immunosuppression rather than IRIS, and hence >40% of graft loss was attributed to rejection [70]. The impressive discordance to the recent Basel report of approximately 10% acute rejection indicates differences in management of BKPyV in KT rather than diagnosis and suggests that treatment by reducing immunosuppression should not be delayed or await a diagnosis of proven PyVAN, but rather be based on probable and presumptive PyVAN defined by plasma BKPyV-DNAemia [37, 44].

Since false-negative histology on biopsies occurs especially with the early PyVAN-A, plasma BKPyV-DNAemia quantification should always be performed concurrently with any allograft biopsy (protocol or for cause), to guide histopathology. The relationship of renal function, BKPyV-DNAemia, PyVAN stage, false-negative biopsies, and predicted graft outcome is illustrated in Fig. 3.

Clinical Evaluation and Diagnostics

Clinical evaluation of KT patients is based on serial renal function using the eGFR, plasma BKPyV-DNAemia, and protocol biopsies and/or anti-donor immunity such

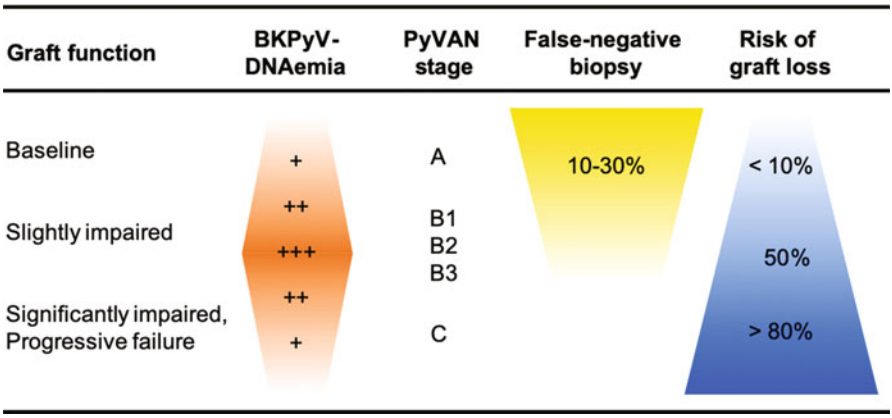


Fig. 3 Kidney graft function gradually declines with progression of PyVAN to later stages. BKPyV-DNAemia reflects viral replication dynamics, and high loads initially correlate with decreasing graft function and a higher risk of graft loss. The rate of false-negative biopsies is up to 30% in early-stage PyVAN with focal involvement (probable and presumptive PyVAN defined by plasma BKPyV loads), for which response to reducing immunosuppression is highest.

as antibody assays. Screening describes testing KT patients without any specific findings and at baseline renal function. The current AST-IDCOP guidelines recommend monthly screening for plasma BKPyV-DNAemia, and to better reflect the continuum of BKPyV replication in the renal allograft, detection of plasma BKPyV loads >3 $\log_{10}$ c/mL sustained for >2 weeks is denoted probable PyVAN and increasing BKPyV-DNAemia to >4 $\log_{10}$ c/mL as presumptive PyVAN (Table 1) [44]. In KT patients with baseline renal function lacking an increased immunological risk, renal allograft biopsy is not required before reducing immunosuppression, which streamlines the timeliness of this intervention. However, any renal allograft biopsy should be accompanied by the less invasive, more sensitive testing for plasma BKPyV-DNAemia to guide histopathology and prime early intervention. In case of SV40-LTag-positive immunohistochemistry and undetectable plasma BKPyV-DNAemia, a rare case of JCPyV-associated nephropathy should be considered and a molecular evaluation of plasma, urine, and biopsy be performed for both viruses [18, 45, 56, 64].

Monthly screening for the first 9 months and thereafter once every 3 months until 2 years post-transplant is strongly recommended, recognizing that detection of PyVAN in early stages generally leads to a much better outcome. The incidence of BKPyV-DNAemia is highest in the first 6 months post-transplant, but in 20–30% of cases, this occurs later, most within the following 3 months [8, 46, 57, 82] as discussed recently [37]. In about 18% of adult KT patients, PyVAN manifests after the first year post-transplant [47].

Longer screening periods may be considered for children because the clinical course may differ from adults, e.g., BKPyV-seronegative status or hyperfiltration caused by donor-recipient size mismatch; the latter may hide a progression to

PyVAN with tissue damage due to a delayed observed increase in serum creatinine. Roughly 10% of new-onset BKPyV-DNAemia in pediatric patients occurred after 2 years post-transplant [46]. BKPyV-DNA QNAT should always be performed if an allograft biopsy is taken to support diagnosis, establish causality, and guide treatment [44].

For the reasons outlined above, the predictive value of BKPyV-DNAemia is 10–50% for proven PyVAN, but increases with duration and decreasing eGFR [18, 44]. High-level viruria (decoy cells or >7 $\log_{10}$ c/mL) has a low positive predictive value but a high negative predictive value (>95%) allowing to rule out BKPyV-DNAemia and proven PyVAN within the next 4 to 8 weeks.

Disease Epidemiology and Risk Factors

Several genetic, immunological, and other factors have been associated with a higher risk of developing BKPyV-DNAemia and PyVAN in KT recipients. Donor factors include deceased donor, HLA mismatch, female gender, donor BKPyV viruria, and high BKPyV-IgG levels, the latter two indicating a risk of higher BKPyV transmission via the renal allograft. Recipient factors include low or undetectable BKPyV-IgG, low or absent BKPyV-specific T cells, higher number of HLA mismatches, AB0 incompatibility, male gender, and older age in the fifth decade of life except for pediatric KT, where younger children presumably due to no previous exposure are considered to be at increased risk [94]. Post-transplant, ureteric stents, acute rejection and antirejection treatment, higher cumulative steroid exposure, maintenance immunosuppression (tacrolimus > cyclosporine A > mTor inhibitors), and retransplantation (especially if BKPyV-DNAemia has not been cleared and allograft still in place) have an increased risk of BKPyV-DNAemia and PyVAN. Most of these factors have been independently observed in several studies, yet translation into clinical decisions is pending, i.e., screening of donor or recipient, living and deceased donor organ allocation, or potential antiviral prophylaxis is not supported by sufficient clinical data evaluating risk/benefit; hence the best approach has not yet been established [49].

Management

Since no specific antiviral treatment is available for BKPyV replication, treatment for probable, presumptive, or proven PyVAN is to promptly reduce immunosuppression. Clearance of BKPyV-DNAemia is then successful in 80–100% of cases [9, 32, 83]. Lower response rates have been observed in patients with prolonged BKPyV-DNAemia and proven PyVAN [48, 83, 87], stressing the benefit of immediate preemptive treatment in patients that are not at risk of acute rejection. Relapse of BKPyV-DNAemia is seen in about 10% of patients who initially cleared [8]. This suggests that plasma BKPyV loads below the lower level of quantification (LLOQ) or even the limit of detection (LOD) still reflect the

negotiated net balance between immunosuppression and immune control. In KT patients with probable or presumptive PyVAN and normal renal function who are not at high risk of acute allograft rejection, lowering immunosuppression can be done without a kidney biopsy. Conversely, in KT recipients with a high risk of rejection and/or significant decline in renal function (e.g., eGFR decrease of >20%), a biopsy may be helpful to rule out acute rejection.

Immunosuppression is typically reduced stepwise, depending on the response, starting with the calcineurin by 25–50% followed by lowering the dose of the antiproliferative drug by 50%. Alternatively, and with no data to support superiority of either approach, this is applied in reverse: first a reduction of the antiproliferative drug, followed by the reduction of the calcineurin inhibitor, and subsequent discontinuation of the antiproliferative drug. Orally co-administered prednisone is tapered to ≤10 mg per day. As a first target, immunosuppressive drug levels should be for tacrolimus <6 ng/mL, cyclosporine <150 ng/mL, mycophenolate mofetil/mycophenolic acid ≤50% of the daily maintenance dose, and sirolimus <6 ng/mL (Table 2). Tacrolimus trough levels of <3 ng/mL and cyclosporine <100 ng/mL may be required in patients not responding by BKPyV-DNAemia decline and sustained clearance or with disease progression. Importantly, immunosuppression reduction is monitored by serum creatinine concentrations and BKPyV-DNAemia, respectively, in at least 2-weekly intervals until BKPyV-DNAemia is cleared. Time to clearance correlates with advanced disease stage and higher plasma viral load, but may occur within 8–12 weeks in early and uncomplicated cases. After successful and stable clearance of plasma BKPyV-DNAemia, standard maintenance immunosuppression may be reinstated to counteract rejection [44]. Although no explicit data are available in this regard, this is usually considered after 6–8 weeks of sustained BKPyV-DNAemia clearance.

Switching from tacrolimus to low-dose cyclosporine A or to mTOR inhibitors (sirolimus, everolimus), from mycophenolic acid to low-dose mTOR inhibitor or leflunomide, has been discussed as an alternative treatment option [43, 59, 60]. Despite these virologically sound and promising outcomes, dedicated randomized control trials are lacking to recommend one treatment strategy over another.

Retransplantation has been performed successfully in over a hundred cases where the renal allograft loss was caused by PyVAN. It is presently unclear, whether or not removal of the first damaged transplant is beneficial to decrease the likelihood of recurrent PyVAN in the new graft. However, sustained clearance of BKPyV-DNAemia for 3 months is tentatively recommended as a marker of sufficient BKPyV-specific immune effectors. Moreover, the source of BKPyV-DNAemia can be attributed to the new allograft if the damaged transplant had been removed [36]. Preemptive retransplantation in patients failing their kidney transplant due to PyVAN should be evaluated with caution. An at least >2 $\log_{10}$ decline of BKPyV-DNAemia and removal of the first allograft should be considered [15, 26, 36]. Finally, a theoretical concern is the risk of superinfecting the new graft, which may be increased due to ureteric stenting and backwash of BKPyV viruria from the old graft following bladder contraction [24, 68, 95].

Antiviral or immune therapy is not established since data on efficacy, risk, and benefit has not been evaluated in proper randomized control trials. However, several alternative or adjunctive treatment options have been reported in mostly small case series. In most cases, the intervention was confounded by simultaneous application of different interventions such as reducing or switching immunosuppression or administration of IVIG [48]. The nucleotide analog cidofovir approved to treat ganciclovir-resistant cytomegalovirus retinitis has been shown to have in vitro activity against BKPyV replication in primary human tubular epithelial cells [5]. No clear benefit could be shown, and the in vitro inhibitory concentration of cidofovir (IC_{90} of 40 μg/mL) may not be reached making its application highly questionable. Moreover, nephrotoxicity and uveitis are feared side effects that were observed in about a fifth of patients receiving intravenous cidofovir. High-dose intravenous cidofovir may not be more efficient due to the need for probenecid preventing uptake in the renal tubule cells, which is supported by mostly failures of treating rare cases of PyVAN in non-kidney SOT [3]. Brincidofovir (CMX001), the lipid-ester prodrug of cidofovir, is thought to increase bioavailability and intracellular drug concentrations and may potentially be the safer drug. Brincidofovir shows higher activity against BKPyV replication in vitro both in renal tubular and urothelial cell culture models [5, 77, 79, 91]. Clinical studies are lacking, and a balanced representation of failures is not available to draw conclusions [78].

Fluoroquinolone antibiotics were shown to have some efficacy against BKPyV replication, likely by inhibition of the helicase domain of the viral large T antigen. Initial studies proposed some protective activity when used prophylactically, but a randomized control trial with levofloxacin could not demonstrate a reduction of BKPyV-DNAemia when compared to the placebo control. Importantly, fluoroquinolone-resistant bacteria increasingly emerged [53, 86].

IVIG preparations contain neutralizing antibodies, and several reports showed clearance of BKPyV-DNAemia. Even though the results look promising, the strength of the effect of IVIG is difficult to assess because administration was combined with lowering of immunosuppression and antivirals [48, 93]. Administration of IVIG or monoclonal antibody therapy for prophylaxis has not been tested yet. Thus, caution is required when administering compounds with presumed antiviral activity, leaving possibly only IVIG as an adjunctive, rather expensive therapy with acceptable risk-benefit profile and potentially anti-inflammatory, steroid-saving properties, if administered in high-dose (Table 2).

Polyomavirus-Associated Hemorrhagic Cystitis: PyVHC

Pathology and Clinical Impact

The pathology of PyVHC is not well established due to reluctance of cystoscopy in early and mild cases and, conversely, reluctance for taking biopsies in advanced cases needing urological intervention in order to avoid further injury to the already widely denuded, profusely bleeding bladder surfaces. Otherwise, few histopathology

Table 2 Current management strategies of PyVAN and PyVHC

PyVAN	PyVHC
Strategy 1: Reduction of calcineurin inhibitor: 25–50% in 1–2 steps Reduction of antiproliferative drug: 50% in first step, discontinued in next step *Strategy 2*: Reduction of antiproliferative drug: 50% Reduction of calcineurin inhibitor: 25–50% in 1–2 steps Discontinue antiproliferative drug Prednisone (corticosteroids) adjusted to ≤ 10 mg/day *Recommended trough levels*: Tacrolimus <6 ng/mL (<3 ng/mL in non-response) Cyclosporine <150 ng/mL (<100 ng/mL in non-response) Mycophenolate mofetil/mycophenolic acid ≤50% of the daily maintenance dose or stop Sirolimus <6 ng/mL *Not recommended*: Fluoroquinolones, cidofovir *Prophylaxis*: Not established *Experimental*: Switching from tacrolimus/mycophenolate to low-dose cyclosporine A/mTOR inhibitors (sirolimus, everolimus) Leflunomide Brincidofovir Intravenous immunoglobulins	*Supportive therapy*: Analgesics, (hyper-)hydration, forced diuresis, platelet transfusion *Weakly recommended*: Fibrine glue Hyperbaric oxygen therapy Intravenous cidofovir (No agreement on dose, most experience with: 3–5 mg/kg/ every 1 or 2 weeks with probenecid or 0.5–1.5 mg/kg 1–3 times per week without probenecid) *Not recommended*: Fluoroquinolones, cidofovir (intravesicular); reducing immunosuppression *Prophylaxis*: Hyperhydration and bladder irrigation during conditioning regimen to prevent extensive urotoxicity of drugs and/or irradiation *Experimental*: Brincidofovir Virus-specific T-cells (mesenchymal stoma cells)

studies are available from cystectomy as the ultimate intervention to control anemic bleeding, leaving little hence not representative histology information. Thus, more emphasis needs to be placed on a stringent case definition of PyVHC. The clinical definition of PyVHC requires the triad of (i) symptoms of cystitis such as dysuria, lower abdominal pain, urge, and/or frequency; (ii) macroscopic hematuria (grade ≥2); and (iii) high-level BKPyV viruria defined as >7 $\log_{10}$ c/mL. Each of the markers may occur alone and is not sufficient. Hematuria is graded according to the severity and is classified into four stages that guide diagnosis of PyVHC: grade 1 microscopic or test-derived; grade 2 macroscopic; grade 3 macroscopic with clots; and grade 4 macroscopic with clots and post-renal failure [4].

Notably, high-level BKPyV viruria is very frequent after allogeneic HSCT, without macrohematuria or cystitis [2], and, hence, is by itself not sufficient for a diagnosis of PyVHC. Similarly, painless macrohematuria can be due to a number of etiologies after allogeneic HSCT and is also not sufficient for the diagnosis of PyVHC. Importantly, other potentially coexisting etiologies of cystitis should be

ruled out, the most difficult one being graft-versus-host disease (GVHD) as detailed below.

The pathogenesis of PyVHC is not fully elucidated, but a sequence of events has been elaborated by Hirsch in the year 2000, after reviewing all available data at that time [7]: First, the conditioning regimen, especially with cyclophosphamide, busulfan, or ifosfamide, or full-body irradiation induces cytopathic damage to the urothelial tissue. Hyperhydration with forced diuresis and mesna (given in combination with cyclophosphamide or ifosfamide) is able to reduce toxicity with virtual disappearance of early-onset HC while still causing subclinical urothelial damage and rarefication of the urothelial layers. Second, high-level BKPyV replication is favored due to the ablation of innate and adaptive immune effectors and systemic stress responses, transcriptional activation stimulated by inflammatory responses leading to NFκB signaling of early viral genes [35], and immunosuppression administered as GVHD prophylaxis. Third, as urothelial cell regeneration is impaired following the conditioning, high-level cytopathic BKPyV replication leads to denudation of the rarefied bladder epithelium resulting in urine leakage, chemical inflammation, and hemorrhage. Following engraftment of neutrophils and other immune effectors, inflammation is exacerbated [35]. In this situation, BKPyV-stimulated as well as independent progression to mucosal GVHD can be envisaged, which has been described as a risk factor or confounder of PyVHC. This scenario is complex and difficult to investigate unless significant factors including high-level BKPyV replication can be reliably removed, preferably early and before onset. Conversely, definitions of clinical response may not allow to capture successful antiviral inventions suggesting that viral endpoints need to be part en route to a multitargeted clinical therapy approach.

PyVHC causes significant morbidity in the approximately 10–15% of allogeneic HSCT patients affected and leads to higher health-care costs due to longer hospitalization and the extensive care needed and, in rare cases, renal failure or other complications can be life-threatening and require urological interventions. BKPyV is highly associated with late-onset HC which manifests 2–8 weeks post-HSCT (range 1 week to 6 months), and it needs to be discriminated from early-onset HC that is directly caused by toxicity of the drugs used in the conditioning regimen that usually occurs within 2 days after transplantation [11, 35]. The natural course of PyVHC usually resolves within 3–5 weeks. However, declining kidney function and coexisting PyVAN have been diagnosed in allogeneic HSCT patients suggesting that more extensive renourinary involvement may complicate this disease in an already compromised patient population [1, 61].

To estimate the clinical impact, some simplified estimates should be discussed. In North America, approximately 10,000 allogeneic HSCTs are currently performed each year [12]. The activity survey of the European Society for Blood and Marrow Transplantation reports >17,000 allogeneic HSCTs in the same year [74]. Taken together, at least 27,000 allogeneic HSCTs are currently performed annually in North America and Europe combined, and the indications and actual numbers are increasing. Assuming a PyVHC complication rate of 10–15%, an estimate of 2700–4050 patients are affected each year. Taking the average of 3–5 weeks of significant

manifestations translates into 56,700–141,750 days of annual excess morbidity, a clinical impact not to be neglected. Assuming that only 5% have a very severe clinical course facing prolonged hospitalization and death, still 135–202 persons are at risk annually. Clearly, more data and research is needed to tackle this neglected, unmet clinical need.

Clinical Evaluation and Diagnostics

Diagnosis of PyVHC must fulfill the following three criteria: (1) clinical symptoms of cystitis (dysuria, abdominal pain), (2) macroscopic grade 2 or higher hematuria, and (3) viruria of >7 $\log_{10}$ c/mL determined by QNAT. In addition, other potentially coexisting etiologies must be ruled out, such as viruses including herpes simplex virus, cytomegalovirus, or adenovirus [2, 58], bacterial infection, or mechanical reasons (ureteric stenting and vesical catheters), graft-versus-host disease, metastatic neoplasia, or thrombocytopenia or clotting disorders [11] (see Table 1).

Imaging-based technologies as well as cystoscopy for histology are not usually necessary for the diagnosis, but may be informative when noninvasively evaluating extent and clinical course including urinary retention, intravesical clots, and ureteric involvement, which may even present as pyelonephritis. Of note, BKPyV-DNAemia >3–4 $\log_{10}$ c/mL are seen in approximately 70% of the more severe BKPyV-HC cases [11]. With clinical recovery of cystitis BKPyV-DNAemia and viruria decline. Asymptomatic HSCT recipients are currently not routinely screened for BKPyV because of a lack of effective preemptive treatment options. Thus, while high-level BKPyV viruria is required for the diagnosis PyVHC with the presumed pathophysiologic role in denudation, extensive inflammation and denudation of the bladder wall may also account for the predictive role of BKPyV-DNAemia for the more severe PyVHC cases [21].

Disease Epidemiology and Risk Factors

The PyVHC incidence after allogeneic HSCT in adults is 16% (range, 7–54%) and 18% (range, 8–25%) in children [11]. Children aged <7 have a lower risk than older children [55]. High-level BKPyV viruria is observed in >50% of allogeneic HSCT, indicating that other factors must play a role in progression to PyVHC. The incidence is highest in haplo-HSCT patients that received cyclophosphamide post-transplant. Autologous HSCTs have a much better outcome, and PyVHC is very unlikely to develop, indicating that immunosuppression and GVHD are major contributing factors in disease pathophysiology. Consistently, a lower risk is generally reported if the donor is related (matched or mismatched) than unrelated donors and a higher risk with severity of acute GVHD [28, 67]. High level of viruria of >7 $\log_{10}$ c/mL and BKPyV-DNAemia >3 $\log_{10}$ c/mL are predictive for PyVHC and have a reported sensitivity of 100% and 86%, respectively. As discussed above for this patient population of allogeneic HSCT, the specificity of viruria is lower at 60%, while 86% for BKPyV-DNAemia [10].

The type and intensity of the conditioning regimen has an impact. Conditioning with cyclophosphamide, busulfan, anti-thymocyte globulins, total body irradiation, and myeloablative, compared to reduced intensity conditioning, concurs with a higher risk for PyVHC [25, 28, 62, 81]. However, recently increasing use of haploidentical donor transplants with post-transplant cyclophosphamide application is also reflected in later PyVHC rates. The stem cell source is also a risk factor with bone marrow grafts having a lower risk than cord blood or peripheral blood [27, 81]. Further coinfection with other viruses, such as adenovirus, CMV, or HHV6, has been reported as an independent risk factor although only in a single study each [31, 55].

Management

PyVHC treatment is mainly based on supportive therapy including analgesia, hyperhydration, forced diuresis, erythrocyte and platelet substitution, and bladder irrigation to prevent clots and obstructions in the urinary tract (Table 2). In contrast to PyVAN, reducing immunosuppression is not considered a standard treatment option. The risk/benefit ratio is too high, increasing severe and generalized damage from GVHD from alloreactive donor cells.

Antiviral drugs have been used, but evidence is often weak, and most data come from small uncontrolled studies. In many reports of high clinical and virologic response rates, however, the response kinetics appear rather slow, making discrimination from the spontaneous resolution of PyVHC difficult.

Cidofovir use has been reported in more than 10 studies, mostly retrospective, for PyVHC. Dosing and application differed using intravenous application of 3–5 mg/kg/week with the addition of probenecid, to reduce nephrotoxicity by inhibiting cidofovir uptake into renal tubular epithelial cells, or lower doses of 0.5–1.5 mg/kg/week without probenecid. Other studies dosed 5 mg/kg/week for intravesical administration. Overall, a clinical response rate was reported in approximately 50%, whereby the virologic response, defined as a reduction of at least 1 $\log_{10}$ c/mL, was for viruria between 38% and 62% and for BKPyV-DNAemia between 45% and 84% [11]. Following intravenous application roughly a fifth of patients had increased serum creatinine levels. Despite a lack of evidence from randomized control trials, intravenous administration of cidofovir may be considered as a treatment option [11]. Overall, our experience with intravenous or intravesical cidofovir administration has been rather disappointing and was abandoned.

A case report describes the use of brincidofovir in a HSCT patient that developed PyVAN. Treatment (100 mg, twice weekly), without reduction of immunosuppression, resulted in a decline of BKPyV-DNAemia of almost 4 $\log_{10}$ c/mL, and the renal function remained sufficient until the patient died of unrelated bacterial sepsis [73]. Although only reported in few cases with issues regarding oral bioavailability and gastrointestinal adverse events, brincidofovir may be a safer and more potent drug than intravenous cidofovir. At this time, clinical development of brincidofovir has been halted.

The fluoroquinolone ciprofloxacin was assessed for prophylaxis of PyVHC in three studies (two retrospective, one cohort-prospective). There was a weak effect on BKPyV replication but none on clinically significant PyVHC. A single case study reports complete clinical responses using levofloxacin to treat severe refractory cases of PyVHC in three HSCT patients [90]. But again, it is not clear whether levofloxacin treatment or spontaneous resolution was responsible for the successful outcome. Some treatment success was also reported in several older cases with the adenine analog vidarabine, intravesical sodium hyaluronate, FXIII concentrate, or estrogens. In a more recent study, 6 out of 14 pediatric patients received choreito, a traditional formula from Japanese traditional medicine. The study reported a significantly shorter duration until resolution of hematuria and cystitis, and BKPyV viruria decreased significantly after choreito administration [50]. Similarly, leflunomide used in two studies reported higher clearance and a decline in BKPyV-DNAemia and viruria; however, randomized control trials have not been conducted.

Since no highly effective antivirals are available and given the central role of the immune system in pathology immune cell therapy is another approach that has been investigated. Allogeneic mesenchymal stoma cells were administered by infusion in seven patients with severe PyVHC, with the idea of using their anti-inflammatory, immunomodulatory, as well as tissue-repairing properties to heal the tissue damage. In five patients HC cleared after infusion with mesenchymal stoma cells, and hematuria disappeared after 3 (median, range, 1–14) days [80]. Another study reported a prothrombotic effect of mesenchymal stroma cells potentially jeopardizing its therapeutic use [65].

Promising results have been obtained with adoptively transferred virus-specific T cells (VSTs). VSTs were generated from third-party donors and were exposed to peptide libraries from 12 immunogenic proteins from BKPyV as well as CMV, EBV, ADV, and HHV6 for in vitro stimulation [72, 92]. The major drawbacks are the costs, complexity, and time to produce VSTs. Tzannou and colleagues conducted a phase II trial with cryopreserved, HLA-matched, third-party ("off-the-shelf") VSTs in allogeneic HSCT patients with viral infections. The results were very encouraging reporting cumulative complete or partial response rate of >90% and 100% for BKPyV in all 16 patients after a single-dose infusion. A complete resolution of gross hematuria was reported in 13 (out of 14) patients with PyVHC by week 6 [92]. The readily available banked VSTs could instantly be used for treatment. Moreover, VSTs may protect against five other double-stranded DNA viruses that all are notoriously problematic, occur simultaneously, and have been associated with a higher mortality in HSCT [33].

There is no documented benefit of IVIG for treating PyVHC, although we speculate that high-dose IVIG administered might be more effective than the usual low-dose IVIG applied every 3–4 weeks in many allogeneic HSCT patients to non-specifically protect against infections. Different interventions aimed at regeneration and damage repair of the urothelial mucosa are hyperbaric oxygen therapy [97] and cystoscopic application of fibrin glue [89] to the damaged tissue. Both had complete clinical response rates of 86% and 83%, respectively. BKPyV loads in urine decreased in about two thirds of patients receiving hyperbaric O_2 therapy. Both

offer treatment alternatives in severe or refractory cases of PyVHC; several disadvantages exist though: Hyperbaric O_2 therapy requires special facilities that are often not available, and there is a risk of ear barotrauma, pressure intolerance, and claustrophobia during treatment. Fibrin glue application is invasive requiring cystoscopy.

Preventive measures include aggressive hyperhydration and possibly prophylactic bladder irrigation during the conditioning regimen to reduce or prevent urotoxicity. Mesna is often used prophylactically in patients that receive cyclophosphamide or ifosfamide to reduce drug toxicity. These interventions are also effective for early-onset HC, since its application, the incidence could be lowered to <3%. Hyperhydration bears the risk of fluid overload, and bladder irrigation is invasive, administered through a three-way Foley catheter, and discomforting for patient [11]. Thus, treatment of PyVHC (Table 2) is still largely unsatisfactory with mainly supportive therapy leaving PyVHC a significant and unmet clinical need.

PyV-Associated Urothelial Cancer: PyVUC

PyVs have been linked to different tumors, ever since the first isolated PyV from mouse was found to induce multiple tumors in newborn mice [30], hence the name *poly-* and *-oma* meaning "many tumors" in Greek. Later studies with the monkey SV40 confirmed these findings in newborn or immunodeficient rodents and demonstrated their ability to transform cell lines in vitro. In fact, SV40 research has been instrumental in defining the concept of tumor suppressor inactivation of DNA tumor viruses as opposed to oncogene activation in retroviral tumorigenesis [66]. BKPyV was therefore seen as potentially oncogenic, and initial infection of the nonspecific (non-human) rodent cells, resulted in oncogenic transformation of those cells. Subsequently, oncogenic transformation was linked to the early viral gene region (EVGR) encoding the large and small tumor antigen (LTag and sTag, respectively). LTag and sTag drive the infected host cell into the S-phase before arresting in G2/M to provide an environment with abundant building blocks for viral replication. LTag inhibits the tumor suppressor protein p53 and pRB and recruits the host cell DNA polymerase to the origin of viral replication located in the noncoding control region (NCCR). LTag then promotes expression of the late viral gene region (LVGR)-encoded capsid proteins Vp1, Vp2, and Vp3, as well as the regulatory agnoprotein. This then leads to virus assembly, cell lysis, and release of the viral progeny. Thus, host cell lysis required for efficient release of the non-enveloped BKPyV virions from the nucleus works as an oncolytic safety barrier against cell transformation and cancer. In the absence of LVGR expression and virally induced cell lysis, the transforming properties of LTag increase the risk of further genetic alterations toward malignancy [13, 14]. Merkel cell PyV is the first human PyV clearly linked cancer, namely, Merkel cell carcinoma [22]. In most cases investigated, the MCPyV genome was chromosomally integrated and so that the gene for LTag was truncated, and no late proteins no late proteins were expressed [88].

Urothelial cancers of the bladder have been found to frequently carry alterations in tyrosine receptor kinase-Ras pathway in the case of low-grade papillary tumors, whereas high-grade metastasizing cancers have alterations in p53 and pRB pathways [96]. Recent evidence points to a role of BKPyV in high-grade urothelial cancers occurring in KT patients without pRB or p53 alterations [52, 71]. Several cases of PyVUC in KT patients with a history of PyVAN identified chromosomal integration of BKPyV genomes [52]. Thereby, viral genome replication and LVGR expression was disrupted preventing viral assembly and viral cell lysis, as predicted [13]. In another case, BKPyV-LTag expression was seen in a high-grade PyVUC derived from a 4-year-old deceased donor [69]. The cancer cells carried a chromosomal integration of a truncated BKPyV genome in addition to a full-length episomal BKPyV genome. Both the integrated and episomal BKPyV genome carried an identical 17bp deletion in the viral NCCR, which activated LTag expression, but interfered with LVGR expression. The integration site was not associated with altered oncogenesis-driving genes, and no other known chromosomal mutation was present [69]. Of note, despite surgical removal, the patient suffered two recurrences leading to life-threatening metastasizing disease, which only resolved into complete remission after discontinuing immunosuppression and abandoning the kidney transplant [69]. In summary, the reported cases suggest that long-lasting high-level BKPyV replication in immunosuppressed patients may lead to genetic accidents of non-lytic transformation and PyVUC, which is seen mostly after KT, but may also affect other transplant patients [54]. Thus, besides surgical removal, reducing or discontinuing immunosuppression may be key to permit antimalignancy immunity to BKPyV-encoded tumor antigens, especially in cases of metastasizing PyVUC. However, this option is limited in non-kidney SOT.

Conclusion

PyVAN, PyVHC, and PyVUC are three distinct pathologies caused by BKPyV, which jeopardize otherwise successful transplantation outcomes in KT and HSCT patients. Despite a variety of treatment options, effective validated therapies are not available, and the current management is far from ideal. Reduction of immunosuppression has significant success rates in KT patients, if applied early for probable and presumptive PyVAN, but approximately 10–20% of patients have a prolonged course or do not respond to treatment. Irreversible damage to the allograft feeds on its lifespan even after PyVAN resolution. Reliable discrimination between acute rejection and PyVAN is still unresolved. Reducing immunosuppression is hardly feasible for non-kidney SOT or allogeneic HSCT as the risk and consequences of immune damage due to rejection or GVHD are dire. The current knowledge about risk factors has yet to be translated into the clinics to improve diagnostics and management. Novel therapeutic approaches have reported promising results in "proof-of-concept" studies but await further validation and controlled clinical trial including switching immunosuppression to low-dose cyclosporine/mTOR inhibitor combinations or immune therapies using IVIG, monoclonal antibodies, and/or

VSTs. Research in vaccine development is pending, and peptide-derived viral proteins and a DNA-vaccine targeting CMV and BKPyV are currently in phase 1 trials.

Significant impact would be expected to come from new safe and effective treatments in the form of a small molecule drug to inhibit BKPyV replication. However, drug development for BKPyV is challenging, facing three major issues: (i) there is no simple high-throughput in vitro system for screening compound libraries for selective anti-BKPyV activity; (ii) preclinical animal models do not exist or require advanced humanization efforts, limiting generalizations; and (iii) the "druggable space" of this small DNA virus is very limited relying heavily on host factors for replication. LTag is a multifunctional protein which includes a helicase domain, a known drug target in bacteria. However, LTag is also a potential oncogene which may unfold once LVGR expression and viral host cell lysis are slowed down or abrogated. The emergence of PyVUC indicates that genetic accidents, leading to only partial LTag functions often lacking the helicase following chromosomal integration, may be oncogenic, particularly in immunocompromised transplant patients. Targeting host proteins and functions that are essential for BKPyV may be considered, but sparing renal function may be a huge obstacle in preclinical and clinical evaluation. Thus, the remarkable coevolutionary adaptation of PyVs and BKPyV in particular to the human host underlies the significant challenges of PyVAN, PyVHC, and PyVUC in transplantation, rendering these widely underestimated diseases an unmet clinical need.

Key Points

- BKPyV-associated diseases PyVAN, PyVHC, and PyVUC have emerged as persistent threats to otherwise successful transplantation outcomes in KT and HSCT patients.
- At least 5000 KT patients annually must be considered at risk for premature renal allograft failure due to BKPyV in North America and Europe, not reaching the average graft survival of 15 years.
- At least 3000 allogeneic HSCT patients in North America and Europe suffer from excess morbidity due to PyVHC causing additional 60,000 days of patient care, often as in-patients.
- The impact of PyVUC cannot be estimated at the present time, but assuming rates of 1%, approximately 500 cases may be attributed to BKPyV in KT patients.
- Antiviral drugs for prevention or treatment are lacking and vaccination to booster BKPyV-specific immunity is unavailable.
- Reducing immunosuppression early for KT patients with sustained BKPyV-DNAemia is currently the only option outlined in current recommendations, but approaches are empiric and dependent on center experience.
- Reducing immunosuppression is not a viable option in PyVHC after allogeneic HSCT due to precipitating GVHD leaving prevention with hyperhydration,

symptomatic analgesic treatment, and poorly efficacious intravenous cidofovir for desperate cases.

- PyVUC diagnosis is dependent on clinical signs and a high index of suspicion, and in the case of LTag expression from chromosomal integration, reduced immunosuppression may be tried in addition to conventional oncologic approaches.

References

1. Abudayyeh A, Hamdi A, Lin H, Abdelrahim M, Rondon G, Andersson BS, Afrough A, Martinez CS, Tarrand JJ, Kontoyiannis DP, Marin D, Gaber AO, Salahudeen A, Oran B, Chemaly RF, Olson A, Jones R, Popat U, Champlin RE, Shpall EJ, Winkelmayer WC, Rezvani K. Symptomatic BK virus infection is associated with kidney function decline and poor overall survival in allogeneic hematopoietic stem cell recipients. Am J Transplant. 2016;16:1492–502.
2. Arthur RR, Shah KV, Charache P, Saral R. BK and JC virus infections in recipients of bone marrow transplants. J Infect Dis. 1988;158:563–9.
3. Barber CE, Hewlett TJ, Geldenhuys L, Kiberd BA, Acott PD, Hatchette TF. BK virus nephropathy in a heart transplant recipient: case report and review of the literature. Transpl Infect Dis. 2006;8:113–21.
4. Bedi A, Miller CB, Hanson JL, Goodman S, Ambinder RF, Charache P, Arthur RR, Jones RJ. Association of BK virus with failure of prophylaxis against hemorrhagic cystitis following bone marrow transplantation. J Clin Oncol. 1995;13:1103–9.
5. Bernhoff E, Gutteberg TJ, Sandvik K, Hirsch HH, Rinaldo CH. Cidofovir inhibits polyomavirus BK replication in human renal tubular cells downstream of viral early gene expression. Am J Transplant. 2008;8:1413–22.
6. Binet I, Nickeleit V, Hirsch HH, Prince O, Dalquen P, Gudat F, Mihatsch MJ, Thiel G. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. Transplantation. 1999;67:918–22.
7. Binet I, Nickeleit V, Hirsch HH. Polyomavirus infections in transplant recipients. Curr Opin Organ Transplant. 2000;5:210–6.
8. Bischof N, Hirsch HH, Wehmeier C, Amico P, Dickenmann M, Hirt-Minkowski P, Steiger J, Menter T, Hopfer H, Schaub S. Reducing calcineurin inhibitor-first for treating BK polyomavirus replication after kidney transplantation: long-term outcomes. Nephrol Dial Transplant. 2019 Jul. 1;34(7):1240–1250.
9. Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. Am J Transplant. 2005;5:582–94.
10. Cesaro S, Tridello G, Pillon M, Calore E, Abate D, Tumino M, Carucci N, Varotto S, Cannata E, Pegoraro A, Barzon L, Palu G, Messina C. A prospective study on the predictive value of plasma BK virus-DNA load for hemorrhagic cystitis in pediatric patients after stem cell transplantation. J Pediatric Infect Dis Soc. 2015;4:134–42.
11. Cesaro S, Dalianis T, Rinaldo CH, Koskenvuo M, Pegoraro A, Einsele H, Cordonnier C, Hirsch HH, Ecil- Group. ECIL guidelines for the prevention, diagnosis and treatment of BK polyomavirus-associated haemorrhagic cystitis in haematopoietic stem cell transplant recipients. J Antimicrob Chemother. 2018;73:12–21.
12. D'Souza A, Fretham C. CIBMTR summary slides 2018: current uses and outcomes of hematopoietic cell transplantation (HCT). 43. 2018. https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/pages/index.aspx
13. Dalianis T, Hirsch HH. Chapter 16: BK polyomavirus and transformation. In: Robertson ES, Perelmann School of Medicine, Philadelphia University of Pennsylvania, PA, USA and

erle@upenn.edu, editors. Cancer associated viruses. Springer Sciences & Business Media. New York, NY 10013, USA. 2012.
14. Dalianis T, Hirsch HH. Human polyomaviruses in disease and cancer. Virology. 2013;437:63–72.
15. Dharnidharka VR, Cherikh WS, Neff R, Cheng Y, Abbott KC. Retransplantation after BK virus nephropathy in prior kidney transplant: an OPTN database analysis. Am J Transplant. 2010;10:1312–5.
16. Drachenberg CB, Beskow CO, Cangro CB, Bourquin PM, Simsir A, Fink J, Weir MR, Klassen DK, Bartlett ST, Papadimitriou JC. Human polyoma virus in renal allograft biopsies: morphological findings and correlation with urine cytology. Hum Pathol. 1999; 30:970–7.
17. Drachenberg CB, Papadimitriou JC, Hirsch HH, Wali R, Crowder C, Nogueira J, Cangro CB, Mendley S, Mian A, Ramos E. Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. Am J Transplant. 2004;4:2082–92.
18. Drachenberg CB, Hirsch HH, Papadimitriou JC, Gosert R, Wali RK, Munivenkatappa R, Nogueira J, Cangro CB, Haririan A, Mendley S, Ramos E. Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: a prospective evaluation. Transplantation. 2007;84:323–30.
19. Drachenberg CB, Papadimitriou JC, Chaudhry MR, Ugarte R, Mavanur M, Thomas B, Cangro C, Costa N, Ramos E, Weir MR, Haririan A. Histological evolution of BK virus-associated nephropathy: importance of integrating clinical and pathological findings. Am J Transplant. 2017;17:2078–91.
20. Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. J Infect Dis. 2009;199:837–46.
21. Erard V, Kim HW, Corey L, Limaye A, Huang ML, Myerson D, Davis C, Boeckh M. BK DNA viral load in plasma: evidence for an association with hemorrhagic cystitis in allogeneic hematopoietic cell transplant recipients. Blood. 2005;106:1130–2.
22. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008;319:1096–100.
23. Funk GA, Steiger J, Hirsch HH. Rapid dynamics of polyomavirus type BK in renal transplant recipients. J Infect Dis. 2006;193:80–7.
24. Funk GA, Gosert R, Comoli P, Ginevri F, Hirsch HH. Polyomavirus BK replication dynamics in vivo and in silico to predict cytopathology and viral clearance in kidney transplants. Am J Transplant. 2008;8:2368–77.
25. Gaziev J, Paba P, Miano R, Germani S, Sodani P, Bove P, Perno CF, Marziali M, Gallucci C, Isgro A, Paciaroni K, Roveda A, Simone MD, De Angelis G, Alfieri C, Lucarelli G. Late-onset hemorrhagic cystitis in children after hematopoietic stem cell transplantation for thalassemia and sickle cell anemia: a prospective evaluation of polyoma (BK) virus infection and treatment with cidofovir. Biol Blood Marrow Transplant. 2010;16:662–71.
26. Geetha D, Sozio SM, Ghanta M, Josephson M, Shapiro R, Dadhania D, Hariharan S. Results of repeat renal transplantation after graft loss from BK virus nephropathy. Transplantation. 2011;92:781–6.
27. Gilis L, Morisset S, Billaud G, Ducastelle-Lepretre S, Labussiere-Wallet H, Nicolini FE, Barraco F, Detrait M, Thomas X, Tedone N, Sobh M, Chidiac C, Ferry T, Salles G, Michallet M, Ader F. High burden of BK virus-associated hemorrhagic cystitis in patients undergoing allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2014;49:664–70.
28. Giraud G, Priftakis P, Bogdanovic G, Remberger M, Dubrulle M, Hau A, Gutmark R, Mattson J, Svahn BM, Ringden O, Winiarski J, Ljungman P, Dalianis T. BK-viruria and haemorrhagic cystitis are more frequent in allogeneic haematopoietic stem cell transplant patients receiving full conditioning and unrelated-HLA-mismatched grafts. Bone Marrow Transplant. 2008;41:737–42.
29. Greenlee JE, Hirsch HH. Polyomaviruses. In: ASM American Society for Microbiology, editor. Clinical virology. 4th ed. Washington, DC: ASM Press; 2017. (asmscience.org).

30. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proc Soc Exp Biol Med. 1953;83:414–21.
31. Han TT, Xu LP, Liu DH, Liu KY, Fu HX, Zhao XY, Zhao XS, Huang XJ. Cytomegalovirus is a potential risk factor for late-onset hemorrhagic cystitis following allogeneic hematopoietic stem cell transplantation. Am J Hematol. 2014;89:55–61.
32. Hardinger KL, Koch MJ, Bohl DJ, Storch GA, Brennan DC. BK-virus and the impact of pre-emptive immunosuppression reduction: 5-year results. Am J Transplant. 2010;10:407–15.
33. Hill JA, Mayer BT, Xie H, Leisenring WM, Huang ML, Stevens-Ayers T, Milano F, Delaney C, Sorror ML, Sandmaier BM, Nichols G, Zerr DM, Jerome KR, Schiffer JT, Boeckh M. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. Blood. 2017;129:2316–25.
34. Hirsch HH. Polyomavirus BK nephropathy: a (re-)emerging complication in renal transplantation. Am J Transplant. 2002;2:25–30.
35. Hirsch HH. Human polyomavirus and papillomavirus infection and disease posttransplant. In: Ljungman P, Snydman D, editors. Transplant infections. Cham: Springer; 2016.
36. Hirsch HH, Ramos E. Retransplantation after polyomavirus-associated nephropathy: just do it? Am J Transplant. 2006;6:7–9.
37. Hirsch HH, Randhawa PS. Screening for BK polyomavirus DNAemia: what should be done? Clin Transplant. 2019;33:e13672.
38. Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med. 2002;347:488–96.
39. Hirsch HH, Kaufmann G, Sendi P, Battegay M. Immune reconstitution in HIV-infected patients. Clin Infect Dis. 2004;38:1159–66.
40. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, Mihatsch MJ, Nickeleit V, Ramos E, Randhawa P, Shapiro R, Steiger J, Suthanthiran M, Trofe J. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. Transplantation. 2005;79:1277–86.
41. Hirsch HH, Kardas P, Kranz D, Leboeuf C. The human JC polyomavirus (JCPyV): virological background and clinical implications. APMIS. 2013; 121:685–727.
42. Hirsch HH, Vincenti F, Friman S, Tuncer M, Citterio F, Wiecek A, Scheuermann EH, Klinger M, Russ G, Pescovitz MD, Prestele H. Polyomavirus BK replication in De Novo Kidney transplant patients receiving tacrolimus or cyclosporine: a prospective, randomized, multicenter study. Am J Transplant. 2013; 13:136–45.
43. Hirsch HH, Yakhontova K, Lu M, Manzetti J. BK polyomavirus replication in renal tubular epithelial cells is inhibited by Sirolimus, but activated by Tacrolimus through a pathway involving FKBP-12. Am J Transplant. 2016;16:821–32.
44. Hirsch HH, Randhawa PS, AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13528.
45. Hocker B, Tabatabai J, Schneble L, Oh J, Thiel F, Pape L, Rusai K, Topaloglu R, Kranz B, Klaus G, Printza N, Yavascan O, Fichtner A, Krupka K, Bruckner T, Waldherr R, Pawlita M, Schnitzler P, Hirsch HH, Tonshoff B. JC polyomavirus replication and associated disease in pediatric renal transplantation: an international CERTAIN registry study. Pediatr Nephrol. 2018;33:2343–52.
46. Hocker B, Schneble L, Murer L, Carraro A, Pape L, Kranz B, Oh J, Zimgibl M, Dello Strologo L, Buscher A, Weber LT, Awan A, Pohl M, Bald M, Printza N, Rusai K, Peruzzi L, Topaloglu R, Fichtner A, Krupka K, Koster L, Bruckner T, Schnitzler P, Hirsch HH, Tonshoff B. Epidemiology of and risk factors for BK polyomavirus replication and nephropathy in pediatric renal transplant recipients: an international CERTAIN registry study. Transplantation. 2019;103:1224–33.
47. Imlay H, Whitaker K, Fisher CE, Limaye AP. Clinical characteristics and outcomes of late-onset BK virus nephropathy in kidney and kidney-pancreas transplant recipients. Transpl Infect Dis. 2018;20:e12928.

48. Kable K, Davies CD, O'Connell PJ, Chapman JR, Nankivell BJ. Clearance of BK virus nephropathy by combination antiviral therapy with intravenous immunoglobulin. Transplant Direct. 2017;3:e142.
49. Kaur A, Wilhelm M, Wilk S, Hirsch HH. BK polyomavirus-specific antibody and T-cell responses in kidney transplantation. Curr Opin Infect Dis. 2019;32:1–11.
50. Kawashima N, Ito Y, Sekiya Y, Narita A, Okuno Y, Muramatsu H, Irie M, Hama A, Takahashi Y, Kojima S. Choreito formula for BK virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015;21:319–25.
51. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. PLoS Pathog. 2009;5:e1000363.
52. Kenan DJ, Mieczkowski PA, Burger-Calderon R, Singh HK, Nickeleit V. The oncogenic potential of BK-polyomavirus is linked to viral integration into the human genome. J Pathol. 2015;237:379–89.
53. Knoll GA, Humar A, Fergusson D, Johnston O, House AA, Kim SJ, Ramsay T, Chasse M, Pang X, Zaltzman J, Cockfield S, Cantarovich M, Karpinski M, Lebel L, Gill JS. Levofloxacin for BK virus prophylaxis following kidney transplantation: a randomized clinical trial. JAMA. 2014;312:2106–14.
54. Kuppachi S, Holanda D, Eberlein M, Alexiev B, Tyler AJ, Wissel MC, Kleiboeker SB, Thomas CP. An unexpected surge in plasma BKPyV viral load heralds the development of BKPyV-associated metastatic bladder cancer in a lung transplant recipient with BKPyV nephropathy. Am J Transplant. 2017;17:813–8.
55. Laskin BL, Denburg M, Furth S, Diorio D, Goebel J, Davies SM, Jodele S. BK viremia precedes hemorrhagic cystitis in children undergoing allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19:1175–82.
56. Lautenschlager I, Jahnukainen T, Kardas P, Lohi J, Auvinen E, Mannonen L, Dumoulin A, Hirsch HH, Jalanko H. A case of primary JC polyomavirus infection-associated nephropathy. Am J Transplant. 2014;14:2887–92.
57. Leboeuf C, Wilk S, Achermann R, Binet I, Golshayan D, Hadaya K, Hirzel C, Hoffmann M, Huynh-Do U, Koller MT, Manuel O, Mueller NJ, Mueller TF, Schaub S, van Delden C, Weissbach FH, Hirsch HH, Study Swiss Transplant Cohort. BK polyomavirus-specific 9mer CD8 T cell responses correlate with clearance of BK viremia in kidney transplant recipients: first report from the Swiss Transplant Cohort Study. Am J Transplant. 2017;17:2591–600.
58. Leung AY, Suen CK, Lie AK, Liang RH, Yuen KY, Kwong YL. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. Blood. 2001;98:1971–8.
59. Li YJ, Weng CH, Lai WC, Wu HH, Chen YC, Hung CC, Yang CW, Tian YC. A suppressive effect of cyclosporine A on replication and noncoding control region activation of polyomavirus BK virus. Transplantation. 2010;89:299–306.
60. Liacini A, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. Transplantation. 2010;90:1450–7.
61. Limaye AP, Smith KD, Cook L, Groom DA, Hunt NC, Jerome KR, Boeckh M. Polyomavirus nephropathy in native kidneys of non-renal transplant recipients. Am J Transplant. 2005;5:614–20.
62. Megged O, Stein J, Ben-Meir D, Shulman LM, Yaniv I, Shalit I, Levy I. BK-virus-associated hemorrhagic cystitis in children after hematopoietic stem cell transplantation. J Pediatr Hematol Oncol. 2011;33:190–3.
63. Menter T, Mayr M, Schaub S, Mihatsch MJ, Hirsch HH, Hopfer H. Pathology of resolving polyomavirus-associated nephropathy. Am J Transplant. 2013;13:1474–83.
64. Miettinen J, Lautenschlager I, Weissbach F, Wernli M, Auvinen E, Mannonen L, Lauronen J, Hirsch HH, Jalanko H. BK polyomavirus viremia and antibody responses of pediatric kidney transplant recipients in Finland. Pediatr Transplant. 2018;23:e13324.

65. Moll G, Rasmusson-Duprez I, von Bahr L, Connolly-Andersen AM, Elgue G, Funke L, Hamad OA, Lonnies H, Magnusson PU, Sanchez J, Teramura Y, Nilsson-Ekdahl K, Ringden O, Korsgren O, Nilsson B, Le Blanc K. Are therapeutic human mesenchymal stromal cells compatible with human blood? Stem Cells. 2012;30:1565–74.
66. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer. 2010;10:878–89.
67. Mori Y, Miyamoto T, Kato K, Kamezaki K, Kuriyama T, Oku S, Takenaka K, Iwasaki H, Harada N, Shiratsuchi M, Abe Y, Nagafuji K, Teshima T, Akashi K. Different risk factors related to adenovirus- or BK virus-associated hemorrhagic cystitis following allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2012;18:458–65.
68. Mosli HA, Farsi HM, al-Zimaity MF, Saleh TR, al-Zamzami MM. Vesicoureteral reflux in patients with double pigtail stents. J Urol. 1991;146:966–9.
69. Muller DC, Ramo M, Naegele K, Ribi S, Wetterauer C, Perrina V, Quagliata L, Vlajnic T, Ruiz C, Balitzki B, Grobholz R, Gosert R, Ajuh ET, Hirsch HH, Bubendorf L, Rentsch CA. Donor-derived, metastatic urothelial cancer after kidney transplantation associated with a potentially oncogenic BK polyomavirus. J Pathol. 2018;244:265–70.
70. Nankivell BJ, Renthawa J, Sharma RN, Kable K, O'Connell PJ, Chapman JR. BK virus nephropathy: histological evolution by sequential pathology. Am J Transplant. 2017;17:2065–77.
71. Papadimitriou JC, Randhawa P, Rinaldo CH, Drachenberg CB, Alexiev B, Hirsch HH. BK polyomavirus infection and renourinary tumorigenesis. Am J Transplant. 2016;16:398–406.
72. Papadopoulou A, Gerdemann U, Katari UL, Tzannou I, Liu H, Martinez C, Leung K, Carrum G, Gee AP, Vera JF, Krance RA, Brenner MK, Rooney CM, Heslop HE, Leen AM. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014;6:242ra83.
73. Papanicolaou GA, Lee YJ, Young JW, Seshan SV, Boruchov AM, Chittick G, Mommeja-Marin H, Glezerman IG. Brincidofovir for polyomavirus-associated nephropathy after allogeneic hematopoietic stem cell transplantation. Am J Kidney Dis. 2015;65:780–4.
74. Passweg JR, Baldomero H, Basak GW, Chabannon C, Corbacioglu S, Duarte R, Kuball J, Lankester A, Montoto S, de Latour RP, Snowden JA, Styczynski J, Yakoub-Agha I, Arat M, Mohty M, Kroger N, Blood European Society for, and Transplantation Marrow. The EBMT activity survey report 2017: a focus on allogeneic HCT for nonmalignant indications and on the use of non-HCT cell therapies. Bone Marrow Transplant. 2019;54:1575.
75. Pastrana DV, Brennan DC, Cuburu N, Storch GA, Viscidi RP, Randhawa PS, Buck CB. Neutralization serotyping of BK polyomavirus infection in kidney transplant recipients. PLoS Pathog. 2012;8:e1002650.
76. Randhawa PS, Finkelstein S, Scantlebury V, Shapiro R, Vivas C, Jordan M, Picken MM, Demetris AJ. Human polyoma virus-associated interstitial nephritis in the allograft kidney. Transplantation. 1999;67:103–9.
77. Randhawa P, Farasati NA, Shapiro R, Hostetler KY. Ether lipid ester derivatives of cidofovir inhibit polyomavirus BK replication in vitro. Antimicrob Agents Chemother. 2006;50:1564–6.
78. Reisman L, Habib S, McClure GB, Latiolais LS, Vanchiere JA. Treatment of BK virus-associated nephropathy with CMX001 after kidney transplantation in a young child. Pediatr Transplant. 2014;18:E227–31.
79. Rinaldo CH, Gosert R, Bernhoff E, Finstad S, Hirsch HH. 1-O-hexadecyloxypropyl cidofovir (CMX001) effectively inhibits polyomavirus BK replication in primary human renal tubular epithelial cells. Antimicrob Agents Chemother. 2010;54:4714–22.
80. Ringden O, Uzunel M, Sundberg B, Lonnies L, Nava S, Gustafsson J, Henningsohn L, Le Blanc K. Tissue repair using allogeneic mesenchymal stem cells for hemorrhagic cystitis, pneumomediastinum and perforated colon. Leukemia. 2007;21:2271–6.
81. Rorije NM, Shea MM, Satyanarayana G, Hammond SP, Ho VT, Baden LR, Antin JH, Soiffer RJ, Marty FM. BK virus disease after allogeneic stem cell transplantation: a cohort analysis. Biol Blood Marrow Transplant. 2014;20:564–70.

82. Schachtner T, Babel N, Reinke P. Different risk factor profiles distinguish early-onset from late-onset BKV-replication. Transpl Int. 2015;28:1081–91.
83. Schaub S, Hirsch HH, Dickenmann M, Steiger J, Mihatsch MJ, Hopfer H, Mayr M. Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. Am J Transplant. 2010;10:2615–23.
84. Sester M, Leboeuf C, Schmidt T, Hirsch HH. The "ABC" of virus-specific T cell immunity in solid organ transplantation. Am J Transplant. 2016;16:1697–706.
85. Shah A, Kumar V, Palmer MB, Trofe-Clark J, Laskin B, Sawinski D, Hogan JJ. Native kidney BK virus nephropathy, a systematic review. Transpl Infect Dis. 2019;21:e13083.
86. Sharma BN, Li R, Bernhoff E, Gutteberg TJ, Rinaldo CH. Fluoroquinolones inhibit human polyomavirus BK (BKV) replication in primary human kidney cells. Antiviral Res. 2011;92:115–23.
87. Sood P, Senanayake S, Sujeet K, Medipalli R, Zhu YR, Johnson CP, Hariharan S. Management and outcome of BK viremia in renal transplant recipients: a prospective single-center study. Transplantation. 2012;94:814–21.
88. Spurgeon ME, Lambert PF. Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. Virology. 2013;435:118–30.
89. Tirindelli MC, Flammia GP, Bove P, Cerretti R, Cudillo L, De Angelis G, Picardi A, Annibali O, Nobile C, Cerchiara E, Dentamaro T, De Fabritiis P, Lanti A, Ferraro AS, Sergi F, Di Piazza F, Avvisati G, Arcese W. Fibrin glue therapy for severe hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20:1612–7.
90. Toptas T, Kaygusuz-Atagunduz I, Kani HT, Adiguzel C, Firatli-Tuglular T. Levofloxacin for the treatment of severe refractory BK virus-associated hemorrhagic cystitis in hematopoietic stem cell transplantation recipients: a report of three cases. Oncol Lett. 2014;8:1775–7.
91. Tylden GD, Hirsch HH, Rinaldo CH. Brincidofovir (CMX001) inhibits BK polyomavirus replication in primary human urothelial cells. Antimicrob Agents Chemother. 2015;59:3306–16.
92. Tzannou I, Papadopoulou A, Naik S, Leung K, Martinez CA, Ramos CA, Carrum G, Sasa G, Lulla P, Watanabe A, Kuvalekar M, Gee AP, Wu MF, Liu H, Grilley BJ, Krance RA, Gottschalk S, Brenner MK, Rooney CM, Heslop HE, Leen AM, Omer B. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, Epstein-Barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2017;35:3547–57.
93. Vu D, Shah T, Ansari J, Naraghi R, Min D. Efficacy of intravenous immunoglobulin in the treatment of persistent BK viremia and BK virus nephropathy in renal transplant recipients. Transplant Proc. 2015;47:394–8.
94. Wilhelm M, Wilk S, Kaur A, Hirsch HH. Can HLA-B51 protect against BKPyV-DNAemia? Transplantation. 2019;103:e384.
95. Wingate JT, Brandenberger J, Weiss A, Scovel LG, Kuhr CS. Ureteral stent duration and the risk of BK polyomavirus viremia or bacteriuria after kidney transplantation. Transpl Infect Dis. 2017;19:e12644.
96. Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. Nat Rev Cancer. 2005;5:713–25.
97. Zama D, Masetti R, Vendemini F, Di Donato F, Morelli A, Prete A, Pession A. Clinical effectiveness of early treatment with hyperbaric oxygen therapy for severe late-onset hemorrhagic cystitis after hematopoietic stem cell transplantation in pediatric patients. Pediatr Transplant. 2013;17:86–91.

Challenges of Human Papillomavirus Infection in Solid Organ and Hematopoietic Stem Cell Transplant Recipients

34

Cristina Brickman and Peter Chin-Hong

Contents

Introduction 834
Pathogenesis of HPV Infection 834
Initial Infection 834
Terminology 835
Development of HSIL 837
Immune Mechanisms of HPV Infection 838
Epidemiology of HPV in SOT and HSCT Recipients 838
HPV Infection 838
SIL 839
Progression from HSIL to Invasive Cancer 841
HPV-Related Malignancies in SOT and HSCT Recipients 842
Non-melanomatous Skin Cancer in SOT and HSCT Recipients 843
Prevention of HPV-Related Complications in SOT and HSCT Recipients 844
Cervical Cancer Screening 844
Anal Cancer Screening 845
Other HPV-Related Malignancies 849
Prophylactic HPV Vaccination 850
Conclusion 852
Key Points 852
References 853

C. Brickman · P. Chin-Hong (✉)
University of California San Francisco, San Francisco, CA, USA
e-mail: Cristina.Brickman@ucsf.edu; Peter.Chin-Hong@ucsf.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_70

Introduction

Human papillomaviruses (HPV) are small, non-enveloped DNA viruses that infect epithelial cells [1]. Cutaneous HPV (cHPV) types are found ubiquitously on the skin and are associated with cutaneous warts [2]. Anogenital HPV types specifically infect the anogenital and upper digestive tract and are sexually transmitted [1]. There is a 75–80% lifetime risk of anogenital HPV infection among all sexually active men and women [3], rendering HPV the most common sexually transmitted agent [4].

Of about 40 anogenital HPV types, almost half are considered oncogenic or "high-risk" (hr-HPV) [5]. Infection with these hr-HPV strains is a common precursor to squamous intraepithelial lesions (SIL), and persistent hr-HPV infection may, in some cases, lead to the cancer precursor high-grade SIL (HSIL). A small proportion of HSIL in turn progresses to invasive squamous cell carcinoma (SCC) giving rise to 99.9% of cervical cancers, 90% of anal cancers, 70% of oropharyngeal cancers, and a high percent of penile, vaginal, and vulvar cancers [6]. The hr-HPV type 16 carries the strongest association with invasive cancer: it is detected in over 50% of cervical cancers, 85% of anal cancers, and almost 90% of HPV-positive oropharyngeal cancers [7]. HPV type 18 is the second most common type associated with cervical cancer (detected in approximately 20% of specimens) but is less prevalent at other cancer sites [7].

Infection with low-risk HPV (lr-HPV) anogenital types is associated primarily with low-grade squamous intraepithelial lesions (LSIL), which include genital warts or condyloma acuminata. Lr-HPV types 6 and 11 cause 90% of genital warts [8]; these are of low oncogenic potential but can be difficult to eradicate and often lead to discomfort and psychological distress.

Solid organ transplant (SOT) recipients have a higher prevalence of HPV infection [9–14] and are at greater risk for complications from HPV compared with the general population [15]. While data are more limited in hematopoietic stem cell transplant (HSCT) recipients, they are likely also at higher risk for complications. Thus, it is important to identify transplant recipients at risk for HPV infection and to develop methods of prevention and/or early detection of malignancy in these patients. In this chapter, we review the pathogenesis and epidemiology of HPV infection in SOT and HSCT with particular focus on anogenital HPV. We also discuss the prevention of complications from HPV infection, including cervical and anal cancer, the two most common HPV-related malignancies.

Pathogenesis of HPV Infection

Initial Infection

Anogenital HPV types preferentially infect areas of metaplasia in both the cervix and the anus referred to as transformation zones where columnar glandular epithelium is actively being replaced by stratified squamous epithelium [5, 16]. To initiate

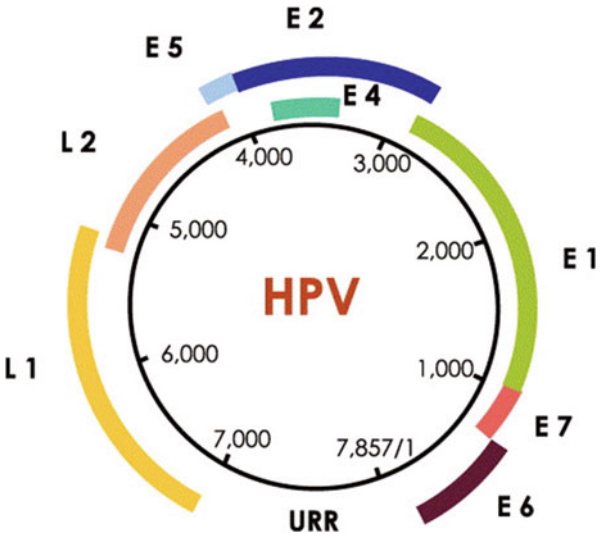


Fig. 1 Schematic presentation of the HPV genome showing the arrangement of the early E or nonstructural genes, the late L capsid structural genes (L1 and L2), and the upstream regulatory region (URR). (Adapted from Ref. [115])

infection, the virus first infects the basal cells of the epithelium, often at sites of microtrauma [17].

The HPV genome is comprised of approximately 8 kb of double-stranded circular DNA, which can be divided into an *early* region, coding for the E1, E2, E4, E5, E6, and E7 proteins, and a *late* region, which encodes two structural capsid proteins termed L1 and L2 [18]. The upstream regulatory region (URR) or long control region (LCR) lays between these two regions and is a non-coding portion that contains numerous binding sites for E2 and multiple transcription factors (Fig. 1).

Once inside the basal layer, the HPV genome is sustained as a quiescent episome. HPV replication and protein expression surge as the infected cells migrate into the suprabasal cell layers and then undergo epithelial differentiation. This may result in changes that are recognized as SIL both clinically and histopathologically. Latently infected cells in the basal cell epithelium likely also persist [19, 20] with the potential to reactivate and lead to SIL.

Terminology

Prior nomenclature reflected a morphologic continuum ranging from low-grade lesions to increasingly higher-grade disease, ending in invasive cancer [21]. Low-grade lesions on cytology were termed LSIL, and on histology they were called intraepithelial neoplasia (IN) grade 1. High-grade lesions were designated HSIL (cytology) and IN grades 2 and 3 (histology). The nomenclature for IN

was further modified depending on site of infection. Thus, IN from the cervix was named cervical intraepithelial neoplasia (CIN) grades 1–3, IN from the anal canal was named anal intraepithelial neoplasia (AIN) grades 1–3, and so forth.

However, it is now known that the majority of low-grade lesions are transient and (even with hr-HPV infection) do not progress to high-grade dysplasia [5, 22]. It is increasingly thought that HPV infection progresses via two distinct pathways, resulting in either high-grade (precancerous) or low-grade (benign) lesions. The College of American Pathologists and the American Society for Colposcopy and Cervical Pathology now recommend the use of LSIL/HSIL terminology for both cytology and histopathologic grading of disease and across all anogenital sites where HPV infection occurs [23]. The use of p16 immunohistochemistry, a biomarker for cellular proliferation, is recommended to clarify the diagnosis of IN 2 by classifying all p16-negative IN 2 lesions as "LSIL" and all p16-positive IN2 and all IN3 lesions as "HSIL" [23]. As such, LSIL reflects active HPV replication and virion formation (i.e., infection), while HSIL reflects HPV-induced transformation (Fig. 2) [24]. The therapeutic implications of this classification are significant: LSIL is not considered pre-cancerous and does not need to be treated, while HSIL is treated in order to reduce the risk of progression to cancer.

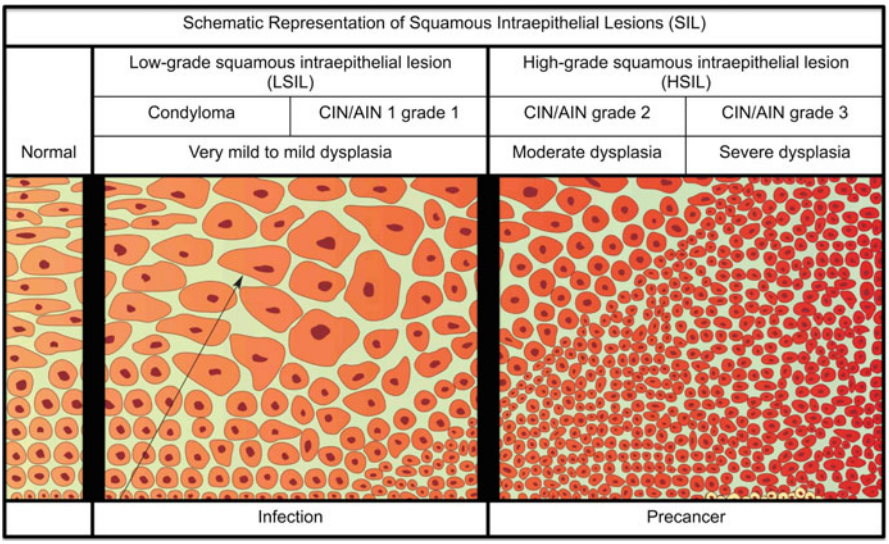


Fig. 2 Schematic representation of the two-tiered system to classify cytology and histology of squamous intraepithelial lesions. Low-grade squamous intraepithelial lesions (LSIL) include cervical intraepithelial neoplasia (CIN) grade 1, anal intraepithelial neoplasia (AIN) grade 1, and condyloma (genital warts). High-grade squamous intraepithelial lesions (HSIL) include CIN/AIN grades 2 and 3. The distinct biology underlying LSIL and HSIL is reflected in this classification. (Adapted from Ref. [116])

Development of HSIL

There are two key HPV oncogenes, E7 and E6, that result in persistent HPV infection and development of HSIL [17]. The proto-oncogene *retinoblastoma* (Rb) is bound by E7, which results in release of the host E2F transcription factor from Rb and cellular entry into the cell cycle S-phase. The oncogene E6 binds and inactivates p53, the tumor suppressor protein, which induces DNA repair enzymes and cellular apoptosis when DNA damage cannot be repaired. Thus, E7 increases the rate of mutations by enhancing replication and E6 allows these mutations to accumulate (Fig. 3). Such progressive genomic instability eventually leads to invasive cancer.

Additionally, E5 protein contributes to malignant transformation by reducing turnover of epidermal growth factor receptors on the cell surface, thus rendering cells more susceptible to the mitogenic effects of epidermal growth factor. Integration of HPV DNA into the cellular genome is another transformative mechanism. The E2 gene codes for a trans-activating protein that binds the URR of the HPV genome and downregulates E6 and E7 expression (Fig. 3). HPV integration disrupts the E2 gene, and the loss of E2 binding to the URR in turn increases E6 and E7 expression [25].

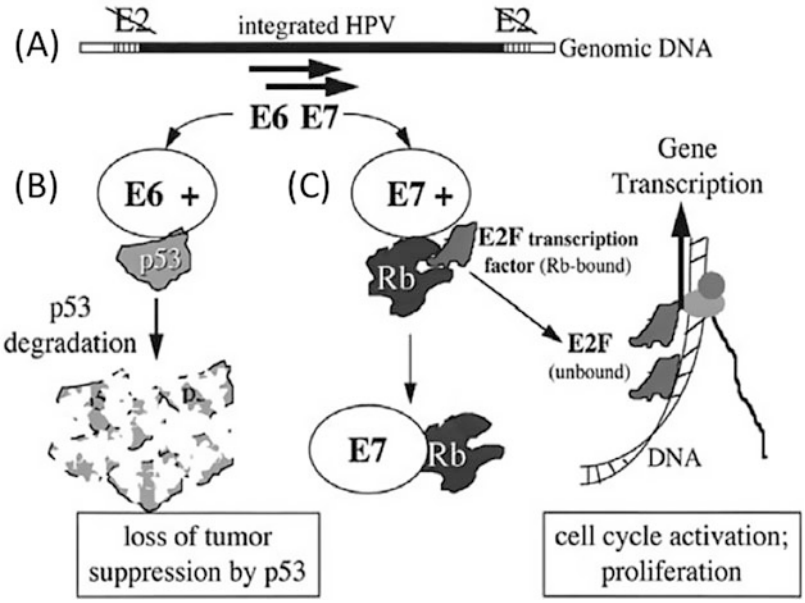


Fig. 3 (**a**) HPV DNA integration disrupts the E2 gene resulting in upregulation of E6 and E7 expression. (**b**) E6 binds and inactivates the tumor suppressor protein p53 resulting in loss of DNA repair and cellular apoptosis when DNA damage cannot be repaired. (**c**) E7 binds to the proto-oncogene *retinoblastoma* (Rb), resulting in release of the host E2F transcription factor from Rb and cellular entry into the cell cycle S-phase. (Adapted from Ref. [117])

The mechanisms determining whether HPV is transforming, resulting in HSIL and cancer, or primarily replicative, leading to LSIL, are not fully understood. HPV type seems to be a major determinant; differences in cellular affinity and function of the E6 and E7 proteins of hr-HPV types compared with lr-HPV types predispose infected cells toward the transformative pathway [26].

Immune Mechanisms of HPV Infection

HPV establishes infection by evading the host innate immune system [27]. Mechanisms of evasion include an exclusively intracellular productive life cycle without viremia or viral-induced cell death as well as viral downregulation of host innate immune signaling pathways resulting in the absence of pro-inflammatory cytokines or recruitment of dendritic cells or macrophages [27].

Despite this, most anogenital HPV infections are eventually cleared [28]. This process is thought to be regulated by adaptive cellular immunity. Longitudinal animal studies of canine oral papillomavirus note the emergence of anti-E2 specific T-cells prior to genital wart regression [29]. In humans, immunohistochemistry studies show that regression of genital warts is preceded by infiltration with CD4 T-cells and Th1 cytokines [30]. E2- and E6-specific T-helper cells are frequently detected in healthy individuals as evidence of previous exposure [31, 32], and the presence of E2-specific T-cells is associated with clearance of cervical SIL in women [33]. Conversely, people with human immunodeficiency virus (HIV) and the associated acquired defects in cellular immunity associated are at increased risk of HPV infection, SIL, and HPV-related malignancies [34]. In transplant recipients, defects in cellular immunity from immunosuppressant drugs and/or myeloablative therapy are similarly associated with increased susceptibility to HPV. The epidemiology of HPV infection specific to SOT and HSCT recipients is discussed in the next section.

Epidemiology of HPV in SOT and HSCT Recipients

HPV Infection

Infection with anogenital HPV in the general population is common and typically occurs soon after sexual debut [35, 36]. Risk factors for cervical HPV infection include early onset of sexual activity, a history of multiple sexual partners, and history of other sexually transmitted infections [5]. In addition to determinants mentioned above, risk factors for anal HPV infection include a history of anal receptive intercourse [34] and, for women, a history of cervical HPV infection or of cervical, vaginal, or vulvar SIL or cancer [37–40].

From cross-sectional studies, the prevalence of cervical hr-HPV infection ranges from 17% to 35% in renal transplant recipients (RTR) [9, 14, 41] and is 10% in liver transplant recipients (LTR) [11]. The largest cross-sectional study involved 218 RTR from the Netherlands where the prevalence of cervical HPV and cervical hr-HPV infection was 27.1% and 17.4%, respectively [41]. The authors compared this to cervical HPV infection prevalence of 9–10% in the general, age-matched Western-European population [42]. Testing for cervical hr-HPV before and after transplantation in a subgroup of 59 women further identified a significant increase in the prevalence of cervical HPV infection post-transplant despite the absence of changes in sexual risk behaviors [43]. This supports the theory that greater HPV prevalence in SOT recipients is due to reactivation of latent infection in the setting of impaired cellular immunity rather than due to a greater number of sexual partners or other traditional risk factors.

Data on anal HPV infection and other anogenital sites is limited for SOT recipients. Small, cross-sectional studies report anal infection rates with any HPV type of 21–48% in RTR [10, 13] and anal infection rates with any HPV type and hr-HPV of 18% and 8%, respectively, in LTR [11]. A larger study is underway to define the prevalence of anal HPV infection in RTR more clearly [44].

For HSCT recipients, the risk of cervical, vaginal, or vulvar HPV infection in female allogeneic stem cell transplant (alloHSCT) recipients increases with time from transplantation, approaching 40% at 10–20 years [45]. As with SOT recipients, the high rates of HPV infection are thought to result from the reactivation of latent HPV post-transplant rather than from an increase in high-risk sexual behaviors. AlloHSCT recipients with cutaneous graft-versus-host disease (GVHD), especially those with severe cutaneous or genital GVHD, are at particular risk. This, presumably, is the result of immune dysregulation and delayed immunologic recovery from GVHD. Moreover, patients with chronic GVHD often require years of immunosuppressive therapy which can lead to long-lasting effects on cellular immunity. Information on the prevalence of anal HPV infection in HSCT recipients, and of genital HPV infection in autologous stem cell transplant (autoHSCT) recipients, is limited.

SIL

SOT recipients are more likely to develop genital warts compared with the general population (Fig. 4a, b). In a registry-based study from the Netherlands, RTR had three times the risk of developing genital warts (LSIL) compared with patients without transplants [46], a finding corroborated by cohort studies from the USA [47] and Australia [48].

The US Transplant Cancer Match Study is an ongoing linkage study between the US nationwide organ transplant registry and 15 state and local registries [49]. In

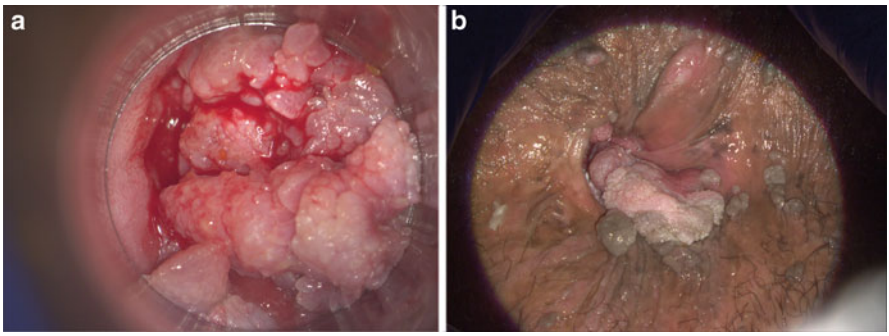


Fig. 4 Bulky condylomata/LSIL in (**a**) anal canal and (**b**) perianus of HIV-negative patient with a history of bilateral orthotopic lung transplant. Thickened micropapillae are typical of LSIL

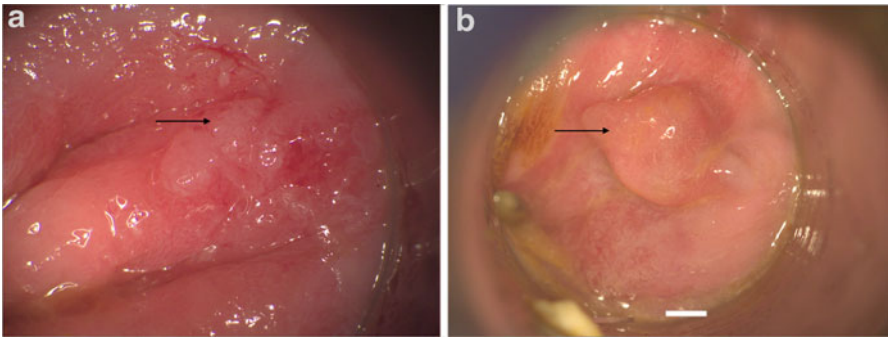


Fig. 5 (**a**) Anal canal HSIL at the right anterior squamo-columnar junction of an HIV-positive patient with a history of renal transplant. Lesion is acetowhite, raised and thickened, with coarse mosaic pattern and punctuation (arrow). (**b**) Follow-up visit with interval development of rubbery polypoid lesion with coarse punctuation suspicious for malignancy (arrow). Surgical excision noted HSIL with features suspicious for superficial invasion

addition to cancer data, this registry captures incidence data for HPV-related in situ *carcinomas,* a term previously used interchangeably with IN 3 and now encompassed by HSIL (Fig. 5a, b) [23]. Data from the US Transplant Cancer Match shows elevated standardized incidence ratios (SIR) for cervical, vaginal, vulvar, anal, and penile HSIL in SOT compared with the general population [49] (Table 1), a finding which is also corroborated by prior smaller and older cohort studies [50, 51].

In HSCT recipients, data on SIL after transplant is limited to several relatively small cohort studies that have evaluated the rates of abnormal cervical cytology before and after transplantation [52–55]. The rates of abnormal cervical cytology in female autoHSCT recipients are similar to that of the age-matched general population [55]. However, a consistent increase in abnormal cervical cytology is seen following alloHSCT [52–56]. As with HPV

Table 1 HPV-related cancers in US organ transplant recipients

	Number	Incidence per 100,000 person years	Standardized incidence ratio	95% confidence interval
In situ cancers (HSIL)				
Cervix	71	144.9	3.3	2.6–4.2
Vagina	34	10.4	10.6	7.4–14.8
Vulva	284	86.5	20.3	18.0–22.8
Anus	53	6.3	11.6	8.7–15.2
Penis	58	11.4	18.6	14.1–24.0
Invasive cancers				
Cervix	52	15.8	1.0	0.8–1.3
Vulva	66	20.1	7.3	5.6–9.2
Anus	103	12.3	5.4	4.4–6.6
Penis	25	4.9	3.9	2.5–5.7
Oropharynx	144	17.2	2.2	1.8–2.5

Source: Adapted from Ref. [49]

infection itself, development of severe cutaneous or genital GVHD is risk factor for abnormal cervical cytology [53, 54]. Data is limited on the actual prevalence of histologic SIL in the cervix as well as on the prevalence of SIL at other genital sites in HSCT recipients.

Progression from HSIL to Invasive Cancer

The rate of progression from HSIL to invasive cancer in SOT and HSCT recipients has not been measured, given that standard of care is to treat HSIL once it is identified. However, data on cervical HSIL progression in the general population and estimates of anal HSIL progression in people living with HIV (PLWH) provide valuable insights into the expected progression of HSIL in SOT and HSCT recipients.

In the general population, the risk of progression to cervical cancer once cervical HSIL is present was studied in New Zealand where cervical HSIL treatment was unethically withheld [57]. For women with persistent HSIL at 24 months, the risk of invasive cervical cancer by 5 years was 20% and by 30 years up to 50%. The study also showed progression of HSIL to invasive cancer after an average of 10–15 years [58].

Although we have not directly measured progression from anal HSIL to invasive anal cancer, combining data on HPV prevalence and anal cancer incidence provides an estimate of 1 in 4196 patients/year in MSM without HIV and 1 in 377 patients/year in MSM with HIV [59], suggesting a possible 10% lifetime risk of anal cancer for those MSM with HIV.

Information on the incidence of progression of HSIL to invasive cancer in other patient populations and at other anatomical sites is unavailable. Given the effects of immunosuppression on cellular immunity, it is reasonable to hypothesize that SOT and certain HSCT recipients (e.g., alloHSCT recipients with GVHD) have rates of HSIL progression to malignancy greater than those of the general population and closer to those of PLWH.

It is worth noting that on a per-HPV infection basis, the estimated rates for progression for cervical HSIL are much higher (approximately 1 in 80 per year) than the measured risk of cancer progression for anal HSIL [57]. While it is unknown as to why the cervix has a higher susceptibility for malignant transformation compared with the anus, contributing factors may include different hormonal milieux and microbiomes.

HPV-Related Malignancies in SOT and HSCT Recipients

The incidence of malignancy in SOT is calculated via the linkage of large transplant and cancer registries. Several such large, population-level studies provide invaluable information about the relationship between SOT and cancer. An Australian study noted a significant increase in the overall incidence of cancer following renal transplantation (SIR 3.27, 95% CI 3.09–3.46) [60]. The SIR was not elevated prior to transplantation, suggesting that immunosuppression itself (rather other traditional risk factors for malignancy like tobacco) was responsible for the results. UK and US linkage studies have similarly noted a twofold increase in the overall risk of malignancy in SOT recipients [15, 61]. Linkage studies also demonstrate a specific increase in the risk of HPV-related malignancies in SOT recipients [15, 49, 60]. In the USA, data from the US Transplant Cancer Match Study showed elevated SIR for vulvar, anal, penile, and oropharyngeal cancer (Table 1) [49]. The SIR of 1 for cervical cancer may reflect the presence of effective screening programs in US SOT recipients rather than an absence of elevated risk for this malignancy.

The risk of vulvar cancer in SOT recipients is particularly elevated with SIRs that range from 7 to 22 [49, 60]. In the general population, vulvar cancers are primarily squamous cell carcinomas (VSCC) and present in two distinct phenotypes. The first occurs in older women and is not associated with HPV infection. The second comprises about 25–40% of cases [62, 63], presents in younger women, and is associated with hr-HPV. A Dutch retrospective cohort of 1253 female RTR detected 6 cases of VSCC of which 5 were associated with HPV: 3 with HPV type 16, 1 with HPV type 53, and 1 with HPV type 58 [12]. Thus, it is plausible and likely that the observed risk for vulvar cancer in SOT recipients is related to a greater proportion of HPV-related VSCC.

Information on solid cancers following HSCT is derived from national and international registries that collect data on health outcomes in HSCT recipients.

Similarly to SOT recipients, HSCT recipients have approximately twice the risk of developing a solid malignancy after transplantation compared with the general, age-matched population [64–66]. However, whether they are at increased risk for HPV-related malignancies has not been clearly established. While smaller studies detected an elevated risk for cervical cancer in female alloHSCT and autoHSCT recipients [67, 68], this has not been replicated in larger studies [64–66, 69, 70]. This discrepancy may result from the smaller sample size in the studies where greater risk was detected. In this setting, a small number of detected cases may drive results toward significance but not be truly representative of the incidence in larger populations [56]. Of interest, registry studies have detected an elevated risk of oral cavity and/or pharyngeal cancer in alloHSCT and combined auto- and alloHSCT recipients, with GVHD as a driving risk factor [64–69]. While it is possible for some of these cases to actually represent oropharyngeal cancer, this has not been confirmed. Only one study specifically calculated the risk for oropharyngeal cancer in alloHSCT recipients and found it to be increased [70]. Two studies have reported increased risk for vulvar cancer in autoHSCT and alloHSCT, respectively [70, 71]; however, these results were only based on a total of one and two cases. Information on risk for anal cancer is limited.

Overall, there is limited evidence for an elevated risk of HPV-related malignancies among HSCT recipients despite greater risk for genital HPV infection and, in females, abnormal cervical cytology post-transplantation [45], although risk for oropharyngeal cancer may be elevated. This may be a result of eventual immune recovery allowing for clearance of HPV, thus decreasing the probability of malignancy. If so, risk likely remains elevated among patients who remain immunocompromised, like alloHSCT recipients with chronic GVHD on immunosuppression. Alternatively, because of the relatively long latent period between HPV infection and development of malignancy [57], patients with HSCT may die from complications related to transplantation before developing HPV-related malignancies. Finally, unlike SOT cancer studies, data from HSCT registries is not linked to cancer registries, and hence detection of malignancies may be undereported.

Non-melanomatous Skin Cancer in SOT and HSCT Recipients

SOT recipients and alloHSCT recipients are also at risk for non-melanoma skin cancer (NMSC) [72–74]. The incidence of NMSC increases with the duration of immunosuppression, ultimately affecting almost 50% of white SOT recipients [72]. While basal cell carcinomas predominate in the general population, cutaneous SCC (cSCC) comprise the majority of NMSC in SOT recipients [72] and an increased proportion of NMSC in alloHSCT recipients [74].

A role for non-anogenital cHPV in the pathogenesis of cSCC has been investigated but not conclusively proven in the healthy, general population [2]. While

cHPV is detected in 30–40% of cSCC from immunocompetent patients, no single HPV type predominates in these specimens, and cHPV is also found ubiquitously on the healthy skin and hair follicles and healthy skin [2]. In SOT recipients, however, cHPV is found in as many as 75–80% of cSCC samples [75, 76], with detection of HPV types 5 and 8 in 50% of specimens [76]. HPV 5 and 8 belong to the beta genus of the papillomaviridae family and are classified as potential carcinogens given their association with epidermodysplasia verruciformis, a rare hereditary skin disorder characterized by susceptibility to cHPV, cutaneous warts, and cSCC [2]. Thus, the greater prevalence of cHPV, particularly of HPV types 5 and 8, suggests a causal association between cHPV and cSCC in SOT recipients. However, most cHPV in cSCC are not transcriptionally active, and, similar to the general population, cHPV are also ubiquitious in healthy skin and hair follicles of SOT recipients [2]. If it is playing a role, cHPV is thought to potentiate the effects of ultraviolet radiation, which remains by far the strongest risk factor for cSCC [2]. The prevalence of cHPV in cSCC of alloHSCT recipients is unknown.

Prevention of HPV-Related Complications in SOT and HSCT Recipients

Until recently, prevention of HPV-related complications consisted of screening to detect and eliminate cervical and anal HSIL before progression to invasive cancer. HPV vaccines were introduced in 2006 to prevent initial HPV infection. Today, both screening and vaccination are important and complementary tools to decrease the risk of complications from HPV infection.

Cervical Cancer Screening

The cervical Papanicolaou (Pap) test, or cervical cytology, was developed by George Papanicolaou in the early 1940s to detect cervical cancer and precancerous lesions like HSIL and is still widely used today [77]. Women with abnormal cytology are referred for colposcopy, where a colposcope microscope and acetic acid are used to examine the cervix and identify HSIL. Affected areas are removed by loop electroexcisional procedure (LEEP), which can be done in the office, or cone conization, which is performed in the operating room [5]. Although no randomized controlled trials exist to establish the efficacy of cervical cytology screening and treatment of HSIL, the rapid decline in US cervical cancer incidence after the 1940s provides compelling evidence of its effectiveness [77]. Molecular techniques to test for hr-HPV have further improved the sensitivity and specificity of cervical cancer screening. Hr-HPV testing can be performed as a “reflex test” (to further risk-stratify in response to an abnormal cervical cytology), a “co-test” (automatically with cervical cytology), or a primary standalone test.

While detailed cervical cancer screening guidelines exist for immunocompetent women (Table 2), it is unclear how well these apply to women at higher risk for cervical cancer like SOT and HSCT recipients. Experts, including the American Society of Transplantation Infectious Diseases Community of Practice, recommend applying screening recommendations for women living with HIV (WLWH) to female SOT recipients [78, 79]. These guidelines are generally more stringent compared with those for the general population; differences include initiation of screening prior to age 21 if women are sexually active, shorter screening intervals, and the extension of screening beyond age 65 even in the absence of risk factors other than immunosuppression itself (Table 2). As with immunocompetent women, hr-HPV testing can be used as a "co-test" in immunocompromised women $\geq$30 years old. However, there is insufficient data to recommend it as a standalone test.

While data on the uptake of cervical cancer screening in SOT recipients from the USA is lacking, international studies suggest that adherence to screening guidelines in SOT recipients is low [80, 81]. A large, population-based cohort study of SOT recipients from Ontario, Canada, determined that only 30% of eligible women were adherent to general cervical cancer screening guidelines from 2000 to 2013 [80]. Furthermore, 31% of eligible women did not undergo any cervical cancer screening during this time period. A previous, smaller study from Northern Ireland in renal SOT recipients had similarly low rates of adherence to general screening guidelines [81].

For HSCT recipients, a large, nationwide, US insurance claims study reported that only 49.1% of alloHSCT and 42.6% of autoHSCT female recipients underwent cervical cytology within 2 years of transplantation [82]. The rate of testing was lower for alloHSCT recipients with GVHD compared with alloHSCT recipients without GHHD (42.5% versus 55.4%, $p \leq 0.01$), perhaps reflecting a sicker patient population less likely to adhere to additional testing, including screening. Uptake was better, but still inferior to that of controls, in an older, cross-sectional survey of HSCT from 40 North American transplant centers, in which 72.6% of women with HSCT reported a cervical cytology within the last year compared with 82.6% of controls ($p = 0.034$) [83]. These numbers are comparable to those reported in a cross-sectional Australian survey of HSCT survivors in New South Wales [84].

Despite variability in methods and results, these studies strongly suggest that SOT and HSCT recipients are less likely to receive cervical cancer screening compared with the general population and highlight the need to improve adherence to guidelines.

Anal Cancer Screening

There is interest in the early detection and treatment of anal HSIL in at-risk individuals. Analogous to the cervical cytology, anal cytology can be used to identify individuals requiring further evaluation. To obtain a specimen for anal

Table 2 US guidelines for cervical cancer screening

	Immunocompetent women						
	American Cancer Society, American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology, 2012 [120]	American College of Obstetricians and Gynecologists, 2016 [121]	Society of Gynecologic Oncology and ASCCP: Interim clinical guidance for primary hr-HPV testing, 2015 [122]	US Preventive Services Task Forces, 2018 [123]	Women living with HIV [119]	Women with solid organ transplant [78, 79]	Women with hematopoietic stem cell transplant [79]
When to start	Age 21	Age 21		Age 21	Within 1 year of onset of sexual activity but no later than age 21	Within 1 year of onset of sexual activity but no later than age 21	Within 1 year of onset of sexual activity but no later than age 21
Screening method and interval							
Cytology alone	Every 3 years	Every 3 years		Every 3 years	Annually, optional every 6 months for first year from HIV diagnosis. Every 3 years after three consecutive normal cytologies	Annually, optional every 6 months for first year from SOT or anti-lymphocyte antibody. Every 3 years after three consecutive normal cytologies	Annually, then every 3 years after three consecutive normal cytologies. Resume annual testing for 3 years if new diagnosis of genital or chronic GvHD

HPV co-test (cytology + HPV)							
21–29 years old	Not recommended	Not recommended		Not recommended	Not recommended	Not recommended	Not recommended
30–65 years old	Every 5 years; preferred	Every 5 years; preferred		For women who want to extend screening interval, HPV co-testing every 5 years is an option	Every 3 years	Every 3 years; preferred	Every 3 years; preferred. Repeat if new diagnosis of genital or chronic GvHD
Primary hr-HPV testing	Not recommended	Every 3 years for women ≥25 years old, based on SGO and ASCCP interim guidelines	Every 3 years for women ≥25 years old	Every 5 years for women 30–65 years old	Insufficient data	Insufficient data	Insufficient data
When to stop	>65 years old with adequate prior screening and no recent HSIL	>65 years old with adequate prior screening and no history of HSIL		>65 years old with adequate prior screening and not at high risk for cervical cancer	Continue indefinitely	Continue indefinitely	Continue indefinitely

cytology testing, a Dacron swab is moistened with water and gently inserted into the anal canal until it hits the wall of the rectum. The swab is then extracted while rotating it in a spiral motion and gently applying mild pressure to the anal wall, after which it is immersed in preservative solution [85]. Digital anorectal examination (DARE) is performed following anal cytology to detect palpable, early invasive cancers.

The presence of any abnormality on DARE or anal cytology is followed by referral for high-resolution anoscopy (HRA). HRA involves use of a colposcope, the same instrument used to examine the cervix, to carefully examine the anal canal and perianus following the application of 5% acetic acid. The colposcope provides magnification and light, while acetic acid leads to dehydration of the intracellular compartment making cells more refractive to light. Lesions suspicious for HSIL or malignancy are biopsied. Treatment of anal HSIL differs from cervical HSIL in that deep excision of the anal canal causes significant morbidity and is not generally feasible. Instead, therapy typically involves superficial ablation with electrocautery (hyfrecation) or infrared coagulation. These can usually be performed in the office with local anesthesia. Alternatively, topical 5-fluorouracil can be applied to the anal canal and/or perianus for patients with widely diffuse disease. Surgical excision is reserved primarily for diagnosis of suspected malignancy [16].

However, despite our ability to detect and treat anal HSIL, it is unclear which specific patients benefit from this. There are presently no national guidelines for anal cancer screening, largely because of the absence of studies demonstrating that treatment of anal HSIL decreases the incidence of anal cancer, although a large, multi-site randomized-controlled trial is underway to answer this question in PLWH [86]. The HIV Medicine Association (HIVMA) recommends yearly anal cytology testing for the following PLWH: men who have sex with men (MSM), women with a history of receptive anal intercourse or of abnormal cervical cytology, and any individual with a history of genital warts [87]. The New York State Department of Health is the only other health organization to recommend anal HSIL screening in PLWH [85]. Its recommendations are similar to those of the HIVMA in terms of specific subgroups of PLWH who should receive screening [85].

Data to guide screening in SOT and HSCT recipients is lacking. The performance of anal cytology in detecting anal HSIL is unknown, and there is no data on the efficacy or effectiveness of therapy in SOT or HSCT recipients. However, given the elevated risk for anal cancer in SOT recipients and the presence of techniques that may prevent its development, the American Society of Transplantation Infectious Diseases Community of Practice does recommend screening SOT recipients with anal cytology (Fig. 6) [78]. Similar to PLWH, screening efforts should focus on SOT recipients at particular risk for anal cancer like MSM; women with a history of receptive anal intercourse; women with a history of cervical, vulvar, or vaginal SIL; and both men and women with a history of genital warts. It is reasonable to similarly screen HSCT recipients with the above criteria, especially those alloHSCT recipients with chronic GVHD.

Although the test characteristics for anal cytology in SOT and HSCT recipients are unknown, it is worth noting that abnormal anal cytology results are common in

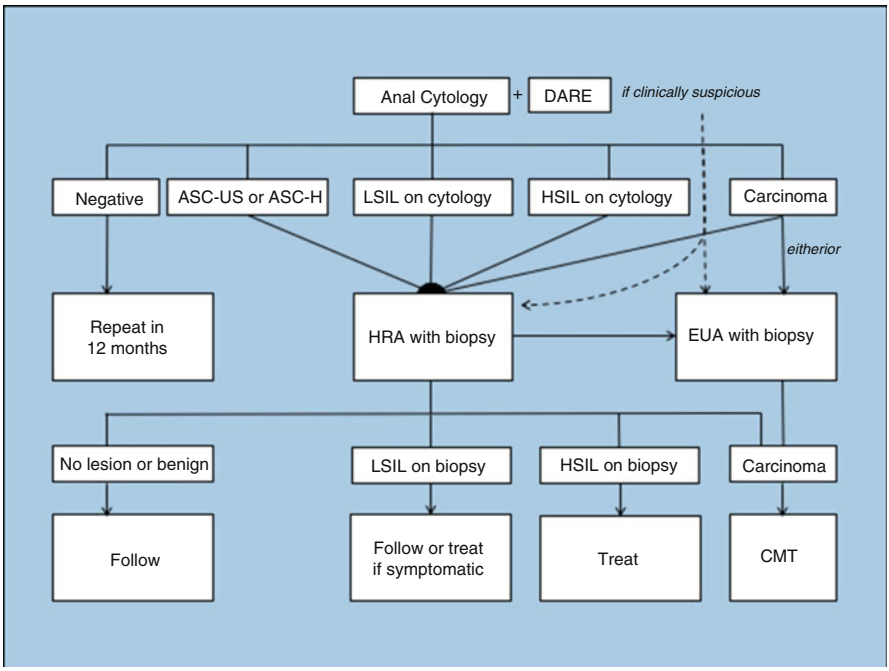


Fig. 6 Anal cancer/HSIL screening algorithm. Abbreviations as follows: atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H); combined modality therapy (CMT,); digital anorectal examination (DARE); examination under anesthesia (EUA); high-resolution anoscopy (HRA); high-grade intraepithelial lesions (HSIL); low-grade squamous intraepithelial lesions (LSIL). (Adapted from Ref. [118])

the non-transplant population, ranging from 20% in low-risk women to approximately 70% in MSM living with HIV [88–91]. Therefore, it is likely that a substantial proportion of screened transplant recipients will require further evaluation when anal cytology-based screening is offered. Before cytology is performed, patients should be counseled about the high probability of referral for HRA as well as the potential risks and benefits of treating anal HSIL. Cytology should be avoided if the infrastructure to identify and treat anal HSIL is unavailable, and instead, yearly DARE to detect early palpable invasive anal cancer can be performed.

Other HPV-Related Malignancies

No recommendations exist to screen for HSIL at sites other than the cervix or anus in either immunocompetent or immunocompromised patients. Given the elevated risk of vulvar cancer in female SOT recipients, the vagina and vulva should be carefully examined at the time of cervical cytology, with a low threshold to perform vaginal and vulvar colposcopy, particularly if genital warts are noted [78]. This approach is

also reasonable for female alloHSCT recipients with a history of cervical or vulvar SIL or with severe cutaneous or genital GVHD.

Prophylactic HPV Vaccination

Three different vaccines exist to prevent initial HPV infection although only one is currently available in the USA. All three contain virus-like particles (VLP) made up of recombinant L1 capsid subunits but differ in the types they cover. Vaccination generates high levels of neutralizing anti-L1 VLP antibodies, which prevent HPV attachment to the basal cell membrane and, thus, initial infection [1].

Gardasil®, the first HPV vaccine, was approved in 2006. It is a quadrivalent vaccine (HPV4) produced by Merck & Co. that confers protection against hr-HPV types 16 and 18 and lr-HPV types 6 and 11. As previously noted, hr-HPV types 16 and 18 account for 70% of cervical cancers and 85% of anal cancers [7], while lr-HPV types 6 and 11 account for 90% of genital warts [8]. Two large, randomized controlled trials in young women demonstrated excellent efficacy of HPV4 in preventing type-specific cervical HSIL and genital warts in the per-protocol analysis (98% and 100%, respectively) [92, 93]. The per-protocol analysis included women who remained HPV-negative 1 month after completion of a three-dose vaccination series given at 0, 1, and 6 months; efficacy was lower in the intention-to-treat analysis (44% for cervical HSIL and 73% for genital warts). In 2009, GlaxoSmithKline introduced a bivalent vaccine (HPV2), Cervarix®, which protects against HPV 16 and 18. Following a similar three-dose vaccination series, its efficacy against type-specific cervical HSIL is comparable to HPV4 [94]. Subsequent studies also established vaccine efficacy of HPV4 in genital wart prevention among young men [95] and anal HSIL prevention in MSM [96]. Merck introduced a nonavalent vaccine (HPV9), Gardasil®9, in 2014. In addition to covering strains 6, 11, 16, and 18, HPV9 confers additional protection from hr-HPV types 31, 33, 45, 52, and 58 [97] which increases the coverage of cervical cancer to 90%. As of late 2016, only HPV9 is available in the USA.

The lower efficacy in the intention-to-treat analyses of HPV2 and HPV4 highlights a key characteristic of prophylactic HPV vaccines: they work when administered *prior* to HPV exposure. Because initial HPV infection occurs soon after sexual debut [35, 98], vaccination is most effective when given before recipients are sexually active. The US Advisory Committee on Immunization Practices (ACIP) consequently recommends routine vaccination of all males and females 11–12 years old, with immunization starting as early as age 9 years through age 26 years [99]. These recommendations extend to all immunocompromised persons through age 26 years [100]. Based on immunogenicity studies, a reduced, two-dose vaccination series with immunizations at 0 and 6–12 months is recommended for non-immunocompromised individuals less than 15 years old [101].

In October 2018, the FDA approved use of HPV9 in men and women ages 27 through 45 years old [102]. This approval was based on a randomized controlled trial of women aged 27–45 years old that showed vaccine efficacy in preventing

type-specific disease [103, 104]. As with studies in younger women, efficacy was higher in per-protocol analyses, which consisted of women without evidence of HPV exposure after completion of the vaccination series. In the USA, 50% of women and 25% of men aged 27–45 years old have serological evidence of infection by at least one of the HPV types in HPV9; 20–28% of women and 7–14% of men aged 27–45 years have serological evidence of infection by HPV types 16 and/or 18 [105]. Rates of actual infection are presumably much higher given that serological response to infection only occurs in 50–70% of women [106] and 4–36% of men [107]. Thus, a substantial proportion of potential vaccine recipients aged 27–45 years have been exposed to HPV, and it is unclear whether widespread vaccination of this age group would be cost-effective. The most recent ACIP guidelines have not extended catch-up vaccination through age 45. Instead, the ACIP acknowledges that some persons aged >26 years may benefit from vaccination and recommends shared clinical decision-making between these persons and their providers [99].

Data on the efficacy of HPV vaccination in SOT and HSCT is limited. One immunogenicity study of 47 SOT recipients ages 18–35 years old who received HPV4 showed a 47% response rate to all four vaccine HPV types [108]. Response rates were higher in patients who received all three vaccine doses. Factors associated with decreased response included lung transplantation and transplant less than 1 year prior to receipt of vaccine. However, a separate immunogenicity study of 14 RT and 3 liver transplant recipients ages 9–17 years old who also received HPV4 showed a 100% response rate to all 4 vaccine types [109]. This rate is comparable to seroconversion rates in the general, immunocompetent population [110]. The reasons for this discrepancy are unknown and may be due to the variability inherent to small cohort studies. However, it may also reflect real differences in vaccine response due to age given that there was a younger median age in the study with the higher vaccine response. Of note, 6 of 14 RT recipients within the adolescent cohort developed acute rejection during the study period. While this rate was higher than expected, there is no evidence that this was related to HPV vaccination. Neither study evaluated the efficacy of the vaccine in preventing SIL.

For HSCT recipients, there are encouraging preliminary results from a phase 1 study of female alloHSCT recipients who underwent HPV4 vaccination at least 90 days post-transplantation [111]. Response rate to all four vaccine types was 78.3% for female alloHSCT recipients receiving immunosuppression for GVHD, 95.2% for female alloHSCT recipients not receiving immunosuppression, and 100% for healthy female controls. A separate study to assess the immunogenicity of HPV9 in patients after alloHSCT is actively recruiting [112].

Despite the possibility of a suboptimal immune response, we recommend proceeding with HPV vaccination of SOT and HSCT recipients who meet age-specific criteria, particularly since HPV vaccines do not contain live virus and have excellent safety records [78, 113, 114]. Both SOT and HSCT recipients should receive the three-dose vaccination series, ideally prior to transplantation. If vaccination is not performed prior to transplantation, it is reasonable to wait at least 3–6 months in SOT recipients and 6–12 months in HSCT recipients after transplantation to potentially improve seroconversion [113, 114]. Whether or not to vaccinate SOT and HSCT

recipients ages 27–45 years old is unclear; however, vaccination in this age group may help decrease the risk of HPV-related malignancies with relatively little downside. Lastly, vaccination does not substitute for ongoing cervical and anal cancer screening.

Conclusion

In summary, SOT recipients are at high risk for infection with anogenital HPV, development of precancerous lesions (HSIL), and progression to invasive malignancy. This process is thought to be mediated via reactivation of latent HPV virus in the setting of impaired cellular immunity. While there is less data available for HSCT recipients, studies suggest that alloHSCT recipients with a history of GVHD are at particular risk for complications from HPV infection, likely from a mechanism similar to that postulated in SOT. Screening and vaccination are two methods to decrease the risk of HPV-related malignancies. There are specific guidelines for cervical cancer screening for female SOT and HSCT recipients; special efforts are needed to improve their uptake. More studies are needed to determine whether anal cancer screening is effective in SOT and HSCT recipients and whether widespread HPV vaccination should be pursued in these groups. In the interim, anal cancer screening is recommended for high-risk SOT recipients when an adequate infrastructure is available and should be considered in high-risk HSCT recipients. ACIP vaccination guidelines should be followed, with additional consideration of extending HPV vaccination to SOT and HSCT recipients ages 27–45 years old.

Key Points

- Infection with high-risk human papillomavirus (hr-HPV) is associated with cervical cancers (99.9%), anal cancers (90%), and oropharyngeal cancers (70%), as well as a high proportion of vaginal, penile, and vulvar cancers.
- Defects in cellular immunity make solid organ transplant (SOT) recipients particularly susceptible to persistent hr-HPV infection, development of cancer precursor lesions, and progression to malignancy.
- AlloHSCT recipients with GVHD are likely at increased risk for HPV-related malignancies.
- Specific cervical cancer screening recommendations exist for SOT and HSCT recipients, while specific anal cancer screening recommendations exist for SOT recipients. It is reasonable to apply anal cancer screening recommendations for SOT to HSCT recipients, particularly alloHSCT recipients with a history of GVHD.
- HPV vaccination may decrease the incidence of HPV-related complications in SOT and HSCT recipients, particularly when administered prior to HPV exposure and transplantation.

References

1. Stanley M, Lowy DR, Frazer I. Chapter 12. Prophylactic HPV vaccines: underlying mechanisms. Vaccine. 2006;24(Suppl 3):S3/106–13.
2. Harwood CA, Proby CM. Human papillomaviruses and non-melanoma skin cancer. Curr Opin Infect Dis. 2002;15(2):101–14.
3. Weaver BA. Epidemiology and natural history of genital human papillomavirus infection. J Am Osteopath Assoc. 2006;106(3 Suppl 1):S2–8.
4. Satterwhite CL, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187–93.
5. Schiffman M, et al. Human papillomavirus and cervical cancer. Lancet. 2007;370 (9590):890–907.
6. Centers for Disease Control and Prevention. Human papillomavirus-associated cancers – United States, 2004–2008. MMWR Morb Mortal Wkly Rep. 2012;61:258–61.
7. Saraiya M, et al. US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. J Natl Cancer Inst. 2015;107(6):djv086.
8. Lacey CJ, Lowndes CM, Shah KV. Chapter 4. Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. Vaccine. 2006;24(Suppl 3):S3/35–41.
9. Aggarwal R, et al. Prevalence and genotypes of HPV in female renal transplant recipients in North India. Int J Gynecol Pathol. 2014;33(5):537–42.
10. Eleuterio J Jr, et al. Prevalence of high-risk HPV and atypia in liquid-based cytology of cervical and intra-anal specimens from kidney-transplanted women. Diagn Cytopathol. 2019;47(8):783–7.
11. Grat M, et al. Initial prevalence of anal human papilloma virus infection in liver transplant recipients. Transpl Int. 2014;27(8):816–23.
12. Meeuwis KA, et al. Anogenital malignancies in women after renal transplantation over 40 years in a single center. Transplantation. 2012;93(9):914–22.
13. Patel HS, et al. Human papillomavirus infection and anal dysplasia in renal transplant recipients. Br J Surg. 2010;97(11):1716–21.
14. Pietrzak B, et al. Prevalence of high-risk human papillomavirus cervical infection in female kidney graft recipients: an observational study. Virol J. 2012;9:117.
15. Collett D, et al. Comparison of the incidence of malignancy in recipients of different types of organ: a UK Registry audit. Am J Transplant. 2010;10(8):1889–96.
16. Darragh T, et al. The anal canal and perianus: HPV-related disease. In: Mayeaux EJ, Cox JT, editors. Modern colposcopy: textbook and atlas. Baltimore: Lippincott, Williams, & Wilkins; 2012.
17. Doorbar J. The papillomavirus life cycle. J Clin Virol. 2005;32(Suppl 1):S7–15.
18. Zheng ZM, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci. 2006;11:2286–302.
19. Selvakumar R, et al. Regression of papillomas induced by cottontail rabbit papillomavirus is associated with infiltration of CD8+ cells and persistence of viral DNA after regression. J Virol. 1997;71(7):5540–8.
20. Maglennon GA, McIntosh P, Doorbar J. Persistence of viral DNA in the epithelial basal layer suggests a model for papillomavirus latency following immune regression. Virology. 2011; 414(2):153–63.
21. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol. 1969;105(3):386–93.
22. Rodriguez AC, et al. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008;100(7):513–7.
23. Darragh TM, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012;16(3):205–42.

24. Wright TC, Kurman RJ, Ferenczy A. Precancerous lesions of the cervix. In: Kurman RJ, editor. Blaustein's pathology of the female genital tract. New York: Springer; 2002. p. 277.
25. Fang J, Zhang H, Jin S. Epigenetics and cervical cancer: from pathogenesis to therapy. Tumour Biol. 2014;35(6):5083–93.
26. Barrow-Laing L, Chen W, Roman A. Low- and high-risk human papillomavirus E7 proteins regulate p130 differently. Virology. 2010;400(2):233–9.
27. Doorbar J, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012; 30(Suppl 5):F55–70.
28. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev. 2012;25(2):215–22.
29. Jain S, et al. Cell-mediated immune responses to COPV early proteins. Virology. 2006;356 (1–2):23–34.
30. Coleman N, et al. Immunological events in regressing genital warts. Am J Clin Pathol. 1994;102(6):768–74.
31. de Jong A, et al. Frequent detection of human papillomavirus 16 E2-specific T-helper immunity in healthy subjects. Cancer Res. 2002;62(2):472–9.
32. Welters MJ, et al. Frequent display of human papillomavirus type 16 E6-specific memory t-Helper cells in the healthy population as witness of previous viral encounter. Cancer Res. 2003;63(3):636–41.
33. Woo YL, et al. A prospective study on the natural course of low-grade squamous intraepithelial lesions and the presence of HPV16 E2-, E6- and E7-specific T-cell responses. Int J Cancer. 2010;126(1):133–41.
34. Brickman C, Palefsky JM. Human papillomavirus in the HIV-infected host: epidemiology and pathogenesis in the antiretroviral era. Curr HIV/AIDS Rep. 2015;12(1):6–15.
35. Dunne EF, et al. Prevalence of HPV infection among females in the United States. JAMA. 2007;297(8):813–9.
36. Han JJ, et al. Prevalence of genital human papillomavirus infection and human papillomavirus vaccination rates among US adult men: National Health and Nutrition Examination Survey (NHANES) 2013–2014. JAMA Oncol. 2017;3(6):810–6.
37. Scholefield JH, et al. Anal intraepithelial neoplasia: part of a multifocal disease process. Lancet. 1992;340(8830):1271–3.
38. Palefsky JM, et al. Prevalence and risk factors for anal human papillomavirus infection in human immunodeficiency virus (HIV)-positive and high-risk HIV-negative women. J Infect Dis. 2001;183(3):383–91.
39. Ogunbiyi OA, et al. Anal human papillomavirus infection and squamous neoplasia in patients with invasive vulvar cancer. Obstet Gynecol. 1994;83(2):212–6.
40. Hernandez BY, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomark Prev. 2005;14(11 Pt 1):2550–6.
41. Meeuwis KA, et al. Cervicovaginal HPV infection in female renal transplant recipients: an observational, self-sampling based, cohort study. Am J Transplant. 2015;15(3):723–33.
42. Forman D, et al. Global burden of human papillomavirus and related diseases. Vaccine. 2012;30(Suppl 5):F12–23.
43. Hinten F, et al. Reactivation of latent HPV infections after renal transplantation. Am J Transplant. 2017;17(6):1563–73.
44. Rosales BM, et al. Transplant recipients and anal neoplasia study: design, methods, and participant characteristics of a prevalence study. Transplant Direct. 2019;5(4):e434.
45. Shanis D, et al. Risks factors and timing of genital human papillomavirus (HPV) infection in female stem cell transplant survivors: a longitudinal study. Bone Marrow Transplant. 2018; 53(1):78–83.
46. Larsen HK, et al. Risk of genital warts in renal transplant recipients – a registry-based, prospective cohort study. Am J Transplant. 2019;19(1):156–65.
47. Nadhan KS, et al. Prevalence and types of genital lesions in organ transplant recipients. JAMA Dermatol. 2018;154(3):323–9.

48. Dyall-Smith D, Trowell H, Dyall-Smith ML. Benign human papillomavirus infection in renal transplant recipients. Int J Dermatol. 1991;30(11):785–9.
49. Madeleine MM, et al. HPV-related cancers after solid organ transplantation in the United States. Am J Transplant. 2013;13(12):3202–9.
50. Alloub MI, et al. Human papillomavirus infection and cervical intraepithelial neoplasia in women with renal allografts. BMJ. 1989;298(6667):153–6.
51. Ogunbiyi OA, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. Br J Surg. 1994;81(3):365–7.
52. Negri G, et al. Abnormal cervical cytology after allogeneic bone marrow transplantation. Am J Clin Pathol. 2014;142(2):222–6.
53. Savani BN, et al. Increased risk of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation – implications for screening and HPV vaccination. Biol Blood Marrow Transplant. 2008;14(9):1072–5.
54. Wang Y, et al. A clinical study of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2012;18(5):747–53.
55. Sasadeusz J, et al. Abnormal cervical cytology in bone marrow transplant recipients. Bone Marrow Transplant. 2001;28(4):393–7.
56. Chang HA, Armenian SH, Dellinger TH. Secondary neoplasms of the female lower genital tract after hematopoietic cell transplantation. J Natl Compr Cancer Netw. 2018;16(2):211–8.
57. McCredie MR, et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol. 2008; 9(5):425–34.
58. Holowaty P, et al. Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst. 1999; 91(3):252–8.
59. Machalek DA, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012; 13(5):487–500.
60. Vajdic CM, et al. Cancer incidence before and after kidney transplantation. JAMA. 2006; 296(23):2823–31.
61. Engels EA, et al. Spectrum of cancer risk among US solid organ transplant recipients. JAMA. 2011;306(17):1891–901.
62. de Sanjose S, et al. Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. Eur J Cancer. 2013;49(16):3450–61.
63. de Martel C, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol. 2012;13(6):607–15.
64. Atsuta Y, et al. Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease. Ann Oncol. 2014;25(2):435–41.
65. Curtis RE, et al. Solid cancers after bone marrow transplantation. N Engl J Med. 1997; 336(13):897–904.
66. Rizzo JD, et al. Solid cancers after allogeneic hematopoietic cell transplantation. Blood. 2009;113(5):1175–83.
67. Bhatia S, et al. Solid cancers after bone marrow transplantation. J Clin Oncol. 2001; 19(2):464–71.
68. Shimada K, et al. Solid tumors after hematopoietic stem cell transplantation in Japan: incidence, risk factors and prognosis. Bone Marrow Transplant. 2005;36(2):115–21.
69. Majhail NS, et al. Secondary solid cancers after allogeneic hematopoietic cell transplantation using busulfan-cyclophosphamide conditioning. Blood. 2011;117(1):316–22.
70. Ringden O, et al. Second solid cancers after allogeneic hematopoietic cell transplantation using reduced-intensity conditioning. Biol Blood Marrow Transplant. 2014;20(11):1777–84.
71. Oddou S, et al. Second neoplasms following high-dose chemotherapy and autologous stem cell transplantation for malignant lymphomas: a report of six cases in a cohort of 171 patients from a single institution. Leuk Lymphoma. 1998;31(1–2):187–94.

72. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. N Engl J Med. 2003;348(17):1681–91.
73. Hampras SS, et al. Prevalence of cutaneous viral infections in incident cutaneous squamous cell carcinoma detected among chronic lymphocytic leukemia and hematopoietic stem cell transplant patients. Leuk Lymphoma. 2018;59(4):911–7.
74. Omland SH, et al. Skin cancer risk in hematopoietic stem-cell transplant recipients compared with background population and renal transplant recipients: a population-based cohort study. JAMA Dermatol. 2016;152(2):177–83.
75. Harwood CA, et al. Human papillomavirus infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. J Med Virol. 2000;61(3):289–97.
76. Stockfleth E, et al. Human papillomaviruses in transplant-associated skin cancers. Dermatol Surg. 2004;30(4 Pt 2):604–9.
77. Tambouret RH. The evolution of the Papanicolaou smear. Clin Obstet Gynecol. 2013; 56(1):3–9.
78. Chin-Hong PV, Reid GE, American Society of Transplantation Infectious Diseases Community of Practice. Human papillomavirus infection in solid organ transplant recipients – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13590.
79. Moscicki AB, et al. Guidelines for cervical cancer screening in immunosuppressed women without HIV infection. J Low Genit Tract Dis. 2019;23(2):87–101.
80. Acuna SA, et al. Uptake of cancer screening tests among recipients of solid organ transplantation. Am J Transplant. 2017;17(9):2434–43.
81. Courtney AE, et al. The uptake of cervical cancer screening by renal transplant recipients. Nephrol Dial Transplant. 2009;24(2):647–52.
82. Hwang JP, et al. Low rate of cervical cancer screening among women with hematologic malignancies after stem cell transplant. Biol Blood Marrow Transplant. 2018;24(5):1094–8.
83. Bishop MM, et al. The preventive health behaviors of long-term survivors of cancer and hematopoietic stem cell transplantation compared with matched controls. Biol Blood Marrow Transplant. 2010;16(2):207–14.
84. Dyer G, et al. Adherence to cancer screening guidelines in Australian survivors of allogeneic blood and marrow transplantation (BMT). Cancer Med. 2016;5(7):1702–16.
85. New York State Department of Health AIDS Institute. HIV clinical resource: anal dysplasia and cancer. New York: New York State Department of Health; 2007. Available from: http://www.hivguidelines.org/clinical-guidelines/adults/anal-dysplasia-and-cancer/. Accessed 7 Aug 2016.
86. AIDS Malignancy Consortium. Topical or ablative treatment in preventing anal cancer in patients with HIV and anal high-grade squamous intraepithelial lesions (ANCHOR). 2014. Available from: http://www.clinicaltrials.gov/
87. Aberg JA, et al. Primary care guidelines for the management of persons infected with HIV: 2013 update by the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis. 2014;58(1):1–10.
88. Kojic EM, et al. Human papillomavirus infection and cytologic abnormalities of the anus and cervix among HIV-infected women in the study to understand the natural history of HIV/AIDS in the era of effective therapy (the SUN study). Sex Transm Dis. 2011;38(4):253–9.
89. Piketty C, et al. High prevalence of anal human papillomavirus infection and anal cancer precursors among HIV-infected persons in the absence of anal intercourse. Ann Intern Med. 2003;138(6):453–9.
90. Robison K, et al. Anal cytology and human papillomavirus genotyping in women with a history of lower genital tract neoplasia compared with low-risk women. Obstet Gynecol. 2015;126(6):1294–300.
91. Schofield AM, et al. A prospective study of anal cancer screening in HIV-positive and negative MSM. AIDS. 2016;30(9):1375–83.
92. Garland SM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med. 2007;356(19):1928–43.

93. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007;356(19):1915–27.
94. Paavonen J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. 2009;374(9686):301–14.
95. Giuliano AR, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med. 2011;364(5):401–11.
96. Palefsky JM, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. N Engl J Med. 2011;365(17):1576–85.
97. Joura EA, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med. 2015;372(8):711–23.
98. Chin-Hong PV, et al. Age-specific prevalence of anal human papillomavirus infection in HIV-negative sexually active men who have sex with men: the EXPLORE study. J Infect Dis. 2004;190(12):2070–6.
99. Meites E, et al. Human papillomavirus vaccination for adults: updated recommendations of the Advisory Committee on Immunization Practices. MMWR Morb Mortal Wkly Rep. 2019; 68(32):698–702.
100. Wheaton AG, et al. Employment and activity limitations among adults with chronic obstructive pulmonary disease – United States, 2013. MMWR Morb Mortal Wkly Rep. 2015; 64(11):289–95.
101. Meites E, Kempe A, Markowitz LE. Use of a 2-dose schedule for human papillomavirus vaccination – updated recommendations of the Advisory Committee on Immunization Practices. MMWR Morb Mortal Wkly Rep. 2016;65(49):1405–8.
102. FDA. FDA approves expanded use of Gardasil 9 to include invidivudals 27 through 45 years old (press release). 2018. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-expanded-use-gardasil-9-include-individuals-27-through-45-years-old
103. Luna J, et al. Long-term follow-up observation of the safety, immunogenicity, and effectiveness of Gardasil in adult women. PLoS One. 2013;8(12):e83431.
104. Munoz N, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. Lancet. 2009;373(9679):1949–57.
105. Liu G, et al. Seroprevalence of 9 human papillomavirus types in the United States, 2005–2006. J Infect Dis. 2016;213(2):191–8.
106. Carter JJ, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis. 2000;181(6):1911–9.
107. Edelstein ZR, et al. Serum antibody response following genital {alpha}9 human papillomavirus infection in young men. J Infect Dis. 2011;204(2):209–16.
108. Kumar D, et al. Immunogenicity of quadrivalent human papillomavirus vaccine in organ transplant recipients. Am J Transplant. 2013;13(9):2411–7.
109. Gomez-Lobo V, et al. Immunogenicity of a prophylactic quadrivalent human papillomavirus L1 virus-like particle vaccine in male and female adolescent transplant recipients. Pediatr Transplant. 2014;18(3):310–5.
110. Block SL, et al. Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. Pediatrics. 2006;118(5):2135–45.
111. ClinicalTrials.gov (Internet). Gardasil vaccination in post stem cell transplant patients, identifier NCT01092195. Bethesda: National Library of Medicine (US); 2010. Available from: https://clinicaltrials.gov/ct2/show/record/NCT01092195. Cited 17 Nov 2019
112. ClinicalTrials.gov [Internet]. Gardasil 9 vaccine in preventing HPV infection in patients with hematologic malignancies undergoing donor stem cell transplant, identifier NCT03023631. Bethesda: National Library of Medicine (US); 2017. Available from: https://clinicaltrials.gov/ct2/show/record/NCT03023631
113. Rubin LG, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58(3):e44–100.

114. Danziger-Isakov L, Kumar D, AST ID Community of Practice. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13563.
115. Munoz N, et al. Chapter 1. HPV in the etiology of human cancer. Vaccine. 2006;24(Suppl 3): S3/1–10.
116. Kurman RJ, editor. Blaustein's pathology of the female genital tract. 5th ed. New York: Springer; 2002.
117. Janicek MF, Averette HE. Cervical cancer: prevention, diagnosis, and therapeutics. CA Cancer J Clin. 2001;51(2):92–114. Quiz 115–8.
118. Park IU, Palefsky JM. Evaluation and management of anal intraepithelial neoplasia in HIV-negative and HIV-positive men who have sex with men. Curr Infect Dis Rep. 2010; 12(2):126–33.
119. Adolescents, P.o.O.I.i.H.-I.A.a. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health and the HIV Medicine Association of the Infectious Diseases Society of America. 2019. Available from: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Cited 12 May 2019.
120. Saslow D, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. J Low Genit Tract Dis. 2012;16(3):175–204.
121. Committee on Practice Bulletins – Gynecology. Practice bulletin no. 168: cervical cancer screening and prevention. Obstet Gynecol. 2016;128(4):e111–30.
122. Huh WK, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Obstet Gynecol. 2015;125(2):330–7.
123. US Preventive Services Task Force, et al. Screening for cervical cancer: US preventive services task force recommendation statement. JAMA. 2018;320(7):674–86.

Solid Organ Transplantation in HIV Positive Patients

35

Elmi Muller and Peter Stock

Contents

Introduction 860
Pathophysiology and Organ Involvement 861
Kidney 861
Liver 861
Patient Selection for Transplantation for a HIV Positive Transplant 863
Preoperative Investigations 863
Contraindications to Transplantation in the HIV Positive Recipient 863
Donor Options 864
HIV Negative Donors 864
HIV Positive Donors 865
Considerations When Utilizing a HIV Positive Donor 866
Antiretroviral Therapy 867
Immunosuppression Choices 868
Rejection 868
Recurrence of HIVAN 869
Second Viral Strain 870
Opportunistic Infection 871
Prophylactic Therapy Against Common Infections 872
Vaccinations Prior to Transplant 873
Vaccinations Post-transplant 873

E. Muller (✉)
Department of Surgery, University of Cape Town, Cape Town, South Africa
e-mail: elmi.muller@uct.ac.za

P. Stock
Department of Surgery, University of California, San Francisco, San Francisco, CA, USA
e-mail: Peter.Stock@ucsf.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_34

Conclusion 873
Key Points 874
References 874

Abbreviations

cART	Combination antiretroviral therapy
CMV	Cytomegalovirus
HHV8	Human herpes 8 virus
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
KS	Kaposi sarcoma
MELD	Model of end-stage liver disease
PCP	Pneumocystis pneumonia
PCV	Pneumococcal conjugate vaccine
PPSV	Pneumococcal polysaccharide vaccine
TOR	Mechanistic target of rapamycin
VEGF	Vascular endothelial growth factor

Introduction

Access to solid organ transplantation for the HIV positive recipient has gained acceptance with several studies demonstrating safety and efficacy comparable to HIV negative recipients [1–3]. The early success reported in HIV-infected kidney transplant recipient has subsequently been reported following liver transplantation as well, although early results were compromised by the absence of successful treatment of the HIV positive recipient coinfected with Hepatitis C [4, 5]. With the advent of direct acting agents to treat hepatitis C, this last hurdle to successful liver transplantation has been eliminated. Excellent long-term outcomes following simultaneous pancreas and kidney transplantation have also been reported in people with Type 1 diabetes and HIV [1], despite the more aggressive immunosuppressive regimens required in this group of recipients.

Along with the increased interest in solid organ transplantation in people infected with HIV, waiting lists have expanded. The waiting times to receive a deceased donor kidney are in excess of 7–8 years in many parts of the USA [6]. In most countries, liver recipients require very high MELD scores for organ allocation. Unfortunately, HIV positive recipients have rapid progression to end-stage liver disease and cannot tolerate the high MELD scores required for liver allocation. The use of HIV positive donors has expedited the time to kidney transplant and facilitated liver allocation at significantly lower MELD scores. In South Africa, a transplant

program utilizing HIV positive donors was started in 2008 [7]. In the USA, transplant programs have been using HIV negative donors for approximately two decades, and recently HIV positive donors have been utilized as part of the Hope Act [8].

The evolution of transplantation in people infected with HIV has been remarkable, and gone from HIV positivity being a strict contraindication to solid organ transplantation to broad acceptance. The dramatic evolution is further highlighted by the current practices to use HIV positive organs in HIV recipients to facilitate transplantation and minimize the time on expanding waiting lists. This chapter will provide an overview on strategies which have resulted in successful transplantation in the HIV positive recipient.

Pathophysiology and Organ Involvement

Kidney

The kidney is a known reservoir for HIV, but there are also indirect causes for renal failure associated with HIV infection. The etiology of kidney disease associated with HIV infection has implications for both the recurrence of nephropathy following transplantation, as well as for the choice to utilize an HIV-infected donor kidney. Particularly if a donor has proteinuria, or collapsing FSGS, they are typically not utilized. The spectrum of renal pathology related to HIV infection is diverse, and include lesions directly related to intrarenal HIV gene expression as well as lesions related to co-morbidities, drug effects, immune dysregulation, and other co-infections. Podocytopathy or immune complex-mediated glomerular disease are two common manifestations of HIV in the kidney. A useful approach to classification is based on the major tissue compartment affected by the virus (Table 1).

Liver

Mortality from liver disease impacts up to 18% of people infected with HIV in the current anti-retroviral era and remains a major cause of death in this population [4]. There is no question that HIV infection exacerbates liver inflammation and progression of fibrosis in people with concomitant causes of liver disease. The multiple mechanisms by which this occurs are beyond the scope of this chapter, but are in part related to dysregulation of the immune system as well the direct effect of HIV on activation of hepatic stellate cells [9]. Although the most common etiology remains viral coinfection with either hepatitis C or hepatitis B, enhanced progression of cirrhosis is also seen with liver disease mediated by alcohol abuse as well as non-alcoholic hepatic steatohepatitis.

Table 1 Pathologic classification of HIV-related kidney diseases

I. Glomerular dominant[a]
a. Podocytopathies (all characterized by extensive foot process effacement)[b]
i. Classic HIVAN
ii. FSGS (NOS) in the setting of HIV
iii. Minimal change disease in the setting of HIV
iv. Diffuse mesangial hypercellularity in the setting of HIV
v. Other podocytopathy in the setting of HIV
b. Immune complex-mediated glomerular disease[a]
i. IgA nephropathy in the setting of HIV
ii. Lupus-like glomerulonephritis in the setting of HIV
iii. Lupus nephritis in the setting of HIV
iv. Membranous nephropathy in the setting of HIV
Indicate whether HBV positive, HCV positive, PLA2R positive (should not preclude workup for other secondary causes)
v. Membranoproliferative pattern glomerulonephritis in the setting of HIV
Indicate whether HCV positive (should not preclude workup for other secondary causes)
vi. Endocapillary proliferative and exudative glomerulonephritis in the setting of HIV
Post-streptococcal, staphylococcal-associated, other
vii. Fibrillary or immunotactoid glomerulonephritis in the setting of HIV
viii. Other immune-complex disease in the setting of HIV
II. Tubulointerstitial dominant[a]
a. Tubulointerstitial injury in the setting of classic HIVAN
i. Hyaline droplet tubulopathy
ii. Tubular microcysts
iii. Tubulointerstitial inflammation
b. Acute tubular injury/acute tubular necrosis
i. Ischemic
ii. Toxic (associated with cART vs. other)
c. Drug-induced tubulointerstitial nephritis (other than cART)
i. Antibiotics
ii. Proton pump inhibitors
iii. NSAIDs
iv. Other
d. Direct renal parenchymal infection by pathogens (bacterial, viral, fungal, protozoal, etc.)
e. Immunologic dysfunction-related tubulointerstitial inflammation
i. Diffuse infiltrative lymphocytosis syndrome (DILS)
ii. Immune restoration inflammatory syndrome (IRIS)
f. Other tubulointerstitial inflammation in the setting of HIV
III. Vascular dominant[a]
a. Thrombotic microangiopathy in the setting of HIV
b. Arteriosclerosis
IV. Other, in the setting of HIV infection
a. Diabetic nephropathy
b. Age-related nephrosclerosis

cART, antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; FSGS, focal segmental glomerulosclerosis; HIV, human immunodeficiency virus; HIVAN, HIV-associated nephropathy; NSAID, nonsteroidal anti-inflammatory drug

[a]Indicate the likelihood of HIV causality

[b] Indicate association with APOL1 risk allele genotype

Patient Selection for Transplantation for a HIV Positive Transplant

Preoperative Investigations

Most transplant centers require HIV-infected transplant candidates to maintain a CD4+ T-cell count of 200 or more cells/mm^3 and to be on a stable antiretroviral regimen. The CD4+ T cell requirements are decreased to 100 cells/mm^3 for liver transplant candidates, because patients with cirrhosis often have splenic sequestration secondary to splenomegaly and portal hypertension. In order to qualify for a kidney transplant, most centers require the patient to be on a stable antiretroviral regimen, and for the HIV viral load to be consistently less than 50 copies/ml, or at worst, if viral "blips" are occurring, that they are small and readily explainable by concurrent illness. Although undetectable HIV viral loads are preferable for liver transplant candidates, most centers will consider liver transplantation with a detectable viral load if the etiology of the liver disease is related to toxicity from the antiretroviral agents. Especially with more antiquated antiretroviral regimens, they will often have to be stopped because of an exacerbated progression of liver disease. In these cases, the HIV provider needs to review the antiretroviral history and make sure that the virus can be suppressed following the liver transplant. It should be noted that the minimal CD4+ T-cell requirements are based on the higher frequencies of opportunistic infections that were observed in prior eras, and with better prophylactic regimens now available, some centers are beginning to push the boundaries in terms of minimal CD4+ requirements for transplantation.

Someone with a previous history of opportunistic infections or HIV-associated neoplasms may be allowed to progress to transplant if the patient received appropriate acute and maintenance therapy and has no evidence of active disease. A list of these generally acceptable conditions are depicted in Table 2.

All potential recipients must be on a stable cART for 3 months prior to the transplant. For potential liver transplant recipients with hepatotoxicity secondary to cART, an antiretroviral regimen is not required prior to the transplant, provided that the HIV physicians can assure the availability of a suppressive antiretroviral regimen post-transplant. For recipients considering an HIV+ donor, viable alternative cART regimens should be discussed pre-transplant so as to best understand the implications of the viral resistance history of any donor.

Contraindications to Transplantation in the HIV Positive Recipient

Contraindications to transplantation in the HIV positive recipient are outlined in Table 3. Many centers will not treat HCV so that the potential recipient can receive a donor organ from an HCV positive recipient, and elect to treat the HCV post-transplant. With the current direct antivirals available to Hepatitis C positive patients,

Table 2 Opportunistic infections that may be allowed for HIV positive patients on the waiting list

Condition	Comment
Candidiasis of esophagus or vagina	Response to treatment with no recurrence in the last 6 months on cART
Encephalopathy (HIV related)	Diagnosed prior to cART and resolved on cART and no evidence of progression
Isosporiasis (chronic intestinal)	Good response to treatment without history of recurrence in the last 6 months on cART
Human Herpes Virus 8	Potential recipients with a history of Kaposi's sarcoma (KS) can be included, provided that the KS has resolved either with reconstitution of the immune system with antiretroviral therapy or treatment with TOR inhibitors. Based on successful treatment of KS with TOR inhibitors, KS is no longer considered a strict contraindication to transplantation
Mycobacterium tuberculosis	Active disease: Completed treatment with clinical radiological and microbiological response, with no recurrent in >6 months, on cART Latent disease: Ok to progress to transplant, provided INH prophylaxis initiated, ideally at least a month before hand Of note, in hyperendemic areas, such as South Africa, secondary LTBi prophylaxis is universally administered, out of concern for high reactivation
Nontuberculous mycobacteria	Completed treatment with clinical, radiological, and microbiological response, with no recurrence in the last 6 months on cART
Pneumocystis pneumonia	Completed treatment with clinical and radiological response. Must be on/restart cotrimoxazole prophylaxis
Nontyphoidal Salmonella septicemia	Completed treatment with clinical response and no recurrence in the last 6 months on cART
Toxoplasmosis (CNS)	Completed therapy and CT/MRI shows signs of resolution without active disease and is on secondary cotrimoxazole prophylaxis

it might be better to transplant the patient first with a hepatitis C positive donor organ, as the donor pool for this group of patients is bigger.

Donor Options

HIV Negative Donors

Most centers in the USA and South Africa accept HIV positive patients on the waiting list for HIV negative organs, as outcomes have been reported as similar to HIV negative recipients [2]. HIV positive recipients should have access to kidneys and livers from HIV negative living and deceased donors using the standard acceptance criteria. For HIV positive patients co-infected with hepatitis C (HCV), the use of HCV positive kidneys and livers are considered by some centers to expedite the time to transplant. However, with the availability of HIV positive organs for use in HIV positive patients, the demand for the HCV positive donor organs in HIV positive recipients has decreased. Experience with HIV

Table 3 Contraindications for transplantation

Any active opportunistic infection	
Any other current neoplasm *except* for:	Anogenital carcinoma in situ Adequately treated BCC or SCC of skin
A history of a solid tumor between 2 and 5 years ago	
Cytomegalovirus disease	Retinitis or drug resistant disseminated infection, where treatment options may be limited in the event of reactivation
Currently active deep fungal infection	Cryptococcus Histoplasmosis Candidiasis at a site other than the esophagus or vagina Sporotrichosis, Aspergillosis, Fusariosis Any other disseminated fungal infections
Disseminated herpes simplex virus infection	
Tuberculosis	Multi-drug resistant (MDR) or extensively drug resistant (XDR) tuberculosis
Progressive multifocal leukoencephalopathy, at any time	
Hepatitis B and C	Untreated hepatitis B co-infection would be a contraindication for most centers, outside of extreme circumstances Untreated hepatitis C should be treated, either ahead of transplant if viremic control was urgent, or post-transplant with direct acting antivirals. HIV/HCV co-infection is not a contraindication to SOT
Parasitic infections	Chronic intestinal cryptosporidiosis of >1 month duration

transplantation in thoracic organ transplant remains more limited although preliminary experience suggests with modern cART regimens survival mirrors HIV-negative counterparts [10].

HIV Positive Donors

The utilization of HIV positive donors in HIV positive recipients has had a marked effect on expediting the time to transplantation. The use of HIV positive donors, as well as the nearly simultaneous expanded safe use of HCV viremic donors, opened the door to utilize all increased infectious risk donors, for instance, donors who die of intravenous drug usage or have a history of high-risk sexual activities. Furthermore, Nucleic Acid Testing (NAT) as well as HIV antibody assays, both have small percentage of false positive tests results as depicted in Table 4. Recognizing some donors might falsely test positive for HIV, the allocation of these organs to HIV positive patients removes any concern that a recipient contract HIV through transplantation and makes utilizing further donors possible [11]. HIV positive donor organs had been utilized in South Africa for the last 10 years with good clinical results [7].

Based on the success in South Africa, the USA has initiated a similar strategy, and the safety and efficacy of using HIV positive donors is being studied in a NIH multicenter trial (Hope Act) [12]. This strategy has decreased waiting times for HIV infected kidney recipients from greater than 8 years to less than 1 year in parts of the

Table 4 False positive tests amongst different screening tests for HIV

Assay	Company	Sensitivity (%)	Specificity (%)	False positive rate (%)
HIV antibody assays:				
ABBOTT PRISM HIV O PLUS	Abbott	100	99.94	0.06
HIVAB HIV-1/HIV-2 (rDNA) EIA	Abbott	100	99.90	0.1
HIV NAT assays:				
COBAS Amplicreen HIV-1 test v 1.5	Roche	99.1	99.7	0.3
Procleix HIV-1/HCV	Gen-Probe	99.8	99.87	0.13
Procleix Ultrio	Gen-Probe	99.6	99.8	0.2

USA, provided the potential recipients have consented to receiving a HIV positive donor [8]. Early experience with HIV positive donors in HIV positive liver recipients is promising, and suggests the utilization of livers from HIV positive donors will also be a valuable source of donor organs for the HIV positive recipient.

Considerations When Utilizing a HIV Positive Donor

History of HIV Disease

A history of HIV disease (if known) should be obtained from the family member/durable power of attorney giving consent for the transplant. If possible, previous medical records should be obtained using the usual protocols of donor networks. Donors who have not been on previous combination antiretroviral therapy (cART) carry a lower risk of harboring a resistant HIV strain. In resource-rich countries, like the USA, where approximately 10% of the population carry transmitted virus, yet approximately 1 in 7 patients are unaware of their HIV status, and may only be diagnosed during their terminal predonation illness, then transmitted HIV resistance is possible even in the absence of donors taking antiretrovirals. Transplanting a recipient with a resistant viral strain is a major concern. South Africa, where HIV positive donors had been used since 2008, has a unique situation in view of the fact that there are very low ART resistance rates [13]. Most patients who failed second-line ART in South Africa have resistance rates of less than 5% [14]. This unique situation is an important consideration, making the scenario in South Africa for using HIV positive donors more favorable than in many other parts of the world. In the USA, most of the HIV donors are not naïve to antiretroviral therapy. In these cases, the donor networks try to get a detailed history of the ART usage of the donor, and get some sense as to the risk of transmitting a resistant strain. Fortunately, in the early experience of using HIV positive donors, the transmission of a resistant strain has not occurred [8].

Renal Impairment in the Donor

Similar to work-up for HIV negative donors, standard blood tests to assess kidney function should be performed. The propensity of HIV to coexist with other conditions – hypertension, smoking, or viral hepatitis, for example – can amplify subtle renal disease risk. Consequently, urine should be tested for protein, and if more than 2+ proteinuria is present on the dipstick, the kidney should probably not be used because of the risk of HIV nephropathy in the donor. A renal biopsy should be performed on all HIV+ donor kidneys, both to rule out HIV nephropathy but also to assess nephrosclerosis and to establish a baseline for comparison for future biopsy samples. In addition, it is useful to store blood from the donor for resistance testing for donor virus if necessary for later investigations.

Donor Screening for Other Infections

Screening for deceased HIV positive donors should include screening for strongyloidiasis in endemic areas, as well as latent Tuberculosis infection, sexually transmitted diseases like Syphilis, West Nile Virus, and Chagas parasitic disease.

For a more extensive discussion on transplant associated diseases listed here, refer to chapters ▶ Chaps. 18, "*Mycobacterium tuberculosis* in Transplantation," ▶ 40, "West Nile Virus and Transplantation," and ▶ 53, "Strongyloides: Emerging Challenges and Prevention."

Antiretroviral Therapy

The drug–drug interactions between anti-retrovirals and immunosuppressive agents are significant, and attention to these interactions is essential for maintaining sufficient exposure to immunosuppressive drugs, as well as sufficient antiviral activity. The most problematic drug–drug interactions result from the fact that the protease inhibitors utilized in many cART regimens inhibit the cytochrome p450 system, whereas the non-nucleoside inhibitors stimulate the same pathway. The cytochrome p450 system is also the system that metabolizes the calcineurin and TOR inhibitors used in almost all maintenance regimens, as well as many other drugs commonly used in transplant, such as statins, azoles, and neuropsychiatric medications. For that reason, it is very challenging to obtain adequate exposure to the commonly used agents used for maintenance immunosuppression. When feasible, integrase inhibitor (INSTi) based antiretroviral regimens should be preferred to regimens including protease and non-nucleoside reverse transcriptase inhibitors, since the integrase inhibitors do not impact the cytochrome p450 system [15]. Similarly, INSTi resistance in the HIV+ donor population is predicted to be very low, likely $<1\%$. In general, rejection rates in patients who are on PIs are reported to be higher than when on alternative ART, in part related to poor exposure to maintenance immunosuppression [16]. Sufficient exposure to appropriate levels of immunosuppressive agents is facilitated by avoiding the protease inhibitors, and it is for the reason that most US centers are now switching to integrase-based cART regimens for HIV positive recipients on waiting lists for solid organ transplantation. Of course, recipients with resistant strains of HIV

based may require regimens dependent on protease inhibitors, and in these cases close monitoring of drug levels is imperative.

Immunosuppression Choices

Despite the theoretical concerns about HIV+ recipients being functionally more immunocompromised than their negative counterparts, rejection rates following both liver and kidney transplantation in the HIV positive recipient have been unexpectedly high. Although part of the higher rejection rates is undoubtedly related to reduced exposure to immunosuppressive medications based on the drug–drug interactions, part of the higher rates may also be related to immune dysregulation. The reasons behind these higher than expected rates of rejection are beyond the scope of this chapter. With the reality of a twofold higher risk of rejection, many centers choose to give all their HIV positive kidney recipients aggressive induction immunosuppression with lymphodepleting agents, most commonly anti-thymocyte globulin (ATG). Of note, lymphodepleting agents are not recommended in HIV positive liver recipients based on adverse outcomes in the earlier experience, although contemporaneous data are lacking. Although the risk/benefit ratio of lymphocyte depleting regimens has been favorable in the kidney transplant recipients, caution should also be used [17]. Thymoglobulin use were associated with an increased kidney recipient mortality in selected kidney transplant recipients [2].

In some studies HIV-positive patients receiving sirolimus-based therapy had higher acute rejection rates when compared to patients receiving calcineurin inhibitor-based regimens [17]. TOR inhibitors possess anti-HIV properties both in vitro and in vivo. RAPA represses HIV-1 replication in vitro through different mechanisms including, but not limited, to down regulation of CCR5 [18].

CCR5 blockade (maraviroc) is also a logical choice for anti-HIV therapy as it not only blocks the entry port for the HIV virion into the lymphocyte, but also blocks the alloimmune response. Homozygotic carriers of an allele of the gene for the CC chemokine receptor 5 (CCR5) with a 32 bp deletion (CCR5Delta32) leads to an inactive receptor. During acute and chronic transplant rejection, ligands for CCR5 are upregulated, and the graft is infiltrated by CCR5-positive mononuclear cells. Therefore, patients who are homozygous for CCR5Delta32 have a lower risk for graft rejection and the literature reports improved outcomes in terms of rejection rates as well as graft survival compared to homozygous wild type or heterozygous CCR5Delta32 genotype [19, 20]. There is a current NIH trial studying the efficacy of CCR5 blockade in terms of blocking the immune response following kidney transplantation.

Rejection

Rejection remains an important clinical issue in HIV positive patients. The reason for high rejection rates in HIV positive patients is still unclear. As previously discussed, it has been speculated that the rejection rates are related to the drug

interactions affecting the cytochrome P450 enzyme resulting in poor exposure to immunosuppressive medications [21, 22]. In some studies, superior outcomes had been reported with integrase strand transfer inhibitor-based antiretroviral regimens. As these regimens have no interaction with calcineurin inhibitors, it should be preferred to protease and non-nucleoside reverse transcriptase inhibitors [15, 23].

However, a more complex immunological situation might also be responsible for high rejection rates in HIV positive patients. Chronic immune activation and inflammation is known to contribute to the risk of cardiovascular diseases in HIV positive patients [24]. The exposure to co-pathogens may result in heterologous immunity, leading to immunologic memory for HLA antigen in the absence of direct exposure. Furthermore, the loss of T helper cell function in response to antigens and the critical reduction in CD4+ T cell numbers might be responsible for a dysregulated immune response in HIV positive patients [25].

In studies that compared rejection rates between HIV positive and negative recipients, not only the rejection rate was increased, but also the time to first rejection episode was shorter [3]. One important consideration is the impact on the long-term renal function after a rejection episode, clearly visible when comparing the eGFR in patients who did not have rejection with those that did experience one or more episode of rejection in the USA multicenter study [3] (Fig. 1).

Recurrence of HIVAN

The incidence of recurrent HIV nephropathy following transplantation has been reported with variable frequencies. In the South African program, 51 patients received 150 protocol as well as "for cause" biopsies over a 10-year period [26].

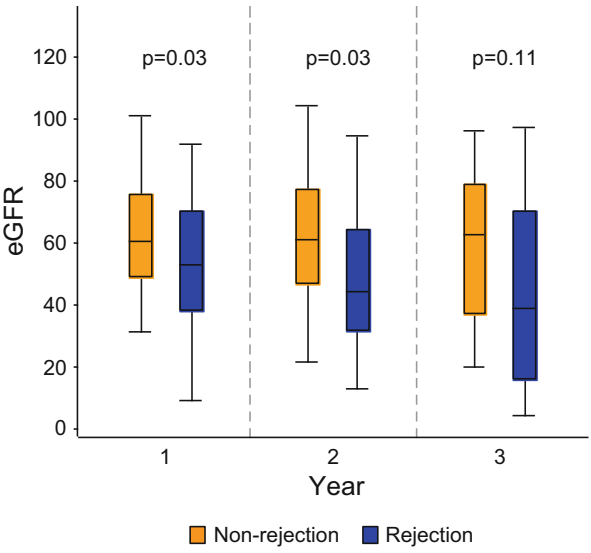


Fig. 1 Comparative eGFR in patients who did not experience rejection with those that had one or more rejection episode

Twelve patients had some features of HIVAN on a protocol biopsy. This included glomerular, interstitial, and tubular findings. In general, the collapsing form of focal segmental glomerular sclerosis (FSGS) is the most common glomerular lesion in HIVAN [27]. Figure 2 represents a histology sample of a South African transplant patient who was diagnosed with plasma cell infiltration, podocyte hypertrophy, as well as FSGS on a protocol biopsy 3 years post-transplant. Despite these changes in the kidney, the patient still had excellent graft function and no proteinuria.

The US NIH multicenter trial reported a 1–2% incidence of HIV nephropathy in the 175 subjects who underwent kidney transplantation. However, biopsies were only performed in this trial for deterioration in the renal function or the development of proteinuria. In this study, proteinuria was an extremely rare event [3]. Unlike the US trial, the French trial reported by Canaud et al. [28] found a much higher incidence of HIVAN in the 19 patients transplanted in this trial. Furthermore, if HIV was detected in the podocytes (5/19), patients had significant deterioration in renal function that often resulted in graft loss. The reasons for the vast disparities between the US and French trials remain unclear, and are the focus of ongoing studies.

Second Viral Strain

A significant concern in the utilization of HIV positive donors is the potential for superinfection with a more virulent or more drug resistant strain than the HIV positive recipient has on board. In a South African study, 24 HIV positive donor and recipient samples were evaluated for HIV drug-resistant mutations pre- and/or post-transplant [26]. Although drug-resistant mutations were found in six recipients, none of these mutations was donor derived. Although a donor virus inoculum was detected in the plasma of eight recipients (32%) at the earliest available time point post-transplant, a long-term sustainable superinfection was absent in all recipients. The ability to detect superinfection was limited by the inherently low proviral loads

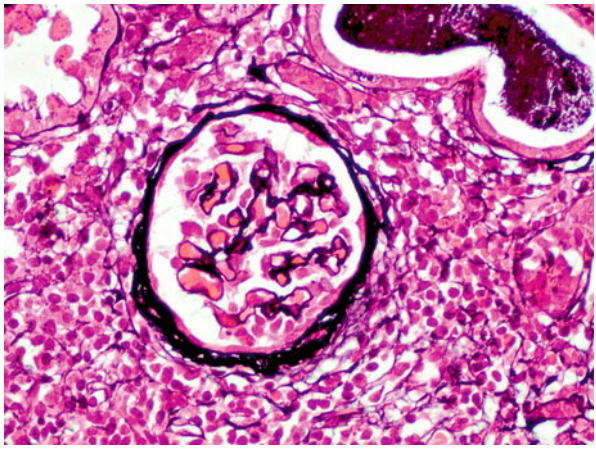


Fig. 2 Example of plasma cell infiltrate, podocyte hypertrophy, end-stage glomeruli, and focal segmental glomerulosclerosis. Despite these changes, patient has excellent graft function

found in ART-suppressed patients, as well as by the ATG-induced T-cell depletion post-transplant. However, superinfection could be detected down to a median of 1% of the viral population. A transient, subclinical superinfection immediately after transplantation suggested lingering viral inoculum and shedding of infected donor cells from the kidney into the recipient's blood. No increases in viral load or new viral resistance have been observed in any of these transplant patients. The favorable clinical outcomes, lack of transmitted drug resistance, as well as the lack of evidence for sustained donor-derived superinfection provides support for the use of HIV+ to HIV+ renal transplants as a treatment option for HIV-infected patients requiring renal-replacement therapy.

Opportunistic Infection

Even in patients with a pre-transplant history of opportunistic infections, recurrent opportunistic infections are not associated with decreased survival. In a large multicenter study of 125 liver and 175 kidney transplants, 55% of liver and 50% of kidney recipients experienced an opportunistic infection. However, these infections did not impact long-term outcomes. Most opportunistic infections occur early, and patients are at the highest risk in the first 6 months after the transplant. Bacterial infections are the most common infective occurrence after a transplant [2]. In some geographical regions, the incidence of tuberculosis and fungal infections seem to be higher in HIV positive recipients than in HIV negative recipients [29]. Prophylaxis strategies against opportunistic infections should provide HIV positive recipients with adequate protection from opportunistic infections, and should be designed with consideration of local donor and recipient epidemiology in mind.

HHV8-related disease, particularly KS, may be exacerbated or reactivated by post-transplant immunosuppression. Importantly, recurrence of effectively treated pre-transplant KS was not observed in the large US multicenter trial [2]. However, the same series reported three cases of de novo cutaneous Kaposi's sarcoma post-transplant, all successfully treated by conversion to TOR inhibitors for immunosuppression. TOR inhibitors down regulate VEGF, and effectively block these highly vascularized lesions from developing. Converting HIV positive recipients to maintenance immunotherapy that includes TOR inhibition has proven to be effective therapy for treating de novo KS in HIV positive transplant recipients. For this reason, KS is no longer considered a contraindication to transplantation, especially if cutaneous only, and especially if it appeared many years prior during phases of more substantial CD4 immunosuppression.

HPV-related cervical and anorectal disease, already accelerated in people with HIV infection, may be exacerbated by post-transplant immunosuppression. It has become apparent that close follow-up with anal and cervical PAP smears is particularly important in the HIV positive recipient, and early detection of atypical cells with aggressive treatment can prevent the need for a major surgical resection. Establishing patients into clinics with robust HPV anogenital screening programs may be beneficial pre-transplant, although they are less commonly available for male patients.

In pediatric patients who had a positive EBV donor, but is negative for EBV before the transplant, EBV viral load monitoring should be done to prevent an opportunistic EBV infection. This is generally not done in adults.

Prophylactic Therapy Against Common Infections

Prophylaxis against fungus, PCP, and CMV is similar to regimens used for HIV negative recipients, with the caveat that *Mycobacterium avium* Complex (MAC) prophylaxis is applied when CD4 counts drop below 75 cells/mm^3, something typically not done in routine solid organ transplantation. A summary of prophylactic therapy is presented in Table 5.

Table 5 Prophylactic therapy recommended for HIV positive recipients of solid organ transplants

Infection	Recommended prophylactic therapy
Pneumocystis pneumonia (PCP)	PCP prophylaxis with trimethoprim/sulfamethoxazole (TMP-SMX) should start immediately post-transplant and continued for 1 year at the discretion of the treating physician. An alternative would be dapsone. If G6PD negative atovaquone is recommended. Some centers use inhaled pentamidine monthly as an alternative approach
Toxoplasmosis	Trimethoprim/sulfamethoxazole (TMP-SMX) prophylaxis should be continued for 1 year at the discretion of the treating physician. An alternative would be Dapsone + Pyrimethamine + Leucovorin if allergic to TMP-SMX
Cytomegalovirus (CMV)	Prophylaxis should continue for 3–6 months after the transplant. Oral valgancyclovir is recommended for 3–6 months after transplant and should be considered again during the treatment of acute rejection for 1–3 months following completion of acute rejection therapy. Donor positive, recipient negative patients should continue prophylaxis for 6 months. Donor negative recipient negative can get Acyclovir as an alternative. Preemptive monitoring with CMV viral loads is less frequently used, but may be acceptable according to center-specific practice
Candidiasis	Center-specific approach, but most centers use prophylaxis with fluconazole 100 mg once a week or nystatin swish/swallow for the first 2–3 months after transplant
Tuberculosis	In endemic areas, all patients receive prophylaxis with INH for 6–12 months after transplant. This can be continued at the discretion of the treating physician
Mycobacterium avium	Azithromycin is initiated when the CD4 counts drop below 75 cells/mm^3 In the general public, if ART is going to be started in a patient with a low CD4 count, clinicians often choose to withhold MAC prophylaxis. In the light of this, a transplant patient with a CD4 count below 50, but with adequate virological control might not need MAC prophylactic therapy
Coccidioidomycosis	Fluconazole is used in endemic areas for 6 months after transplant

Vaccinations Prior to Transplant

Akin to solid organ transplant in HIV negative populations, care should be taken to protect patients before transplant, in line with vaccination recommendations for HIV positive adults. Pneumococcal Polysaccharide Vaccine (PPSV) and Pneumococcal Conjugate Vaccine (PCV) should be given to all patients on the waiting list pre-transplant. Hepatitis A and B immunity should be checked, and if a patient is not immunized, this vaccine should be given before the transplant when the immune response is more robust, and seroconversion more likely. Influenza vaccine should be given once yearly. Recombinant herpes zoster vaccination (Shingrix vaccine) is usually given pre-transplant in selected high-risk patient groups, especially patients over 50 years old. Emerging data suggests it is safe and effective post-transplant also, although not specific to the HIV-infected recipient [30]. Human papillomavirus (HPV) vaccine should be given in selected patients, usually under the age of 45 years. All patients have to be up to date with tetanus, diphtheria, pertussis (Tdap), as well as measles, mumps, and rubella (MMR) vaccinations before transplantation.

Vaccinations Post-transplant

Annual influenza vaccination is recommended post-transplant in all patients. It is usually not recommended to use any live virus vaccine post-transplant. Therefore MMR should not be given post-transplant. It is recommended to wait 3–6 months after transplant before giving vaccines, appreciating theoretical concerns around allostimulation being triggered toward the graft. When a COVID-19 vaccine becomes available, this should be given as well. If a patient received treatment for rejection, vaccination should be avoided for 6 months after this.

Conclusion

Transplantation should be offered to patients living with HIV. Most experience exists in kidney transplant**;** however, liver, heart, lungs, and pancreas may also be safely used. Higher rates of rejection have been observed following liver and kidney transplants in the HIV positive recipient. The drug–drug interactions between immunosuppressive agents and some of the antiretroviral agents have resulted in reduced exposure to the immunosuppressive agents. These interactions can be minimized by switching potential recipients to integrase inhibitor-based regimens before transplant if deemed safe by an HIV provider. Further studies are necessary to determine the additional contribution of the dysregulated immune system to the high rejection rates that have been observed in the HIV positive recipient.

Recurrence of HIV nephropathy was not problematic in the US series, but reports of recurrent disease in France have prompted a re-examination of tissue from biopsies from the US multicenter NIH and Cape Town series. Progression of HIV to AIDS has not been an issue, provided patients remained on a stable antiretroviral

regimen. Prophylaxis against opportunistic infections during the early post-transplant period is essential. Oncogenic viruses mediating cancer development (e.g., Kaposi sarcoma, lymphoma) remain problematic, and HIV positive transplant recipients should undergo screening cervical and anal PAP smears regularly after transplantation. Some anti-retroviral regimens (CCR5 blockade) block the immune response, and some immunosuppressive agents (TOR inhibitors) have an antiretroviral effect and decrease the HIV viral reservoir. Both of these serendipitous findings are being studied in prospective trials in the USA.

Key Points

- HIV positive patients have graft and survival outcome results compatible with HIV negative patients after transplantation.
- Using HIV positive donors have increased the amount of available organs to the HIV positive patient group.
- Rejection rates in HIV positive patients remain higher than in HIV negative patients. Reasons for this include drug interactions as well as inherent immunological differences between HIV positive and negative patients.

References

1. Roll GR, et al. Long-term follow-up of beta cell replacement therapy in 10 HIV-infected patients with renal failure secondary to type 1 diabetes mellitus. Am J Transplant. 2020;20:2091.
2. Roland ME, et al. Survival in HIV-positive transplant recipients compared with transplant candidates and with HIV-negative controls. AIDS. 2016;30(3):435–44.
3. Stock PG, et al. Outcomes of kidney transplantation in HIV-infected recipients. N Engl J Med. 2010;363(21):2004–14.
4. Campos-Varela I, et al. Temporal trends and outcomes in liver transplantation for recipients with human immunodeficiency virus infection in Europe and United States. Transplantation. 2019. https://doi.org/10.1097/TP.0000000000003107.
5. Castells L, et al. Pegylated interferon plus ribavirin in HIV-infected patients with recurrent hepatitis C after liver transplantation: a prospective cohort study. J Hepatol. 2015;62(1):92–100.
6. Tourret J, et al. Access to the waiting list and to kidney transplantation for people living with HIV: a national registry study. Am J Transplant. 2019;19(12):3345–55.
7. Muller E, et al. HIV-positive-to-HIV-positive kidney transplantation – results at 3 to 5 years. N Engl J Med. 2015;372(7):613–20.
8. Durand CM, Segev D, Sugarman J. Realizing HOPE: the ethics of organ transplantation from HIV-positive donors. Ann Intern Med. 2016;165(2):138–42.
9. Chamroonkul N, Bansal MB. HIV and the liver. Nat Rev Gastroenterol Hepatol. 2019;16(1):1–2.
10. Koval CE, Farr M, Krisl J, et al. Heart or lung transplant outcomes in HIV-infected recipients. J Heart Lung Transplant. 2019;38(12):1296–305.
11. Durand CM, et al. Clarifying the HOPE Act landscape: the challenge of donors with false-positive HIV results. Am J Transplant. 2020;20(2):617–9.
12. Boyarsky BJ, et al. The future of HIV Organ Policy Equity Act is now: the state of HIV+ to HIV+ kidney transplantation in the United States. Curr Opin Organ Transplant. 2019;24(4):434–40.

13. Muller E, Barday Z. HIV-positive kidney donor selection for HIV-positive transplant recipients. J Am Soc Nephrol. 2018;29:1090.
14. Pillay V, et al. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. Antivir Ther. 2008;13(Suppl 2):101–7.
15. Spagnuolo V, Uberti-Foppa C, Castagna A. Pharmacotherapeutic management of HIV in transplant patients. Expert Opin Pharmacother. 2019;20(10):1235–1250. https://doi.org/10.1080/14656566.
16. Rollins B, et al. Higher rates of rejection in HIV-infected kidney transplant recipients on ritonavir-boosted protease inhibitors: 3-year follow-up study. Clin Transplant. 2019;33(6): e13534.
17. Locke JE, et al. Immunosuppression regimen and the risk of acute rejection in HIV-infected kidney transplant recipients. Transplantation. 2014;97(4):446–50.
18. Dufty NE, et al. Pharmacokinetic interaction of maraviroc with tacrolimus in a patient coinfected with HIV and hepatitis B virus following hepatic transplant due to hepatocellular carcinoma. J Antimicrob Chemother. 2013;68(4):972–4.
19. Reshef R, et al. Blockade of lymphocyte chemotaxis in visceral graft-versus-host disease. N Engl J Med. 2012;367(2):135–45.
20. Fischereder M, et al. CC chemokine receptor 5 and renal-transplant survival. Lancet. 2001;357 (9270):1758–61.
21. Flentge CA, et al. Synthesis and evaluation of inhibitors of cytochrome P450 3A (CYP3A) for pharmacokinetic enhancement of drugs. Bioorg Med Chem Lett. 2009;19(18):5444–8.
22. Frassetto L, et al. Changes in clearance, volume and bioavailability of immunuosuppressants when given with HAART in HIV-1 infected liver and kidney transplant recipients. Biopharm Drug Dispos. 2013;34:442.
23. Rosa R, et al. Impact of antiretroviral therapy on clinical outcomes in HIV (+) kidney transplant recipients: review of 58 cases. F1000Res. 2016;5:2893.
24. Tingstedt JL, et al. The impact of concurrent HIV and type II diabetes on immune maturation, immune regulation and immune activation. APMIS. 2019;127:529.
25. Hearps AC, et al. HIV infection and aging of the innate immune system. Sex Health. 2011;8(4): 453–64.
26. Selhorst P, et al. Longer-term outcomes of HIV-positive-to-HIV-positive renal transplantation. N Engl J Med. 2019;381(14):1387–9.
27. Wearne N, Okpechi IG. HIV-associated renal disease – an overview. Clin Nephrol. 2016;86(13): 41–7.
28. Canaud G, et al. The kidney as a reservoir for HIV-1 after renal transplantation. J Am Soc Nephrol. 2014;25(2):407–19.
29. Miro JM, et al. Infections in solid organ transplant HIV-infected patients. Clin Microbiol Infect. 2014;20(Suppl 7):119–30.
30. Vink P, et al. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in chronically immunosuppressed adults following renal transplant: a phase 3, randomized clinical trial. Clin Infect Dis. 2020;70(2):181–90. https://doi.org/10.1093/cid/ciz177.

Hepatitis A Infections in Transplant

36

Rebekah J. Lane

Contents

Introduction 877
Epidemiology 878
Structure 880
Transmission 880
Diagnosis 882
Clinical Presentation 883
Transplant for HAV 886
Prevention 887
Food Precautions 887
Immunization 888
Conclusion 897
Key Points 897
References 899

Introduction

Though the majority of deaths related to viral hepatitis are attributable to hepatitis B and hepatitis C, hepatitis A (HAV) continues to contribute to a large burden of morbidity. Tens of millions come in contact with HAV annually [1]. In the past it has been one of the most frequently notified vaccine preventable diseases in the USA [2]. In 2016, the Centers for Disease Control and Prevention (CDC) was notified of 2007 cases of acute HAV in the USA, likely to represent only a portion of new cases, with actual estimates likely to be over twice the number of reported cases in any year. In that same year, over 70 death certificates listed HAV as the cause of death [3]. Despite this, most recent publications regarding viral hepatitis focus on hepatitis

R. J. Lane (✉)
Departments of General Medicine and Infectious Disease, Auckland City Hospital, Auckland, New Zealand
e-mail: rebekahl@adhb.govt.nz

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_35

B (HBV) and hepatitis C (HCV) and those coinfected with human immunodeficiency virus (HIV), as endorsed by World Health Organization member states and Sustainable Development Goals [4].

In areas of higher endemicity, improving health and hygiene (environment, water supply, and food sanitation) led to a decline in infection in children, resulting in a population of more susceptible teenagers, young, and even older adults at risk of contracting disease [5–7].

This vulnerable population is also increasingly receiving immunosuppressive therapies for various reasons, including solid organ and hematological transplants, making them prone to infections. This, when combined with improved post-transplant morbidity, mortality, quality of life, and increasingly available travel to countries further afield, is a risk for HAV infection. Indeed, immunocompromised travelers are tending to travel to similar destinations and for durations comparable to immunocompetent travelers, highlighting the importance of pretravel care and education to this group [8–11].

While studies on HAV and vaccination have been performed in the immunocompromised host, most data is from those who are healthy. Studies are small, heterogeneous, and variable in results. More robust data from those who are undergoing, or have received a transplant, is needed.

Epidemiology

In countries of high endemicity, seroprevalence rapidly rises in young children, who often have asymptomatic disease [6]. This leads to a difficulty in disease surveillance. As the incidence of HAV decreases in a country, the average age of infection increases [1]. Therefore, as symptomatic disease is more common with older age, there is an increase in case detection despite a decreased overall incidence [12].

Around the world, the prevalence of HAV infection varies and is related to income. Lower-income countries such as Africa and South Asia have higher seroprevalence, while higher-income countries, such as Western Europe, Australia, New Zealand, Canada, the USA, Japan, Korea, and Singapore, have lower rates [6].

Disease continues to occur in the areas of lower endemicity as a result of outbreaks and high-risk groups. Globalization of trade and travel remain important drivers of epidemiological change in HAV infection. Travel to an area of high or intermediate endemicity remains a significant risk for HAV infection. Case reporting from 13 European countries from 2009 to 2015 showed that 28% of cases were associated with travel, and of these 40% were in children less than 15 years of age [13]. The risk of infection is approximately 300 per 100,000 travelers per month in tourist areas in developing countries, and it is five to seven times higher for those traveling off the usual tourist routes [14].

In addition to travel-related risk, other factors for acquiring HAV include close contact with someone known to have the infection, men who have sex with men, and practices associated with intravenous drug use. Exposure to contaminated food and water can lead to common-source outbreaks (See Table 1). Occupational risk

Table 1 Categories of persons with increased risk for hepatitis A virus (HAV) infection or increased risk for complications in the event of exposure to HAV

Type of risk	Risk category	Examples
Increased risk for HAV infection	Close contacts of persons with HAV infection[a]	Household contacts Caretakers Sexual contacts Persons working with nonhuman primates Persons working HAV in a research laboratory
Increased risk for HAV-associated complications	Immunocompromised persons	Congenital or acquired immunodeficiency HIV infection Chronic renal failure/undergoing dialysis Solid organ, bone marrow, or stem cell transplant recipients Persons with diseases requiring treatment with immunosuppressive drugs/biologics (e.g., tumor necrosis alpha inhibitors), long-term systemic steroids, radiation therapy
	Chronic liver disease	Hepatitis B infection Hepatitis C infection Cirrhosis (any etiology) Fatty liver disease (hepatic steatosis) Alcoholic liver disease Autoimmune hepatitis Alanine aminotransferase (ALT) or aspartate amino transferase (AST) level more than twice the upper limit of normal or persistently elevated for 6 months

From Nelson et al. [15]
Abbreviation: HIV = human immunodeficiency virus
[a]Excludes health care personnel using appropriate personal protective equipment

includes those working in research laboratories studying HAV, including those using nonhuman primates [15]. Despite this, the majority of cases have no known or identifiable risk [16].

Sources of foodborne infection and outbreaks include contaminated produce such as fresh fruits and vegetables and shellfish or contamination by food-handlers, at the point of sale, restaurants or social events. Distinguishing a foodborne outbreak can be difficult due to the long incubation time and variability in clinical presentation and location. Some outbreaks have been linked to local and imported foods such as sundried tomatoes, pomegranate arils, frozen strawberries, iceberg lettuce, green onions, sandwiches, bakery goods, oysters, and clams [17–25].

Unfortunately, there is no data on the national rates of HAV infection in transplanted or immunocompromised hosts, particularly in those who travel or may be at risk for other reasons. Reported data is limited to case reports. Despite incremental changes in HAV vaccination recommendations 1996–2006 by the Advisory Committee on Immunization Practices (ACIP) to increase coverage for children and persons at high risk for HAV infection, since early 2017, the CDC has observed an

increase in the number of community-wide HAV outbreaks in multiple states [26] indicating ongoing risk for in the pre- and posttransplant setting but also for potential donor-derived infection.

Structure

Hepatitis A is a 27-nm, single-stranded, non-enveloped RNA virus that is the only member of the genus *Hepatovirus* in the family *Picornaviridae* [27]. Of the seven known genotypes, types I and III are most commonly involved in human infection [27]. Despite genetic heterogeneity of nucleotide sequences, there is only one known serotype [28].

Like other members of the *Picornaviridae* family, the positive sense, linear virus of 7478 nucleotides has a long 5′ untranslated region (UTR) lacking a cap structure, with an additional small covalently bound protein termed VPg.

A polyprotein coding region of 2227 amino acid residues (P1 region) codes for the primary structural proteins in the virion, consisting of, from the N terminus, 3 major viral capsid proteins – VP0, VP3, and VP1-2A (Px). This section of the RNA is followed by a region that translates into nonstructural proteins in the open reading frame (ORF) and finally a short 3′ UTR with a virus-coded poly(A) tail [29, 30]. These nonstructural proteins, P2 and P3, are thought to be involved in RNA synthesis and are termed replicative proteins, with the latter containing a viral protease, 3C, which cleaves the capsid proteins from the precursor polyprotein [27].

The capsid proteins VP0, VP3, and VP1-2A form monomers that combine into pentamers. A provirion is formed when 12 pentamers associate with viral RNA. VP4 is derived from cleavage of VP0 and is essential for capsid maturation from provirion to virion, but is not found in the mature virion [31] (see Fig. 1).

Transmission

Transmission of HAV is predominantly via fecal-oral spread. Presumed enterohepatic circulation after ingestion of virions leads to replication in the hepatocytes with secretion of infectious virions into the bile and subsequently stool. Replication is thought to be limited to hepatocytes and epithelial cells of the gastrointestinal tract, but cell culture has been performed in other cell lines in a laboratory setting [27]. Release of a quasi-enveloped virions [32] from the basolateral surface of hepatocytes leads to contact with dendritic cells, eliciting type I interferon(IFN) release and adaptive cellular responses [33].

Transmission has also been documented via exposure to infectious blood and tissue. Because the presentation of clinical signs and development of IgM lag behind viremia in HAV, donor-transmitted infection may not be detected by routine screening tests. There are documented transfusion-transmitted HAV infections with red

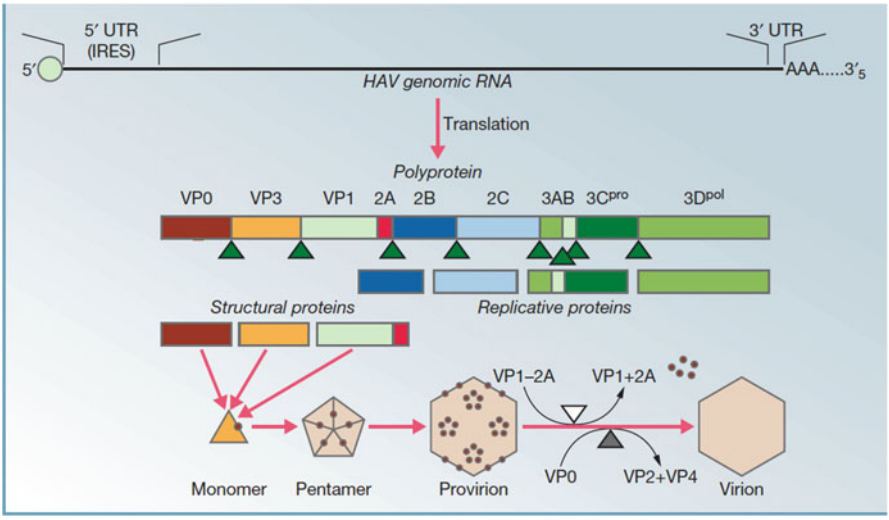


Fig. 1 Organization of the RNA genome of hepatitis A virus (HAV), polyprotein cleavage, and viral assembly. The 7.5-kb positive-strand RNA is covalently attached to VPg (5′ end) and has a poly(A) tail. The 5′ untranslated region (UTR) of 734 nucleotides functions as an internal ribosome entry site (IRES) to initiate translation (*vertical arrow*) of the precursor polyprotein of 2227 amino acids. The single viral protease, $3C^{pro}$, cleaves itself from the polyprotein and subsequently cleaves elsewhere in the polyprotein to yield the structural protein precursors VP0, VP3, and VP1–2A (PX) and replicative proteins 2B, 2 C, 3A, 3B (VPg), and 3D (RNA-dependent RNA polymerase). VP0, VP3, and VP1–2A probably remain associated as a monomer and then form pentamers that are a stable precursor in capsid formation. Assembly of 12 pentamers together with RNA forms the provirion, after which 2A is susceptible to cleavage by host cell protease(s) *(open arrowhead)*. The final maturation cleavage of VP0 to VP2 and VP4 *(solid arrowhead)* is dependent on the encapsidated viral RNA. (Figure 176-3 from Averhoff et al. [179])

blood cells [34, 35], platelets [35], fresh frozen plasma [36], and blood factor donations [37]. HAV has also been transmitted from a whole blood donation and platelets despite the presence of HAV-IgG in the recipient [38, 39]. This highlights the importance of education in donors to ensure notification of later development of clinical symptoms following blood product donation, to allow tracing of recipients potentially exposed.

Various techniques have been utilized to reduce the infectivity of HAV in blood and factor products, particularly factor IX. These include precipitation, diethylaminoethyl cellulose (DEAE-C) absorption and elution, heparin affinity chromatography, and nanofiltration [40]. The virucidal method of solvent detergent fails to effectively inactivate non-enveloped viruses [41] and was the reason for transmission of disease in hemophiliacs [42, 43].

Donor-derived HAV infection has also occurred in both solid organ and stem cell transplantation. There has been one documented case of HAV infection in a 7-year-old recipient of a combined liver-small intestine-pancreas transplant [44]. Interestingly, the recipient was documented to be seropositive prior to transplantation from

prior vaccination. Serum and fecal HAV-RNA was detected at 10 months post-transplantation, but earlier admissions showing elevated ALT results and concerns for acute rejection indicated a likely protracted course of disease. Donor-transmitted infection was confirmed by genetically identical HAV-RNA sequences. The recipients of the donor heart and kidneys were also seropositive prior to transplantation but did not develop infection.

Despite best efforts to minimize transmission, a stem cell transplantation (HSCT) has also been shown to result in HAV infection in the recipient [45]. Due to limited options, an HLA-matched allogeneic HSCT proceeded, despite the donor presenting with acute HAV infection, confirmed by the presence of HAV-RNA. The transplant was delayed for 4 days while the donor biochemical liver tests improved, and the recipient received high-dose intravenous immunoglobulin (IVIG) which was continued on a weekly basis for 2 months posttransplant. Despite the recipient having a transient increase in bilirubin and ALT in the first 2 weeks posttransplant, attributed to the conditioning regime, there were never any signs of symptomatic hepatitis. Detectable HAV virus was found in stools, but not blood, as measured by reverse transcriptase polymerase chain reaction (RT-PCR) at day 32 and was correlated with a transient increase in ALT day 35 to 55. From 15 to 90 days posttransplant, there was detectable HAV-IgG and IgM, of which the latter could not be attributed to IVIG administration. It was postulated that the IgM may have been produced by donor B lymphocytes, therefore suggesting that the transplanted immune system may retain the ability to produce antibodies despite immunosuppression in the early post-transplant period.

Diagnosis

The diagnosis of HAV is made by the detection of infection, by either the presence of HAV-IgM (indicative of recent exposure) or the presence of HAV-RNA. It is not possible to distinguish HAV infection from other types of viral hepatitis on the basis of clinical and epidemiological features alone and therefore requires confirmatory laboratory testing.

Historically, direct examination of the virus occurred through electron microscopy, a difficult and somewhat insensitive technique. Currently, HAV viral antigen may be detected in stools using enzyme-linked immunosorbent assays (ELISA). Detection of antigen in blood using immunoassays is difficult due to fibronectin binding and masking of antigenic determinants [46].

Serological testing remains the mainstay of diagnosis and is very sensitive in the context of clinical cases of acute hepatitis or those recently exposed. Both HAV-IgM and HAV-IgG against capsid proteins are typically present at the onset of symptoms which occurs after a 4-week incubation. IgM, the primary marker for acute infection, typically wanes about 4–6 months postexposure [47]. Numerous commercially available tests rely on either manual or automated enzyme immunoassay platform result. In the absence of a positive IgM result, HAV-IgG corresponds with past infection or seroconversion following vaccination. Some commercially available

assays do not distinguish between IgG and IgM and rely on total HAV antibody levels. A positive result in this circumstance may represent current or prior infection or an immunization response [48].

The arbitrary cutoff for seroconversion or development of immunity has been determined to be HAV-IgG levels of ≥20 IU/mL as determined by ELISA. More recent studies have used a higher cutoff of ≥33 IU/mL, thought to be similar to peak titers of antibodies achieved with a passive immunization strategy utilizing immune globulin (IG) [49, 50]. However, there may be some differences in the affinity of the antibodies between passive and active strategies [51] which may result in differences in resultant immune response. Immunogenicity is measured by the elicited seroconversion rate and geometric mean concentrations (GMC), representing the mean antibody concentration of the group being studied. GMC of antibody to HAV antigen can measured at intervals postvaccine administration and are typically utilized in a research setting [52]. The lower limit of antibody requirement to prevent disease is unknown [53].

Nucleic acid detection is most widely performed by reverse transcriptase polymerase chain reaction (RT-PCR) and may be detectable in blood prior to the presence of HAV-IgM in the so-called window period. It is the most sensitive detection technique and can be both qualitative and quantitative. Nucleic acid detection by other techniques have been utilized, such as restriction fragment length polymorphisms, single-strand conformational polymorphism, southern blotting, nucleic acid sequencing-based amplification, and hybridization and antigen capture RT-PCR [48]. RT-PCR can also detect species-specific viral strains implicated in parenteral transmission of virus [54].

Multiplex RT-PCR platforms provide efficient ways to detect multiple potential causative viral organisms with common simultaneous detection of HAV, hepatitis E (HEV), rotavirus, and poliovirus [48].

Liver biopsies performed in the context of acute HAV show the cardinal features of hepatocellular degeneration, characterized as either ballooning or acidophilic (apoptotic) change, with various degrees of portal and lobular inflammation and hepatocyte regeneration [27].

To date, there are no studies that determine the sensitivity and specificity of any of these diagnostic modalities in the posttransplant setting.

Clinical Presentation

In healthy hosts, typically less than 10–15% of children under the age of 6 years are symptomatic with infection [55, 56], while in adults clinical disease is more common, with symptoms present in over 75% and jaundice in 40–70% [57, 58]. In the immunocompetent, infection is generally self-limiting and does not result in chronic liver disease. As there is only one known serotype, immunity is lifelong following initial infection [28].

Those with symptomatic infection may experience jaundice, nausea, vomiting, diarrhea, anorexia, or abdominal pain accompanied by elevation of the

aminotransferase liver enzymes. Non-hepatic complications are uncommon but include nephrotic syndrome, glomerulonephritis, vasculitis, pancreatitis, Guillain-Barre syndrome, thrombocytopenia, and aplastic anemia [59]. Evanescent rashes, transient arthralgias, and rare cryoglobulinemia complications (cutaneous vasculitis and arthritis) have been documented [60]. Extrahepatic manifestations were seen in around 8% of cases in a US outbreak [61]. Complications more typically occur in those who are older [57] and comorbid, particularly those with chronic liver disease [61–63].

Although the virions replicate in hepatocytes, liver damage is thought to be a consequence of host cytotoxic-T cell response, rather than direct virion cytotoxicity [64, 65]. There have been contrasting studies supporting or refuting the fact that liver damage is related to the degree of viremia [66, 67]. The ambiguity of abnormal liver tests can complicate the diagnosis in the posttransplant setting, as there is often a myriad of reasons for liver abnormalities including organ rejection, graft versus host disease, sinusoidal obstruction syndrome, hepatotoxicity from drugs, and other infections. Despite immunosuppression of T cell responses, there is no current evidence to support reduced severity of liver damage in transplant recipients.

The incubation period from exposure until development of symptomatic infection is thought to be around 28 days (range 15–50 days) [68]. In experimentally infected healthy male subjects, the average duration of viremia prior to peak ALT as determined by PCR was 17 days. Viremia persisted on average for 79 days after this with an average total duration of 95 days [69]. In those with clinical infection, viremia as determined by real-time RT-PCR was found to persist for at least 60 days, with viral load declining with time [70]. Some studies have suggested that peak viremia corresponds with the peak biochemical abnormalities, although this does not necessarily correspond with peak HAV-IgM or IgG [71]. In patients with acute HAV, viral shedding in the stool continues for a median period of 81 days (range 57 to 127) after disease onset [66]. Viral loads are highest in the stool prior to the onset of symptoms [72] (see Fig. 2).

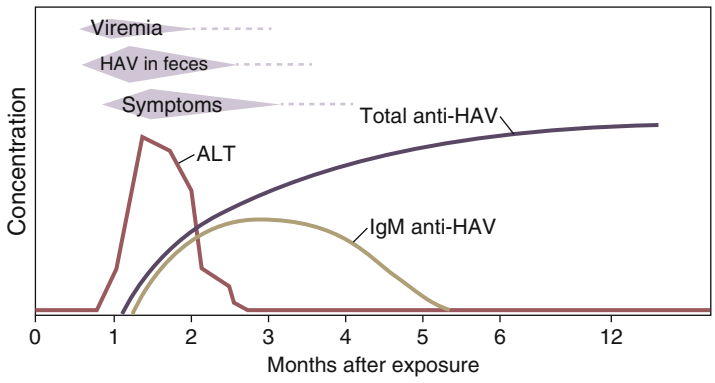


Fig. 2 Clinical, virological, and serological events after hepatitis A virus (HAV) infection. *ALT* alanine aminotransferase, *Ig* immunoglobulin. (Figure 176-5 from Averhoff et al. [179])

It is unclear if those who are immunosuppressed in the transplant setting are viremic, or infectious, for longer durations than immunocompetent hosts. Certainly, those with HIV infection are viremic for a longer period, persisting beyond biochemical and clinical resolution of hepatitis [73]. Children are also known to shed virus for longer periods than adults, at time for periods up to 10 weeks [74]. An infant infected in a nosocomial setting was found to continue to shed virus up to 6 months postinfection [34]. In the transplant setting, there have been two case reports of persistent elevation of HAV-IgM for over 30 months posttransplant and persistent HAV-RNA detection in the stool. Unusually, in both described cases, chronic hepatitis was seen on liver biopsy in the absence of any other hepatitic viral etiology [75, 76]. The lack of cell-mediated immunity, which is important in viral clearance, may contribute to persistence in these scenarios.

A small proportion of patients may develop a relapsing or biphasic course. This biphasic response has been described in infants and the elderly [77, 78]. Typically, these patients initially improve both clinically and biochemically before having a relapse, either with or without symptoms, within 6 months of the initial infection. Incidence of biphasic response varies in reported literature from 3.3% to 20% [79]. Up to 25% of children admitted to hospital may have relapsing disease [80].

Several studies have found that there is a higher case fatality rate in those with underlying chronic liver disease. In a Shanghai outbreak in 1988 of over 310,000 cases, several US outbreaks, and smaller case series, HAV infection in those with chronic HBV was associated with higher peak laboratory abnormalities, more severe disease including fulminant failure, and a higher case fatality rate [81–84]. Fulminant disease is defined by the sudden onset of hepatic encephalopathy of any grade, coagulopathy with international normalized ratio (INR) $\geq$1.5, and jaundice with bilirubin $>$50 μmol/L [38].

One series prospectively followed patients with chronic hepatitis and found that HAV superinfection resulted in increased risk of fulminant disease in patients with pre-existing HCV, compared to those with HBV [63]. Potential reasons for this include coinfection with hepatitis G virus, autoantibodies, or HLA phenotypes.

While increasing age and hepatitis status are associated with worse disease, there is no clear evidence that this is true for immunocompromised hosts [85]. However, in a 2013 outbreak in the USA, a pre-existing chronic medical condition (including pharmacological immunosuppression) was associated with an increased risk of hospitalization [18].

Management of cases is supportive, with a significant role in prevention or spread in the local community. Some early studies have looked at the possibility of the direct acting antiviral (DAA) sofosbuvir being used to prevent relapsing disease posttransplant, but this needs further assessment in both animal and human models [86].

Overall quoted mortality rates vary with the population studied. Historical data from the US states the rate of death associated with HAV is 0.1–0.6% [87, 88]. The most recent data published by the CDC states mortality increases with age, and in those under 40, it is $<$0.01 per 100,000 people, increasing to 0.08 in those over 75 years of age [16]. In a review of the Shanghai outbreak, which predominantly

involved children and young adults, the death rate was 0.015% [89]. In those admitted to hospital in US outbreak in Tennessee, serious illness and death were more common in those over 40 years of age, with a death rate of 5.7%, though the young were not without complicated illness with a mortality rate of 1% [61].

Transplant for HAV

In an attempt to determine risk factors for deterioration and need for referral for transplantation in a pediatric cohort, a single center in France reviewed 24 children admitted with liver failure associated with HAV over the 15-year period from 1980 to 1995. Of these, 11 children either died or had transplantation. Of the nine children who received transplant, two died on the postoperative day with complications of grade 4 encephalopathy, cerebral edema, and subsequent irreversible brain damage. The best predictor of a poor outcome in those with encephalopathy was a prothrombin time 21% below normal and a serum bilirubin >400 μmol/L [80]. Two further cases died within 2 years of transplant with complications of sepsis and chronic rejection.

Prognostic tools can be used to identify the risk of patients requiring consideration for transplantation. The ALFA score is a risk prediction model for death or transplantation associated with HAV-related acute liver failure. It was developed based on the study of a Korean cohort of 294 patients and validated on further patients from Japan, India, and the UK. It utilizes age, INR, bilirubin, ammonia, creatinine, and hemoglobin levels on the day of diagnosis of acute liver failure [90]. The US Acute Liver Failure (US ALF) Study Group reviewed 29 patients enrolled with HAV, a total of 5.6% of total registered. Of those, 55% spontaneously recovered, 31% underwent transplantation, and 14% died. Based on this data, a prognostic index specific for HAV, the HAV-ALFSG, was determined to predict death or transplantation based on four factors on the day that acute fulminant hepatitis is determined – creatinine ≥2.0 mg/dl, ALT ≤2600 IU/L, intubation status, and need for pressor support [91]. Other ALF risk prediction models used include the Kings College Criteria (KCC) for non-paracetamol-related acute liver failure, new ALFSG index, Model for End stage Liver Disease (MELD), and the MELD including sodium (MELD-Na) [92–94]. However, these are not specific for HAV.

Outcomes of transplant for acute liver failure are generally poorer compared with those for chronic liver disease [95, 96]. According to the US ALF Group Registry, HAV-related acute liver failure represents around 3% of cases [97]. Rates of spontaneous recovery range from 25 to 70% [91, 92, 98, 99]. It is suggested that outcomes in those who receive liver transplants for acute liver failure associated with HAV are worse than for those who receive them for HBV. In a single-center review from Korea, 5.6% of patients received liver transplants for acute liver failure. Of these, 14.2% were for HAV, while 16.7% were for HBV [32]. Graft survival was 65.5% for HAV at both 1 and 5 years posttransplant, compared to 88% and 84% for HBV (1 and 5 years posttransplant, respectively). Multivariate analysis showed that

acute pancreatitis and relapse of HAV were independent risk factors for graft failure [100].

Extrahepatic virions, presumed enteric, are suspected to be involved in recurrent HAV infection after liver transplantation. Both protracted and relapsing courses have been identified with detection of either HAV antigens, HAV-RNA, or HAV-specific genomic sequences in graft, serum, or stools, with variable clinical presentation [101–103]. One case even resulted in re-transplantation [104]. Subclinical presentations may suggest recurrent HAV infection following liver transplant is an under-recognized phenomenon, especially when liver test abnormalities are common posttransplant and have a wide variety of causes. The secondary effect of post-transplant immune suppression in limiting the host response to viral infection warrants further investigation.

Prevention

The major goal after both solid organ and stem cell transplants is the resumption of a normal and healthy life, as far as this is possible. Therefore, prevention of any infection, particularly during ongoing maintenance immunosuppression or an immature immune system, is critical. Overall, this involves planning, preparation, and education which commences as early as possible prior to transplantation. The backbone of prevention remains immunization.

In some circumstances immunization must be administered posttransplant, such as emergency solid organ transplant for acute organ failure, or HSCT, which involves the engraftment of a naïve immune system, potentially lacking humoral and cellular immunity. Unexpected travel requirements, or exposure to HAV in an outbreak setting, may also involve unanticipated active immunization or passive immune globulin to prevent disease in the posttransplant setting.

Many regions with active transplant programs tend to have low endemicity of HAV, and later-life infections are usually as a result of outbreaks. In this context, while immunization is the mainstay of HAV prevention, other preventative strategies that target the mechanisms of outbreak should be considered.

Food Precautions

As part of healthy living posttransplant, all recipients should be aware of recommendations for hand hygiene and food preparation to avoid ingestion of HAV virions, as disease occurs predominantly via fecal-oral spread. The recommendations for reducing the risk of HAV infection in the immunocompromised patient are the same as for the immunocompetent individual. Unfortunately, the HAV virion is hardy and survives in the environment for prolonged periods. Virions can survive drying and storage at 25 °C at 42% relative humidity for up to 30 days and still cause infection [105]. Surfaces may be disinfected with a 1:100 dilution of sodium hypochlorite (i.e., household bleach) in tap water [53].

Raw or improperly cooked shellfish cause outbreaks as the virus is not killed unless food is heated to at least 185 °F (85 °C) for 1–2 minutes. This thorough cooking of shellfish is longer than current practices of steaming until the shells open, leaving limited avenues for decontamination [106]. Transplant patients are advised to avoid all raw or undercooked shellfish (oysters, clams, mussels) [107].

For produce, contamination may occur at any step – during cultivation, processing, storage, distribution, or final preparation. Methods to disinfect produce include use of chlorinated water, hydrostatic pressure, and heat [106]. Freezing has little impact on reducing the viability of HAV contamination, particularly seen in frozen produce such as berries and herbs [108]. Rinsing in cold or warm water has little impact on removing enteric viruses from food and chlorine, a common sanitizing agent for produce that has a variable effect on infective risk. Knowledge of uncooked shellfish and produce as source of outbreaks must be included in education posttransplant. However, avoidance of all potential food-based sources of HAV is not possible while maintaining a healthy diet. As a result, immunization remains the key preventative strategy.

Immunization

The backbone of prevention of infection with HAV is vaccination with utilization both before and after exposure to the virus. Inactivated HAV vaccines were first licensed in the USA in 1995 for preexposure prophylaxis. In immunocompetent hosts, seroconversion rates 1 month after a single dose of vaccine are 95.7% rising to 99.8% after a further dose [109]. In 2005 the USA, the Food and Drug Administration (FDA) approved the vaccine in children from age 12–23 months. Initially, HAV vaccines were administered according to a three-dose schedule; however, all vaccines are now given according to a two-dose schedule [110]. Around the world, different countries have adopted varying strategies for vaccination regarding HAV – this approach may be either for universal coverage or based on prevalence or risk stratification [111].

Current vaccine series with doses and intervals that are available on the US market are listed (see Table 2).

In the USA, the ACIP recommends universal vaccination against HAV for children [112]. HAV vaccination has been found to be safe and immunogenic in infants and children from 6 to 21 months independent of the mother's serostatus [113, 114]. Passively transferred maternal antibody persists for at least 6 months and results in a blunted response to HAV vaccination [113], but despite this, infants under 6 months appear to be primed with vaccine despite interference [115, 116]. It is incorporated into the routine schedule, starting from 12 months of age, with either the two-dose series of HAV vaccine or the three-dose series with the combined HAV-HBV vaccine [112].

In adults, vaccination via the same two-dose series is recommended on the basis of risk. This includes persons with chronic liver disease or clotting factor disorders, travelers in countries with high or intermediate endemic HAV, those in close personal

Table 2 Vaccines used to prevent hepatitis A virus (HAV) infection

Vaccine	Trade name (manufacturer)	Age group (years)	Dosage	Route	Schedule	Booster
Hepatitis A vaccine, inactivated	Havrix (GlaxoSmithKline)	1–18	0.5 mL (720 ELU)	IM	0, 6–12 Mos	None
		≥ 19	1 mL (1440 ELU)	IM	0, 6–12 Mos	None
Hepatitis A vaccine, inactivated	VAQTA (Merck and co.)	1–18	0.5 mL (25 U)	IM	0, 6–12 Mos	None
		≥19	1 mL (50 U)	IM	0, 6–12 Mos	None
Combined hepatitis A and B vaccine[a]	Twinrix (GlaxoSmithKline)	≥18 (primary)	1 mL (720 ELU HAV +20 μg HBsAg)	IM	0, 6–12 Mos	None
		≥18 (accelerated)	1 mL (720 ELU HAV +20 μg HBsAg)	IM	0, 7, 21–30 days	12 Mos

From Nelson et al. [15]
Abbreviations: *ELU* ELISA units of inactivated HAV, *HBsAg* hepatitis B surface antigen, *IM* intramuscular, *U* units of HAV antigen
[a]Combined hepatitis A and B vaccine (Twinrix) should not be used for postexposure prophylaxis

contact with an international adoptee in the first 60 days after arrival from a country with high or intermediate endemic HAV, men who have sex with men, homeless persons, persons who use injection or non-injection drugs, and persons who work with HAV in a laboratory or with nonhuman primates infected with the virus. In addition, any person who is not at risk for HAV infection but wants protection against it may be vaccinated [117].

While many studies have given additional doses compared to the recommended schedule, in healthy recipients, booster doses are not thought to be required after a standard two-dose series [110]. A persistent amnestic response lasts up to 12 years postvaccination course [118] with modeling suggesting that antibody levels persist for more than 20 years in immunocompetent adults [119–121]. Similar modeling predicts persistence for an average of 14–20 years for children [122, 123]. Due to the arbitrary threshold for seropositivity, it is unclear if immunity is "lost" despite antibody decline over time and what additional role cellular immunity has in this situation [124]. HAV-proliferative T cell responses have been found to persist for many years postvaccination [125]. There is no difference in recommendations for vaccine dosing for immunocompetent and immunocompromised individuals.

Immunocompromised Hosts

Much of the information regarding HAV vaccination in immunocompromised hosts is based on studies concerning individuals with HIV infection where there is both an impaired response and duration of protection [126]. Much lower rates of HAV

vaccine responsiveness and persistence of antibody response occurred in HIV-positive hemophiliacs compared to those without the infection, with even poorer results in those with evidence of HIV progression [127–129].

In a small systematic review of studies on HAV in immunocompromised hosts, factors that were related to a poorer response to vaccination included the number of immunosuppressive drugs and not completing a vaccine schedule. Those who received the vaccine course with doses only 1 month apart also had poorer responses than those who had 3–6 months between doses [126].

Responses to HAV vaccines were reviewed retrospectively in a heterogeneous group of 85 immunocompromised hosts which included predominantly those on immunosuppressive drugs (n = 65) but also a small cohort post-SCT (n = 13) or with HIV infection who were not on immunosuppression (n = 7). It was found that 76.5% developed HAV-IgG after either one or two vaccine doses. Of the cohort, ten patients had received solid organ transplants and their response rate was 60%. Those on tumor necrosis factor-α (TNF-α) blockers (predominantly those with underlying rheumatic disorders) had better responses, and females were more adherent with postvaccine titer measurements [126].

In HIV patients with hemophilia, use of the standard adult titer vaccine (1440 ELISA units per dose) failed to elicit a vaccine response in those who had already failed to respond following three doses of the lower titer vaccine (720 ELISA units per dose) though numbers were very small [130]. It is unclear the role of booster vaccine in those who fail to respond and knowledge of serostatus postvaccination is important as it highlights a need for those who need protection via passive strategies.

Recommendations of the Infectious Diseases Society of America (IDSA) are that inactivated vaccines should be administered more than 2 weeks prior to immunosuppression [131]. Anticipated immunosuppression should be an indication for HAV vaccination in those at risk.

Pretransplant

Solid Organ Transplant

The American Society of Transplantation (AST) guidelines recommend that both children and adults should undergo a HAV vaccination schedule, ideally completed prior to transplantation [132]. Vaccine titer monitoring is recommended if ongoing risk for exposure, such as planned travel to high-risk areas [132]. Combined HAV-HBV vaccination can be used for solid organ transplant (SOT) candidates aged ≥12 years of age in whom both vaccines are indicated [131]. While it is recommended that children undergo the usual vaccine schedule appropriate for age prior to transplantation, often chronic ill health can impact this, making this population more at risk of vaccine preventable illnesses [133].

HAV vaccine has been extensively studied in candidates with underlying chronic liver disease, particularly those pending liver transplants. It is well tolerated in this population, including those with HBV, HCV, and non-viral etiologies [134]. Though seroconversion after a single HAV vaccine was higher in the healthy adult subgroup, seroconversion rates after two doses, 6 months apart, were comparable in those with

chronic HBV [135]. Safety and immunogenicity have also been determined in a cohort of children who were chronic HBV carriers with rates of seroconversion of 90.0%, 96.9%, and 100%, respectively, after the first, second, and third dose, suggesting a better response than adult counterparts [136], though GMC tended to be lower compared to healthy controls [137].

In those with decompensated liver disease, the antibody response is poorer when compared to the previous group, but better than those who are vaccinated post-transplant. Recommendations therefore support vaccinating as early as possible during the pretransplant course. This is supported by the immunogenicity of the standard vaccine schedule in those with compensated compared with decompensated cirrhosis (Child-Pugh class B/C), with seroconversion rates of compensated patients of 98% compared with 65.7% of those with decompensated disease, 1 month after final dose [138]. In addition, for those on the liver transplant waiting list, the lower the United Network for Organ Sharing (UNOS) score, which is related to the MELD score, the higher the chance of seroconversion with vaccination. However, overall rates were poor with only 48.6% seroconverting [139]. Another small study found that vaccination responses of 24 patients with severe chronic liver disease were compared to those of 8 liver transplant patients vaccinated posttransplant. Using a threshold of $\geq$33mIU/mL for HAV-IgG response, 50% of those with severe liver disease seroconverted (median titer of 34.7mIU/mL), while no liver transplant patients achieved this threshold (median titer of 0.0mIU/mL) [140].

Despite recommendations for vaccinations in those with chronic liver disease, vaccine uptake remains poor [141]. Of a cohort who proceeded to liver transplant, only 45% demonstrated HAV seroconversion when tested after transplantation. Only 26% were vaccinated according to a complete vaccination dose schedule, and these patients had a vaccine response for HAV of 50%. An additional 31% received a single dose of vaccine for HAV-HBV, but not a complete course and unsurprisingly the response rate was much lower among these patients, stressing the importance of completing the vaccination schedule. As supported by prior data, patients seemed to be more likely to respond to a vaccination if they had a lower Child-Pugh score at time of vaccination, further highlighting that patients should be vaccinated as early as possible in the course of their liver disease [142].

Recently, a high-dose accelerated vaccination schedule in those with cirrhosis pretransplant has been found to be effective with a single dose of Havrix 1440 μg repeated after 1 and 2 months and an additional booster for those failing to seroconvert [143]. This has found to be 94.3% effective compared with only 79.5% with the standard schedule of combined HAV-HBV or HAV vaccine.

Stem Cell Transplant

Routine pretransplant testing for HAV in HSCT recipients or donors with normal liver function testing is not necessary as HAV-IgG presence in the absence of IgM has no impact on transplant outcome [144]. One guideline states that there are higher rates of sinusoidal obstruction syndrome (SOS) post-HSCT in recipients who present with HAV-IgM and subsequently undergo myeloablative conditioning. This indicates that if a recipient presents with acute hepatitis prior to transplant, this should be

tested and transplant delayed, though this seems to be based more on expert opinion rather than primary data [144].

Pre-donation vaccination benefit is limited to T cell-dependent vaccines, and a durable humoral and cell-mediated immune response is not seen in the recipients. Overall risk-benefit ratios do not therefore support the use of pretransplant vaccination in clinical practice currently [145, 146]. There are no HAV-specific studies looking at this, and therefore, pre-donation HAV vaccination is not recommended as any potential benefit is unknown [147].

Posttransplant

Solid Organ Transplant

If HAV vaccination did not occur prior to transplant in at-risk recipients, it should be completed posttransplant. This includes all liver and renal transplant patients who have underlying HBV or HCV or are at risk of acquisition by other modalities [148]. Posttransplant vaccination should not be withheld for concerns regarding organ rejection [131].

Posttransplant HAV vaccination has been found to be safe, though studies report conflicting seroconversion compared to healthy controls with widely varied results ranging from 0% to 97% in different studies [140, 148]. Some have found statistically less immunogenicity compared to those vaccinated pretransplant and healthy controls [149], while others have found sufficient vaccine response even in as long as the vaccination series is completed [148]. Studies are often small and based on heterogeneous groups with variations in results between studies, likely due to discrepancies in patient selection, severity of liver disease, immunosuppressive medications, and type of vaccine used [150].

Though well tolerated, after a standard HAV vaccine series following liver transplant, only 8% seroconverted 1 month after the first dose, while 26% seroconverted after the second. Vaccine responders presented with higher total white blood cell and lymphocyte count at vaccination compared to non-responders and started vaccination series further in their posttransplant course [149]. In a similar study after kidney transplantation, only 26.9% seroconverted after a full series, with multivariate analysis showing that higher hemoglobin and lower serum creatinine levels were better prognostic factors for seroconversion. There was a trend for higher response rates with cyclosporine over tacrolimus and with lower prednisone doses [151].

There is no clear data on the level of protection or duration of response to vaccination in the pediatric solid organ transplant population. Historically, this population has low rates of vaccination prior to transplantation [152] . Overall, HAV vaccine response in immunocompromised children is suboptimal [133]. In a cohort of pediatric heart transplant children traveling to the World Transplant Games who had not received either HAV or HBV vaccinations prior, only 25% developed titers consistent with HAV seroconversion following vaccination. However, the type of vaccine and the number of doses administered were heterogeneous across the whole cohort [153].

Some have postulated that there are unique features regarding renal transplantation with poorer seroconversion rates. In a study that had higher levels of immunogenicity, only 72% of renal transplant recipients seroconverted after a standard series compared to 97% of liver recipients and 100% of healthy controls. In addition, the renal recipients also had lower GMC, with an inverse association with the number of immunosuppressive drugs patients were receiving [148]. In healthy donors, immunity is presumed after a single dose because of seroconversion rates of >90%. However, this can't be assumed in transplant recipients where in 68% a single dose did not result in protection [148].

Rates of antibody decline postvaccination are also greater in liver and kidney transplant recipients compared with normal controls [154]. In a follow-on study looking at posttransplant antibody decline, after 2 years, only 59% of liver transplant and 26% of renal transplant patients showed persistent seroconversion compared with 100% of control subjects. In the liver transplant group, decline in antibody level was greatest in those on cyclosporine compared with tacrolimus, even after adjustment for time posttransplant which contrasted to prior studies showing trends for higher responses with cyclosporine [151]. In the renal transplant group, type of immunosuppression did not alter antibody decline [154]. It remains unclear if there is something unique about the transplant type and seroconversion rates or if this is related to the underlying immunosuppression regime.

In those with immunity from prior infection, there is significant loss of HAV-IgG post-liver transplantation. Though numbers were small, one study showed 18% became seronegative at 1-year posttransplant while 29% were seronegative at the 2-year posttransplant mark [155]. In contrast, a study of renal transplant patients with underlying HCV, HAV-IgG levels remained over 90% at a median of 3 years posttransplant. However, pretransplant HAV-IgG levels were not determined, so the degree of loss could not be correlated [156]. The presence of antibodies after natural infection is higher than with immunization, so the decline may be faster in the latter. As in adults, the duration of protection after vaccination is not known in pediatric transplant patients [111].

Further studies are also required to determine if postvaccination antibody decline in immunocompromised patients warrants booster vaccinations. The association of an arbitrary antibody level defining persistence of humoral immunity is even less clear in the immunocompromised, as is the role of maintained T memory cells.

Hematopoietic Stem Cell Transplant

There are a number of studies that follow HSCT patients seropositive for HAV prior to transplantation as a result of infection earlier in life, rather than vaccination. The effects of HSCT upon their immune response to HAV indicate a role for posttransplant vaccination. In Brazil, in a study showing pretransplant seroprevalence of 92.2%, survival analysis showed rates of the probability of becoming seronegative were 4.5% at 1 year, 7.9% at 2 years, 10.1% at 3 years, and 23.4% at 4 years [157]. Loss of antibody was significantly associated with longer follow-up, GVHD, and younger age. An Argentinean cohort of 36 non-vaccinated patients receiving a peripheral-HSCT for heterogeneous indications demonstrated that 31 patients (86%)

remained seropositive on retesting an average of 12 months posttransplant (range 1–51) while the remaining patients became seronegative. There was no clear correlation with age, sex, diagnosis, type of transplant, timing of follow-up testing, or the amount of CD34+ cells that were given on univariate or multivariate analysis [158]. In another high-endemicity setting, the Ankara University Pediatric HSCT Unit evaluated 30 patients ranging in age from 1 to 18 for loss of HAV-IgG posttransplant. Seventy-six percent (n = 23) of patients were seropositive for HAV prior to transplant, and received transplants from donors, of whom 66% were seropositive. Ten patients (43.5%) became seronegative posttransplant with the median time of 12 months (range 12–32 months). Age, sex, diagnosis, GVHD, and follow-up time were not found to have a statistically significant contribution though numbers were very small. Although those with negative donor serology appeared to have a greater loss of antibody (75% vs. 26%), the results were not statistically significant. Interestingly, two of the three recipients who seroconverted posttransplant received transplants from seropositive donors [159]. Overall, recommendations are that those undergoing transplant in countries of high endemicity should undergo pretransplant seroprevalence testing and receive posttransplant vaccination if negative. The role of a booster vaccination in this setting is unclear, given the loss of antibody titers in the months following transplant.

The IDSA suggests that all HSCT recipients are to be considered unvaccinated after transplant with some experts recommending revaccination even if there are protective antibody titers [131]. After HSCT, most vaccinations are recommended based on local vaccine schedules, in the context of travel to endemic areas, occupational or personal risk. Despite regional differences with recommendations, there is often poor adherence to guidelines posttransplant [160]. As HBV vaccination is also required, administering the combined three-dose HAV-HBV vaccine course with an additional booster dose can be considered. If giving the standard monovalent vaccine, a routine two-dose schedule is sufficient. Expedited vaccination schedules using the combined HAV-HBV vaccine on a schedule of 0, 7, and 21 days and 12 months for rapid seroconversion have been studied with evidence that this is as immunogenic as the monovalent vaccine two-dose schedule [161].

Based on expert opinion, in the absence of posttransplant graft versus host disease (GVHD), persistent chemotherapy, and anti-CD20 monoclonal antibody therapy within the past 6 months, routine vaccination schedules may commence from 6 months posttransplant with evidence of immune reconstitution (131, 160). Within local schedules, HAV vaccination is often delayed until 1 year after transplantation, when the immunosuppression is even lower [160]. Vaccines should be delayed in the context of ongoing treatment for GVHD or administration of intravenous immunoglobulin (IVIG) replacement therapy. GVHD is independently associated with reduced T cell and antibody responses to vaccination and is also a marker for infectious risk [144, 162, 163]. IVIG is given in the context of profound hypogammaglobulinemia (IgG <400 mg/L) and recurrent infections, highlighting delayed humoral immune reconstitution [144]. However, recently administered IG products are not thought to inhibit the immune response to inactivated vaccines, unlike live vaccines, and should not be an excuse for a missed opportunity to

administer vaccines to those who are seen infrequently [160]. IVIG is likely to provide a period of protection against HAV, which may allow for later, perhaps more immunogenic, HAV vaccination.

Vaccine responses have been studied in a cohort of patients who underwent autologous HSCT for multiple myeloma, of whom 75% were on lenalidomide maintenance therapy. Of the 120 patients studied, 56 received vaccination post-transplant for HAV. Of the HAV vaccinated patients, 30% ($n = 17$) seroconverted, 29% ($n = 16$) retained immunity measured prevaccination, 21% ($n = 11$) did not respond, while 20% ($n = 11$) were non-evaluable (defined as missing results or titers being performed beyond 6 months postvaccination) [164]. Of those who received HAV vaccination, there was no statistical difference in vaccine response in those on maintenance therapy versus those who were not, though numbers were small. While it appears response rates to vaccination were high with other vaccines, the responses to HAV, HBV, and polio were poor in patients evaluable for response. Further studies are required determining exact response by titer and the rates of decline over time.

Travel Recommendations

Recommendations for HAV prevention in the circumstance of travel into areas of high endemicity varies depending on several factors. The main preventative strategies are the administration of either HAV vaccine or immune globulin (IG).

Ideally travel to HAV risk areas should be delayed until at least 1 year post-SOT and after 2 years in HSCT after resolution of GVHD or intensive immunosuppression – corresponding with the time of highest risk of infection [165]. The optimal approach for the traveling immunocompromised patient is a review at least 6–12 months prior to departure. If indicated based on destination, the first dose of HAV vaccine should be administered with repeat dosing after 6 months as per the routine schedule [131]. This is in comparison with healthy travelers who have high levels of seroconversion after a single dose [166]. Poor seroconversion after a single dose of vaccine has also been seen in patients with rheumatoid arthritis [167]. Use of a double dose, or repeat vaccine 4 weeks apart, gave patients with rheumatoid arthritis similar seroconversion rates to healthy controls after a single vaccine (85), and it may be considered if this approach may be utilized in transplant patients. Ideally, seroconversion should be assessed 4–8 weeks prior to departure with administration of IG (given as intramuscular gamma globulin) if seronegative [168, 169]. If seronegative, and travel is for more than 2 months duration, repeat doses should be given every 2 months [112, 170]. For less than 2 months of protection, a dose of 0.1 mL/kg is recommended; for more than 2 months of protection, a dose of 0.2 mL/kg is recommended and should be repeated every 2 months for long-term travel with no maximum dosage limitations [170]. GamaSTAN S/D, the only IG product approved by the Food and Drug Administration (FDA) for hepatitis A prophylaxis, is supplied in 10 and 2 mL units; therefore, rounding up to avoid wasting of product is recommended [150].

Often pretravel planning is not feasible with emergency travel, so those planning on departing in under two weeks should receive simultaneous administration of HAV vaccine and IG, administered at separate anatomical sites [15]. When

HAV vaccine and IG are administered simultaneously in the immunocompromised, the antibody titer achieved is lower, but not the proportion classified as seropositive, and is therefore not thought to have clinical relevance. How this affects the duration of protection is unclear [171]. In addition to the immunocompromised host, those who are over the age of 40 years and with underlying chronic liver disease should receive similar treatment based on risk [15]. This approach is in contrast to the immunocompetent aged between 12 months and 40 years who can receive a single dose of vaccine prior to travel and complete as per the schedule on return [15]. In immunocompetent children given simultaneous IG and three-dose vaccine strategy (0, 1, 6 months), there was a twofold lower antibody response as determined by GMC than if vaccine was given alone. There was immediate protection as determined by antibody response at 5 days when giving the combined active/passive vaccination strategy. In those who received IG alone, levels waned to around 66% seropositive at 2 months and 11% at 5 months with none of them being seropositive at 6 months [49].

There are no specific different recommendations for immunocompromised children traveling prior to the standard vaccination schedule, but care should be taken in this situation and delayed if possible. In immunocompetent children, if travel is occurring prior to the standard schedule vaccination of commencing at 12 months, HAV vaccine may be administered from 6 to 11 months though this should not be counted toward the routine two-dose series [15]. This approach was developed due to the fact that IG cannot be administered at the same time as MMR due to inhibition of the immune response to this vaccine for up to 3 months [170, 172] with prevention of measles, mumps, and rubella being the priority in traveling infants which can cause more severe illness than HAV in this age group [173]. Infants less than 6 months of age or those in whom vaccination is contraindicated should receive a single dose of IG before travel.

Adequate vaccination requires education of both patients and health practitioners to present or refer to travel clinics or vaccine providers within the adequate time frame. Despite efforts, uptake of recommendations is poor. In a North American transplant clinic survey, 36% of attenders revealed that they had recently traveled outside Canada and the USA (10). Despite 66% of travelers seeking pretravel advice, primarily from their transplant physician, 63% traveled to areas where HAV was endemic, but only 5% had received HAV immunization. This further highlights the importance of the transplant infectious disease physician within the posttransplant setting.

Postexposure Prevention

Postexposure prophylaxis with either HAV vaccine or IG prevents infection when given within 2 weeks of exposure [15, 53, 174]. Initial recommendations supported the primary use of IG which, when administered within 2 weeks of last exposure, is 89–90% effective in preventing clinical disease [175]. However, a large study of over 1090 immunocompetent people in an outbreak setting between the ages of 2–40 years found that vaccine administration was non-inferior [174]. The effectiveness beyond this time period was not determined. The combination HAV-HBV vaccine should not be used as postexposure prophylaxis, due to the duration of the vaccine course. Prior recommendations by the ACIP have determined the

postexposure strategy on the basis of age – those between 1 and 40 years have been recommended vaccination, while, if available, IG has been recommended to those under 1 or over 40 years [166, 176]. But, HAV vaccine has multiple advantages over IG administration, with a longer duration of protection, ease of administration, and greater acceptability and availability [176]. This postexposure prophylactic regimen, however, is not applicable to the transplant recipient or immunocompromised host.

While there are no studies that specifically assess the risk in the immunocompromised host postexposure to HAV, the vaccine is clearly less immunogenic in this population, and therefore a combined IG and vaccine approach is recommended for a potentially more durable response [15]. Transplant recipients with hypogammaglobulinemia or receiving routine immunoglobulin repletion with IVIG for other indications do not need additional protection against HAV (or measles and varicella) [150, 177].

Increased doses of IG (0.1 mL/kg) have been recommended since 2017, due to declining HAV-IgG potency, presumed from reduced prevalence in the donor pool [170, 178]. Dosing strategies for other IG indications such as measles, varicella, or rubella pre- and postexposure are unchanged (see Table 3).

Conclusion

While much progress has been made on a worldwide front in reducing rates of HAV infection, we continue to have a group of susceptible hosts at risk of more severe disease, who are often unprotected despite known risk factors for HAV acquisition. In practice, HAV vaccination rates are poor among the immunocompromised.

While there is obviously limited and heterogeneous information on HAV infection and vaccination in transplanted and immunocompromised hosts, it is clear that much of our information on immunocompetent hosts cannot necessarily be applied to transplant patients. Further study, particularly on improving immunogenicity and durability of both humoral and cellular responses to vaccination in transplant recipients, is required.

From the perspective of those involved in transplantation and the care of immunocompromised hosts, knowledge of the importance of the timing of vaccination remains key. Ensuring our patients are educated and seek further medical opinion prior to travel or other risk factors is critical.

Key Points

- Hepatitis A is non-enveloped RNA virus with predominantly fecal-oral spread.
- It is associated with typically foodborne outbreaks in areas of low endemicity, such as the USA and Western Europe, but there is higher transmission in other areas such as South Asia and Africa.
- Immunity to HAV occurs after infection or vaccination – the persistence of this is unclear after transplantation.

Table 3 Recommendations for postexposure prophylaxis and preexposure protection, by age group and risk category

Indication/age group	Risk category/health status	Hepatitis A vaccine	Immune globulin
Postexposure prophylaxis			
<12mos	Healthy	No	0.1 mL/kg[a]
12 Mos–40 years	Healthy	1 dose[b]	None
>40 years	Healthy	1 dose[b]	0.1 mL/kg[c]
≥12mos	Immunocompromised or chronic liver disease	1 dose[b]	0.1 mL/kg[d]
≥12mos	Vaccine contraindicated[e]	No	0.1 mL/kg
Preexposure prophylaxis[f]			
<6mos	Healthy	No	0.1–0.2 mL/kg[g]
<12mos	Healthy	1 dose[h]	None
12mos–40 years	Healthy	1 dose[i]	None
>40 years	Healthy	1 dose[i]	0.1–0.2 mL/kg[g, j]
All ages	Immunocompromised or chronic liver disease	1 dose[i]	0.1–0.2 mL/kg[g, j]
≥6mos	Persons who elect not to receive vaccine or for whom vaccine is contraindicated	No	0.1–0.2 mL/kg[g]

From Nelson et al. [15]

[a]Measles, mumps, and rubella vaccine should not be administered for at least 3 months after receipt of IG

[b]A second dose is not required for postexposure prophylaxis; however, for long-term immunity, the hepatitis A vaccination series should be completed with a second dose at least 6 months after the first dose

[c]The provider's risk assessment should determine the need for immune globulin administration. If the provider's risk assessment determines that both vaccine and immune globulin are warranted, HAV vaccine and immune globulin should be administered simultaneously at different anatomic sites

[d]Vaccine and immune globulin should be administered simultaneously at different anatomic sites

[e]Life-threatening allergic reaction to a previous dose of hepatitis A vaccine or allergy to any vaccine component

[f]IG should be considered before travel for persons with special risk factors for either HAV infection or increased risk for complications in the event of exposure to HAV

[g]0.1 mL/kg for travel up to 1 month; 0.2 mL/kg for travel up to 2 months, 0.2 mL/kg every 2 months for travel of ≥2 months' duration

[h]This dose should not be counted toward the routine two-dose series, which should be initiated at age 12 months

[i]For persons not previously vaccinated with HAV vaccine, administer dose as soon as travel is considered, and complete series according to routine schedule

[j]May be administered, based on providers' risk assessment

- HAV vaccine is recommended universally for children in the USA and on the basis of risk in adults, which includes many transplant recipients, particularly those with chronic liver disease.
- In SOT recipients, vaccine strategies to prevent HAV should be commenced as early as possibly in the pretransplant period for increased immunogenicity.

- Post-HSCT, recipients should be considered as unvaccinated and receive HAV vaccine as per their local schedules after transplant.
- Solid organ and hematopoietic stem cell transplant recipients are increasingly traveling to high-risk countries, and awareness of this fact before and after transplant can help protect patients from HAV.
- In those traveling to areas with increased HAV endemicity, a full HAV vaccine course must be completed or given with IG if vaccination is unable to occur in a timely manner.

References

1. Rein DB, Stevens A, Flaxman A, Wittenborn JS, Timothy N, Wiktor SZ, et al. P703 the global burden of hepatitis a virus in 1990 and 2005- clinical key. J Hepatol. 2014;60(1):S303.
2. Daniels D, Grytdal S, Wasley A. Surveillance for acute viral hepatitis – United States, 2007. Morb Mortal Wkly Rep Surveill Summ. 2009;58(3):1–27.
3. Viral Hepatitis Surveillance – United States, 2016 – 2016HepSurveillanceRpt.pdf: s of Information National Notifiable Diseases Surveillance System (NNDSS)Adjustments to Reported Cases from the National Notifiable Diseases Surveillance System Enhanced Viral Hepatitis Surveillance Sites Mortality/Death Certificates; 2016. https://www.cdc.gov/hepatitis/statistics/2016surveillance/pdfs/2016HepSurveillanceRpt.pdf
4. Cooke GS, Andrieux-Meyer I, Applegate TL, Atun R, Burry JR, Cheinquer H, et al. Accelerating the elimination of viral hepatitis: a Lancet Gastroenterology & Hepatology Commission. Lancet Gastroenterol Hepatol. 2019;4(2):135–84.
5. Campagna M, Siddu A, Meloni A, Basciu C, Ferrai L, Pettinau A, et al. Changing pattern of hepatitis a virus epidemiology in an area of high endemicity. Hepat Mon. 2012;12(6):382–5.
6. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine. 2010;28(41):6653–7.
7. Ly KN, Klevens RM. Trends in disease and complications of hepatitis A virus infection in the United States, 1999–2011: a new concern for adults. J Infect Dis. 2015;212(2):176–82.
8. Schwartz BS, Rosen J, Han PV, Hynes NA, Hagmann SH, Rao SR, et al. Immunocompromised travelers: demographic characteristics, travel destinations, and pretravel health care from the U.S. Global TravEpiNet consortium. Am J Trop Med Hyg. 2015;93(5):1110–6.
9. Trubiano JA, Johnson D, Sohail A, Torresi J. Travel vaccination recommendations and endemic infection risks in solid organ transplantation recipients. J Travel Med. 2016;23(6):1–15.
10. Boggild AK, Sano M, Humar A, Salit I, Gilman M, Kain KC. Travel patterns and risk behavior in solid organ transplant recipients. J Travel Med. 2004;11(1):37–43.
11. Buchan CA, Kotton CN. Travel medicine, transplant tourism, and the solid organ transplant recipient – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13529.
12. Van Effelterre T, Guignard A, Marano C, Rojas R, Jacobsen KH. Modeling the hepatitis A epidemiological transition in Brazil and Mexico. Hum Vaccin Immunother. 2017;13(8):1942–51.
13. Beaute J, Westrell T, Schmid D, Muller L, Epstein J, Kontio M, et al. Travel-associated hepatitis A in Europe, 2009 to 2015. Euro Surveill. 2018;23(22):1–9.
14. Ryan ET, Kain KC. Health advice and immunizations for travelers. N Engl J Med. 2000;342(23):1716–25.
15. Nelson NP, Link-Gelles R, Hofmeister MG, Romero JR, Moore KL, Ward JW, et al. Update: recommendations of the Advisory Committee on Immunization Practices for use of hepatitis A vaccine for postexposure prophylaxis and for preexposure prophylaxis for international travel. MMWR Morb Mortal Wkly Rep. 2018;67(43):1216–20.

16. Viral Hepatitis Surveillance – United States, 2016 – 2016HepSurveillanceRpt.pdf: Centers for Disease Control and Prevention. https://www.cdc.gov/hepatitis/statistics/2016surveillance/pdfs/2016HepSurveillanceRpt.pdf
17. Donnan EJ, Fielding JE, Gregory JE, Lalor K, Rowe S, Goldsmith P, et al. A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. Clin Infect Dis. 2012;54(6):775–81.
18. Epson EE, Cronquist A, Lamba K, Kimura AC, Hassan R, Selvage D, et al. Risk factors for hospitalisation and associated costs among patients with hepatitis A associated with imported pomegranate arils, United States, 2013. Public Health. 2016;136:144–51.
19. Hutin YJ, Pool V, Cramer EH, Nainan OV, Weth J, Williams IT, et al. A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. New Engl J Med. 1999;340(8):595–602.
20. Lucioni C, Cipriani V, Mazzi S, Panunzio M. Cost of an outbreak of hepatitis A in Puglia, Italy. PharmacoEconomics. 1998;13(2):257–66.
21. Rosenblum LS, Mirkin IR, Allen DT, Safford S, Hadler SC. A multifocal outbreak of hepatitis A traced to commercially distributed lettuce. Am J Public Health. 1990;80(9):1075–9.
22. Hepatitis A outbreak associated with green onions at a restaurant – Monaca, Pennsylvania, 2003. MMWR Morb Mortal Wkly Rep. 2003;52(47):1155–7.
23. Foodborne hepatitis A – Missouri, Wisconsin, and Alaska, 1990–1992. MMWR Morb Mortal Wkly Rep. 1993;42(27):526–34.
24. Weltman AC, Bennett NM, Ackman DA, Misage JH, Campana JJ, Fine LS, et al. An outbreak of hepatitis A associated with a bakery, New York, 1994: the 1968 "west branch, Michigan" outbreak repeated. Epidemiol Infect. 1996;117(2):333–41.
25. Desenclos JC, Klontz KC, Wilder MH, Nainan OV, Margolis HS, Gunn RA. A multistate outbreak of hepatitis A caused by the consumption of raw oysters. Am J Public Health. 1991;81(10):1268–72.
26. CDC alert: hepatitis A outbreaks in multiple states: recommendations and guidance: Infectious Diseases Society of America (IDSA). 2018. https://www.idsociety.org/news%2D%2Dpublications-new/cdc-alerts/cdc-alert-hepatitis-a-outbreaks-in-multiple-states-recommendations-and-guidance/
27. Cuthbert JA. Hepatitis A: old and new. Clin Microbiol Rev. 2001;14(1):38–58.
28. Lemon SM, Jansen RW, Brown EA. Genetic, antigenic and biological differences between strains of hepatitis A virus. Vaccine. 1992;10(Suppl 1):S40–4.
29. Najarian R, Caput D, Gee W, Potter SJ, Renard A, Merryweather J, et al. Primary structure and gene organization of human hepatitis A virus. Proc Natl Acad Sci U S A. 1985;82(9):2627–31.
30. Weitz M, Baroudy BM, Maloy WL, Ticehurst JR, Purcell RH. Detection of a genome-linked protein (VPg) of hepatitis A virus and its comparison with other picornaviral VPgs. J Virol. 1986;60(1):124–30.
31. Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: a summary and update on the molecular virology, epidemiology, pathogenesis and prevention. J Hepatol. 2018;68(1):167–184.
32. Feng Z, Hensley L, McKnight KL, Hu F, Madden V, Ping L, et al. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature. 2013;496(7445):367–71.
33. Feng Z, Li Y, McKnight KL, Hensley L, Lanford RE, Walker CM, et al. Human pDCs preferentially sense enveloped hepatitis A virions. J Clin Invest. 2015;125(1):169–76.
34. Rosenblum LS, Villarino ME, Nainan OV, Melish ME, Hadler SC, Pinsky PP, et al. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. J Infect Dis. 1991;164(3):476–82.
35. da Silva SG, Leon LA, Alves G, Brito SM, Sandes Vde S, Lima MM, et al. A rare case of transfusion transmission of hepatitis A virus to two patients with haematological disease. Transfus Med Hemother. 2016;43(2):137–41.
36. Sherertz RJ, Russell BA, Reuman PD. Transmission of hepatitis A by transfusion of blood products. Arch Intern Med. 1984;144(8):1579–80.

37. Hughes JA, Fontaine MJ, Gonzalez CL, Layon AG, Goodnough LT, Galel SA. Case report of a transfusion-associated hepatitis A infection. Transfusion. 2014;54(9):2202–6.
38. Gowland P, Fontana S, Niederhauser C, Taleghani BM. Molecular and serologic tracing of a transfusion-transmitted hepatitis A virus. Transfusion. 2004;44(11):1555–61.
39. Vestergaard HT, Harritshoj LH, Midgley SE, Ullum H, Kampmann P. Transfusion transmission of hepatitis A virus with fecal shedding in a previously hepatitis A vaccinated recipient. J Infect Chemother. 2018;24(9):766–8.
40. Johnston A, Macgregor A, Borovec S, Hattarki M, Stuckly K, Anderson D, et al. Inactivation and clearance of viruses during the manufacture of high purity factor IX. Biologicals. 2000;28(3):129–36.
41. Mannucci PM, Gdovin S, Gringeri A, Colombo M, Mele A, Schinaia N, et al. Transmission of hepatitis A to patients with hemophilia by factor VIII concentrates treated with organic solvent and detergent to inactivate viruses. The Italian Collaborative Group. Ann Intern Med. 1994;120 (1):1–7.
42. Mannucci PM. Outbreak of hepatitis A among Italian patients with haemophilia. Lancet. 1992;339(8796):819.
43. Temperley IJ, Cotter KP, Walsh TJ, Power J, Hillary IB. Clotting factors and hepatitis A. Lancet. 1992;340(8833):1466.
44. Foster MA, Weil LM, Jin S, Johnson T, Hayden-Mixson TR, Khudyakov Y, et al. Transmission of hepatitis A virus through combined liver-small intestine-pancreas transplantation. Emerg Infect Dis. 2017;23(4):590–6.
45. Ghez D, Nicand E, Ferre V, de Tailly PD, Coppo P, Lenoir B, et al. Allogenic bone marrow transplantation with a donor presenting with an acute hepatitis A. J Hepatol. 2001;34(4):625–30.
46. Seelig R, Pott G, Seelig HP, Liehr H, Metzger P, Waldherr R. Virus-binding activity of fibronectin: masking of hepatitis A virus. J Virol Methods. 1984;8(4):335–47.
47. Kao HW, Ashcavai M, Redeker AG. The persistence of hepatitis A IgM antibody after acute clinical hepatitis A. Hepatology (Baltimore, Md). 1984;4(5):933–6.
48. Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis a virus infection: a molecular approach. Clin Microbiol Rev. 2006;19(1):63–79.
49. Leentvaar-Kuijpers A, Coutinho RA, Brulein V, Safary A. Simultaneous passive and active immunization against hepatitis A. Vaccine. 1992;10(Suppl 1):S138–41.
50. Zaaijer HL, Leentvaar-Kuijpers A, Rotman H, Lelie PN. Hepatitis A antibody titres after infection and immunization: implications for passive and active immunization. J Med Virol. 1993;40(1):22–7.
51. Lemon SM, Murphy PC, Provost PJ, Chalikonda I, Davide JP, Schofield TL, et al. Immunoprecipitation and virus neutralization assays demonstrate qualitative differences between protective antibody responses to inactivated hepatitis A vaccine and passive immunization with immune globulin. J Infect Dis. 1997;176(1):9–19.
52. Andre F, Van Damme P, Safary A, Banatvala J. Inactivated hepatitis A vaccine: immunogenicity, efficacy, safety and review of official recommendations for use. Expert Rev Vaccines. 2002;1 (1):9–23.
53. Fiore AE, Wasley A, Bell BP. Prevention of hepatitis A through active or passive immunization: recommendations of the advisory committee on immunization practices (ACIP). MMWR Recomm Rep. 2006;55(Rr-7):1–23.
54. Wang H, Zheng H, Cao J, Zhou W, Yi Y, Jia Z, et al. Genetic diversity of hepatitis A virus in China: VP3-VP1-2A genes and evidence of quasispecies distribution in the isolates. PLoS One. 2013;8(9):e74752.
55. Gingrich GA, Hadler SC, Elder HA, Ash KO. Serologic investigation of an outbreak of hepatitis A in a rural day-care center. Am J Public Health. 1983;73(10):1190–3.
56. Hadler SC, Webster HM, Erben JJ, Swanson JE, Maynard JE. Hepatitis A in day-care centers. A community-wide assessment. N Engl J Med. 1980;302(22):1222–7.

57. Lednar WM, Lemon SM, Kirkpatrick JW, Redfield RR, Fields ML, Kelley PW. Frequency of illness associated with epidemic hepatitis A virus infections in adults. Am J Epidemiol. 1985;122(2):226–33.
58. Tong MJ, el-Farra NS, Grew MI. Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. J Infect Dis. 1995;171(Suppl 1):S15–8.
59. Averhoff FM, Khudyakov Y, Nelson NP. 24 – Hepatitis A vaccines. In: Plotkin SA, Orenstein WA, Offit PA, Edwards KM, editors. Plotkin's vaccines (7th edn). Philadelphia, PA. Elsevier; 2018. p. 319–41.e15.
60. Schiff ER. Atypical clinical manifestations of hepatitis A. Vaccine. 1992;10(Suppl 1):S18–20.
61. Willner IR, Uhl MD, Howard SC, Williams EQ, Riely CA, Waters B. Serious hepatitis A: an analysis of patients hospitalized during an urban epidemic in the United States. Ann Intern Med. 1998;128(2):111–4.
62. Akriviadis EA, Redeker AG. Fulminant hepatitis A in intravenous drug users with chronic liver disease. Ann Intern Med. 1989;110(10):838–9.
63. Vento S, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, et al. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. N Engl J Med. 1998;338(5):286–90.
64. Vallbracht A, Gabriel P, Maier K, Hartmann F, Steinhardt HJ, Muller C, et al. Cell-mediated cytotoxicity in hepatitis A virus infection. Hepatology (Baltimore, Md). 1986;6(6):1308–14.
65. Stapleton JT. Host immune response to hepatitis A virus. J Infect Dis. 1995;171(Suppl 1): S9–14.
66. Tjon GM, Coutinho RA, van den Hoek A, Esman S, Wijkmans CJ, Hoebe CJ, et al. High and persistent excretion of hepatitis A virus in immunocompetent patients. J Med Virol. 2006;78(11):1398–405.
67. Rezende G, Roque-Afonso AM, Samuel D, Gigou M, Nicand E, Ferre V, et al. Viral and clinical factors associated with the fulminant course of hepatitis A infection. Hepatology (Baltimore, Md). 2003;38(3):613–8.
68. Krugman S, Giles JP. Viral hepatitis. New light on an old disease. JAMA. 1970;212(6):1019–29.
69. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis. 2000;182(1):12–7.
70. Costa-Mattioli M, Monpoeho S, Nicand E, Aleman MH, Billaudel S, Ferre V. Quantification and duration of viraemia during hepatitis A infection as determined by real-time RT-PCR. J Viral Hepat. 2002;9(2):101–6.
71. Normann A, Jung C, Vallbracht A, Flehmig B. Time course of hepatitis A viremia and viral load in the blood of human hepatitis A patients. J Med Virol. 2004;72(1):10–6.
72. Tassopoulos NC, Papaevangelou GJ, Ticehurst JR, Purcell RH. Fecal excretion of Greek strains of hepatitis A virus in patients with hepatitis A and in experimentally infected chimpanzees. J Infect Dis. 1986;154(2):231–7.
73. Ida S, Tachikawa N, Nakajima A, Daikoku M, Yano M, Kikuchi Y, et al. Influence of human immunodeficiency virus type 1 infection on acute hepatitis A virus infection. Clin Infect Dis. 2002;34(3):379–85.
74. Robertson BH, Averhoff F, Cromeans TL, Han X, Khoprasert B, Nainan OV, et al. Genetic relatedness of hepatitis A virus isolates during a community-wide outbreak. J Med Virol. 2000;62(2):144–50.
75. Inoue K, Yoshiba M, Yotsuyanagi H, Otsuka T, Sekiyama K, Fujita R. Chronic hepatitis A with persistent viral replication. J Med Virol. 1996;50(4):322–4.
76. McDonald GS, Courtney MG, Shattock AG, Weir DG. Prolonged IgM antibodies and histopathological evidence of chronicity in hepatitis A. Liver. 1989;9(4):223–8.
77. Gruer LD, McKendrick MW, Beeching NJ, Geddes AM. Relapsing hepatitis associated with hepatitis A virus. Lancet. 1982;2(8290):163.
78. Cornu C, Lamy ME, Geubel A, Galanti L. Persistence of immunoglobulin M antibody to hepatitis A virus and relapse of hepatitis A infection. Eur J Clin Microbiol. 1984;3(1):45–6.

79. Glikson M, Galun E, Oren R, Tur-Kaspa R, Shouval D. Relapsing hepatitis A. review of 14 cases and literature survey. Medicine. 1992;71(1):14–23.
80. Debray D, Cullufi P, Devictor D, Fabre M, Bernard O. Liver failure in children with hepatitis A. Hepatology (Baltimore, Md). 1997;26(4):1018–22.
81. Keeffe EB. Is hepatitis A more severe in patients with chronic hepatitis B and other chronic liver diseases? Am J Gastroenterol. 1995;90(2):201–5.
82. Mele A, Tosti ME, Stroffolini T. Hepatitis associated with hepatitis A superinfection in patients with chronic hepatitis C. N Engl J Med. 1998;338(24):1771; author reply 2–3.
83. Leino T, Pebody R, Leinikki P. Hepatitis associated with hepatitis A superinfection in patients with chronic hepatitis C. N Engl J Med. 1998;338(24):1772; author reply −3.
84. Hasle G, Hoel T, Jensenius M. Mortality of hepatitis A in adults with hepatitis C antibodies. Lancet. 1998;351(9119):1888.
85. Rosdahl A, Herzog C, Frosner G, Noren T, Rombo L, Askling HH. An extra priming dose of hepatitis A vaccine to adult patients with rheumatoid arthritis and drug induced immunosuppression – A prospective, open-label, multi-center study. Travel Med Infect Dis. 2018;21:43–50.
86. Jiang W, Muhammad F, Ma P, Liu X, Long G. Sofosbuvir inhibits hepatitis A virus replication in vitro assessed by a cell-based fluorescent reporter system. Antivir Res. 2018;154:51–7.
87. Melnick JL. History and epidemiology of hepatitis A virus. J Infect Dis. 1995;171(Suppl 1):S2–8.
88. Wasley A, Miller JT, Finelli L. Surveillance for acute viral hepatitis–United States, 2005. Morb Mortal Wkly Rep Surveill Summ. 2007;56(3):1–24.
89. Cooksley WG. What did we learn from the Shanghai hepatitis A epidemic? J Viral Hepat. 2000;7(Suppl 1):1–3.
90. Kim JD, Cho EJ, Ahn C, Park SK, Choi JY, Lee HC, et al. A model to predict 1-month risk of transplant or death in hepatitis A-related acute liver failure. Hepatology 2019;70(2):621–629.
91. Taylor RM, Davern T, Munoz S, Han SH, McGuire B, Larson AM, et al. Fulminant hepatitis A virus infection in the United States: incidence, prognosis, and outcomes. Hepatology (Baltimore, Md). 2006;44(6):1589–97.
92. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology. 1989;97(2):439–45.
93. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. Hepatology (Baltimore, Md). 2001;33(2):464–70.
94. Koch DG, Tillman H, Durkalski V, Lee WM, Reuben A. Development of a model to predict transplant-free survival of patients with acute liver failure. Clin Gastroenterol Hepatol. 2016;14(8):1199–206.e2.
95. Chan G, Taqi A, Marotta P, Levstik M, McAlister V, Wall W, et al. Long-term outcomes of emergency liver transplantation for acute liver failure. Liver Transpl. 2009;15(12):1696–702.
96. Freeman RB Jr, Steffick DE, Guidinger MK, Farmer DG, Berg CL, Merion RM. Liver and intestine transplantation in the United States, 1997–2006. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2008;8(4 Pt 2):958–76.
97. Sedhom D, D'Souza M, John E, Viral Hepatitis RV. Acute liver failure: still a problem. Clin Liver Dis. 2018;22(2):289–300.
98. Schiodt FV, Atillasoy E, Shakil AO, Schiff ER, Caldwell C, Kowdley KV, et al. Etiology and outcome for 295 patients with acute liver failure in the United States. Liver Transpl Surg. 1999;5(1):29–34.
99. Gimson AE, White YS, Eddleston AL, Williams R. Clinical and prognostic differences in fulminant hepatitis type A, B and non-A non-B. Gut. 1983;24(12):1194–8.
100. Jung DH, Hwang S, Lim YS, Kim KH, Ahn CS, Moon DB, et al. Outcome comparison of liver transplantation for hepatitis A-related versus hepatitis B-related acute liver failure in adult recipients. Clin Transpl. 2018;32(1):1–12.
101. Fagan E, Yousef G, Brahm J, Garelick H, Mann G, Wolstenholme A, et al. Persistence of hepatitis A virus in fulminant hepatitis and after liver transplantation. J Med Virol. 1990;30(2):131–6.

102. Gane E, Sallie R, Saleh M, Portmann B, Williams R. Clinical recurrence of hepatitis A following liver transplantation for acute liver failure. J Med Virol. 1995;45(1):35–9.
103. Eisenbach C, Longerich T, Fickenscher H, Schalasta G, Stremmel W, Encke J. Recurrence of clinically significant hepatitis A following liver transplantation for fulminant hepatitis A. J Clin Virol. 2006;35(1):109–12.
104. Park GC, Hwang S, Yu YD, Park PJ, Choi YI, Song GW, et al. Intractable recurrent hepatitis A virus infection requiring repeated liver transplantation: a case report. Transplant Proc. 2010;42(10):4658–60.
105. McCaustland KA, Bond WW, Bradley DW, Ebert JW, Maynard JE. Survival of hepatitis A virus in feces after drying and storage for 1 month. J Clin Microbiol. 1982;16(5):957–8.
106. Fiore AE. Hepatitis A transmitted by food. Clin Infect Dis. 2004;38(5):705–15.
107. Avery RK, Michaels MG. Strategies for safe living following solid organ transplantation-guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13519.
108. Butot S, Putallaz T, Sanchez G. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. Int J Food Microbiol. 2008;126(1–2):30–5.
109. Andre FE, D'Hondt E, Delem A, Safary A. Clinical assessment of the safety and efficacy of an inactivated hepatitis A vaccine: rationale and summary of findings. Vaccine. 1992;10(Suppl 1): S160–8.
110. Van Damme P, Banatvala J, Fay O, Iwarson S, McMahon B, Van Herck K, et al. Hepatitis A booster vaccination: is there a need? Lancet. 2003;362(9389):1065–71.
111. Danziger-Isakov L, Posfay-Barbe KM. Optimal approach to immunization in pediatric solid organ transplantation. Pediatr Transplant. 2012;16(7):680–3.
112. Robinson CL, Bernstein H, Romero JR, Szilagyi P. Advisory committee on immunization practices recommended immunization schedule for children and adolescents aged 18 years or younger – United States, 2019. MMWR Morb Mortal Wkly Rep. 2019;68(5):112–4.
113. Bell BP, Negus S, Fiore AE, Plotnik J, Dhotre KB, Williams J, et al. Immunogenicity of an inactivated hepatitis A vaccine in infants and young children. Pediatr Infect Dis J. 2007;26(2):116–22.
114. Piazza M, Safary A, Vegnente A, Soncini R, Pensati P, Sardo M, et al. Safety and immunogenicity of hepatitis A vaccine in infants: a candidate for inclusion in the childhood vaccination programme. Vaccine. 1999;17(6):585–8.
115. Dagan R, Amir J, Mijalovsky A, Kalmanovitch I, Bar-Yochai A, Thoelen S, et al. Immunization against hepatitis A in the first year of life: priming despite the presence of maternal antibody. Pediatr Infect Dis J. 2000;19(11):1045–52.
116. Letson GW, Shapiro CN, Kuehn D, Gardea C, Welty TK, Krause DS, et al. Effect of maternal antibody on immunogenicity of hepatitis A vaccine in infants. J Pediatr. 2004;144(3):327–32.
117. Kim DK, Hunter P. Recommended adult immunization schedule, United States, 2019. Ann Intern Med. 2019;170(3):182–92.
118. Van Herck K, Van Damme P, Lievens M, Stoffel M. Hepatitis A vaccine: indirect evidence of immune memory 12 years after the primary course. J Med Virol. 2004;72(2):194–6.
119. Van Herck K, Beutels P, Van Damme P, Beutels M, Van den Dries J, Briantais P, et al. Mathematical models for assessment of long-term persistence of antibodies after vaccination with two inactivated hepatitis A vaccines. J Med Virol. 2000;60(1):1–7.
120. Van Damme P, Thoelen S, Cramm M, De Groote K, Safary A, Meheus A. Inactivated hepatitis A vaccine: reactogenicity, immunogenicity, and long-term antibody persistence. J Med Virol. 1994;44(4):446–51.
121. Hens N, Habteab Ghebretinsae A, Hardt K, Van Damme P, Van Herck K. Model based estimates of long-term persistence of inactivated hepatitis A vaccine-induced antibodies in adults. Vaccine. 2014;32(13):1507–13.
122. Chan CY, Lee SD, Yu MI, Wang YJ, Chang FY, Lo KJ. Long-term follow-up of hepatitis A vaccination in children. Vaccine. 1999;17(4):369–72.

123. Fan PC, Chang MH, Lee PI, Safary A, Lee CY. Follow-up immunogenicity of an inactivated hepatitis A virus vaccine in healthy children: results after 5 years. Vaccine. 1998;16(2–3):232–5.
124. Fujiyama S, Odoh K, Tanaka M, Kuramoto I, Tomita K. Evaluation of the timing of the booster injection after a primary vaccination against hepatitis A. J Gastroenterol Hepatol. 1997;12(2):172–5.
125. Cederna JB, Klinzman D, Stapleton JT. Hepatitis A virus-specific humoral and cellular immune responses following immunization with a formalin-inactivated hepatitis A vaccine. Vaccine. 1999;18(9–10):892–8.
126. Garcia Garrido HM, Wieten RW, Grobusch MP, Goorhuis A. Response to hepatitis A vaccination in immunocompromised travelers. J Infect Dis. 2015;212(3):378–85.
127. Santagostino E, Gringeri A, Rocino A, Zanetti A, de Biasi R, Mannucci PM. Patterns of immunogenicity of an inactivated hepatitis A vaccine in anti-HIV positive and negative hemophilic patients. Thromb Haemost. 1994;72(4):508–10.
128. Hess G, Clemens R, Bienzle U, Schonfeld C, Schunck B, Bock HL. Immunogenicity and safety of an inactivated hepatitis A vaccine in anti-HIV positive and negative homosexual men. J Med Virol. 1995;46(1):40–2.
129. Neilsen GA, Bodsworth NJ, Watts N. Response to hepatitis A vaccination in human immunodeficiency virus-infected and -uninfected homosexual men. J Infect Dis. 1997;176(4):1064–7.
130. Tilzey AJ, Palmer SJ, Harrington C, O'Doherty MJ. Hepatitis A vaccine responses in HIV-positive persons with haemophilia. Vaccine. 1996;14(11):1039–41.
131. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58(3):309–18.
132. Danziger-Isakov L, Kumar D. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13563.
133. Verma A, Wade JJ. Immunization issues before and after solid organ transplantation in children. Pediatr Transplant. 2006;10(5):536–48.
134. Keeffe EB, Iwarson S, McMahon BJ, Lindsay KL, Koff RS, Manns M, et al. Safety and immunogenicity of hepatitis A vaccine in patients with chronic liver disease. Hepatology (Baltimore, Md). 1998;27(3):881–6.
135. Tsang SW, Sung JJ. Inactivated hepatitis A vaccine in Chinese patients with chronic hepatitis B infection. Aliment Pharmacol Ther. 1999;13(11):1445–9.
136. Nebbia G, Giacchino R, Soncini R, Ramaccioni V, Timitilli A, Zanetti AR. Hepatitis A vaccination in chronic carriers of hepatitis B virus. J Pediatr. 1999;134(6):784–5.
137. Ferreira CT, da Silveira TR, Vieira SM, Taniguchi A, Pereira-Lima J. Immunogenicity and safety of hepatitis A vaccine in children with chronic liver disease. J Pediatr Gastroenterol Nutr. 2003;37(3):258–61.
138. Arguedas MR, Johnson A, Eloubeidi MA, Fallon MB. Immunogenicity of hepatitis A vaccination in decompensated cirrhotic patients. Hepatology (Baltimore, Md). 2001;34(1):28–31.
139. Smallwood GA, Coloura CT, Martinez E, Stieber AC, Heffron TG. Can patients awaiting liver transplantation elicit an immune response to the hepatitis A vaccine? Transplant Proc. 2002;34(8):3289–90.
140. Dumot JA, Barnes DS, Younossi Z, Gordon SM, Avery RK, Domen RE, et al. Immunogenicity of hepatitis A vaccine in decompensated liver disease. Am J Gastroenterol. 1999;94(6):1601–4.
141. Younossi ZM, Stepanova M. Changes in hepatitis A and B vaccination rates in adult patients with chronic liver diseases and diabetes in the U.S. population. Hepatology (Baltimore, Md). 2011;54(4):1167–78.
142. Andersson D, Castedal M, Friman V. Are liver transplant recipients protected against hepatitis A and B? Transplant Proc. 2013;45(3):1193–7.
143. Wigg AJ, Wundke R, McCormick R, Muller KR, Ramachandran J, Narayana SK, et al. Efficacy of high-dose, rapid, hepatitis A and B vaccination schedules in patients with cirrhosis. Clin Gastroenterol Hepatol. 2019;17(6):1210–1212.

144. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.
145. Storek J, Dawson MA, Lim LC, Burman BE, Stevens-Ayers T, Viganego F, et al. Efficacy of donor vaccination before hematopoietic cell transplantation and recipient vaccination both before and early after transplantation. Bone Marrow Transplant. 2004;33(3):337–46.
146. Wimperis JZ, Gottlieb D, Duncombe AS, Heslop HE, Prentice HG, Brenner MK. Requirements for the adoptive transfer of antibody responses to a priming antigen in man. J Immunol (Baltimore, Md: 1950). 1990;144(2):541–7.
147. Cordonnier C, Einarsdottir S, Cesaro S, Di Blasi R, Mikulska M, Rieger C, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(6):e200–e212.
148. Stark K, Gunther M, Neuhaus R, Reinke P, Schroder K, Linnig S, et al. Immunogenicity and safety of hepatitis A vaccine in liver and renal transplant recipients. J Infect Dis. 1999;180(6):2014–7.
149. Arslan M, Wiesner RH, Poterucha JJ, Zein NN. Safety and efficacy of hepatitis A vaccination in liver transplantation recipients. Transplantation. 2001;72(2):272–6.
150. Kotton CN, Hibberd PL. Travel medicine and the solid organ transplant recipient. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2009;9(Suppl 4):S273–81.
151. Jeon HJ, Ro H, Jeong JC, Koo TY, Han M, Min SI, et al. Efficacy and safety of hepatitis A vaccination in kidney transplant recipients. Transpl Infect Dis. 2014;16(3):511–5.
152. Ginsburg CM, Andrews W. Orthotopic hepatic transplantation for unimmunized children: a paradox of contemporary medical care. Pediatr Infect Dis J. 1987;6(8):764–5.
153. Martin K, Drabble A, Manlhiot C, Dipchand AI. Response to hepatitis A and B vaccination after pediatric heart transplant. Pediatr Transplant. 2012;16(7):699–703.
154. Gunther M, Stark K, Neuhaus R, Reinke P, Schroder K, Bienzle U. Rapid decline of antibodies after hepatitis A immunization in liver and renal transplant recipients. Transplantation. 2001;71 (3):477–9.
155. Arslan M, Wiesner RH, Poterucha JJ, Gross JB Jr, Zein NN. Hepatitis A antibodies in liver transplant recipients: evidence for loss of immunity posttransplantation. Liver Transpl. 2000;6(2):191–5.
156. Mariya FA, de Mello Perez R, Silva AE, Pestana JO, Ferraz ML. Prevalence of previous hepatitis A virus infection in renal transplant patients with hepatitis C: evidence of persistent anti-hepatitis A virus immune response. Transplant Proc. 2004;36(5):1500–1.
157. Godoi ER, de Souza VA, Cakmak S, Machado AF, Vilas Boas LS, Machado CM. Loss of hepatitis A virus antibodies after bone marrow transplantation. Bone Marrow Transplant. 2006;38(1):37–40.
158. Dignani MC, Miceli MH, Rosa CM, Gatica J, Martinez-Rolon J, Pizzolato M. Loss of hepatitis A virus (HAV) antibodies after peripheral stem cell transplantation (PSCT). Bone Marrow Transplant. 2003;31(9):809–12.
159. Unal Ince E, Ertem M, Ileri T, Sayili A, Belgemen T, Uysal Z. Significant loss of hepatitis A Ab after allogeneic hematopoietic SCT in pediatric patients. Bone Marrow Transplant. 2010;45 (1):171–5.
160. Carpenter PA, Englund JA. How I vaccinate blood and marrow transplant recipients. Blood. 2016;127(23):2824–32.
161. Connor BA, Blatter MM, Beran J, Zou B, Trofa AF. Rapid and sustained immune response against hepatitis A and B achieved with combined vaccine using an accelerated administration schedule. J Travel Med. 2007;14(1):9–15.
162. Ljungman P, Cordonnier C, Einsele H, Englund J, Machado CM, Storek J, et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant. 2009;44(8):521–6.
163. Storek J, Wells D, Dawson MA, Storer B, Maloney DG. Factors influencing B lymphopoiesis after allogeneic hematopoietic cell transplantation. Blood. 2001;98(2):489–91.
164. Palazzo M, Shah GL, Copelan O, Seier K, Devlin SM, Maloy M, et al. Revaccination after autologous hematopoietic stem cell transplantation is safe and effective in patients with

multiple myeloma receiving lenalidomide maintenance. Biol Blood Marrow Transplant. 2018;24(4):871–6.
165. Kotton CN, Kroger AT, Freedman DO. Immunocompromised travelers – Chapter 5 – 2020 Yellow Book|Travelers' Health|CDC. 2019. https://wwwnc.cdc.gov/travel/yellowbook/2020/travelers-with-additional-considerations/immunocompromised-travelers
166. Update: prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 2007;56(41):1080–4.
167. Askling HH, Rombo L, van Vollenhoven R, Hallen I, Thorner A, Nordin M, et al. Hepatitis A vaccine for immunosuppressed patients with rheumatoid arthritis: a prospective, open-label, multi-centre study. Travel Med Infect Dis. 2014;12(2):134–42.
168. Askling HH, Dalm VA. The medically immunocompromised adult traveler and pre-travel counseling: status quo 2014. Travel Med Infect Dis. 2014;12(3):219–28.
169. Rosen J. Travel medicine and the solid-organ transplant recipient. Infect Dis Clin N Am. 2013;27(2):429–57.
170. Nelson NP. Updated dosing instructions for immune globulin (human) GamaSTAN S/D for hepatitis A virus prophylaxis. MMWR Morb Mortal Wkly Rep. 2017;66(36):959–60.
171. Kroger AT, Robinson CL. Vaccination & immunoprophylaxis: general recommendations – Chapter 2 – 2020 Yellow Book|Travelers' Health|CDC. 2019. https://wwwnc.cdc.gov/travel/yellowbook/2020/preparing-international-travelers/vaccination-and-immunoprophylaxis-general-recommendations
172. Siber GR, Werner BG, Halsey NA, Reid R, Almeido-Hill J, Garrett SC, et al. Interference of immune globulin with measles and rubella immunization. J Pediatr. 1993;122(2):204–11.
173. Kroger AT, Duchin J, Vázquez M. General best practice guidelines for immunization. Best practices guidance of the Advisory Committee on Immunization Practices (ACIP). https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/downloads/general-recs.pdf
174. Victor JC, Monto AS, Surdina TY, Suleimenova SZ, Vaughan G, Nainan OV, et al. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. N Engl J Med. 2007;357(17):1685–94.
175. Landrigan PJ, Huber DH, Murphy GD 3rd, Creech WB, Bryan JA. The protective efficacy of immune serum globulin in hepatitis A: a statistical approach. JAMA. 1973;223(1):74–5.
176. Link-Gelles R, Hofmeister MG, Nelson NP. Use of hepatitis A vaccine for post-exposure prophylaxis in individuals over 40 years of age: a systematic review of published studies and recommendations for vaccine use. Vaccine. 2018;36(20):2745–50.
177. Lee S, Kim HW, Kim KH. Antibodies against hepatitis A and hepatitis B virus in intravenous immunoglobulin products. J Korean Med Sci. 2016;31(12):1937–42.
178. Tejada-Strop A, Costafreda MI, Dimitrova Z, Kaplan GG, Teo CG. Evaluation of potencies of immune globulin products against hepatitis A. JAMA Intern Med. 2017;177(3):430–2.
179. Averhoff F, Khudyakov Y, Bell BP. 176 – Hepatitis A Virus. In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (8th edn). Philadelphia, PA. Elsevier; 2015. p2095–112.e5

Hepatitis E Infections in Transplants

37

Olivier Marion and Nassim Kamar

Contents

Introduction 910
Disease Epidemiology: Transmission 911
Clinical Impact on Transplant: Both Solid Organ and Bone Marrow/Stem Cell 913
Clinical Manifestations 913
Chronic Infections 913
Extrahepatic Manifestations 914
Diagnosis Including Clinical Evaluation and Available/Emerging Diagnostics 915
Serological Tests 915
Antigen Detection 915
Molecular Diagnosis 915
Natural History and Diagnostic Algorithm 916
Management Including Emerging Treatment Modalities and Options for Prophylaxis/Prevention 916
Immunosuppressive Regimen Management 916
Ribavirin Monotherapy 917
Other Antivirals 918
Prevention 920
Conclusion 920
Key Points 920
References 920

Abbreviations

HEV	Hepatitis E virus
SVR	Sustained virological response

O. Marion · N. Kamar (✉)
Department of Nephrology, Dialysis and Organs Transplantation, CHU Rangueil, INSERM U1043, IFR –BMT, Paul Sabatier University, Toulouse, France
e-mail: marion-olivier@hotmail.fr; kamar.n@chu-toulouse.fr

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_36

Introduction

The hepatitis E virus (HEV) is a leading agent of viral hepatitis worldwide [1]. HEV is a member of *Hepeviridae* family. The strains that infect humans belong to *Orthohepevirus A* species for which eight genotypes have been identified [2]. Genotypes 1 and 2, which are restricted to humans, are responsible for acute hepatitis transmitted by fecal-oral route, principally via contaminated drinking water in low-income countries. Both genotypes are involved in epidemics and sporadic cases in resource-poor areas and are associated with severe manifestations in pregnant woman and patients with a preexisting liver disease [1]. In industrialized countries, HEV genotypes 3 and 4 are predominately observed, with a zoonotic reservoir mainly composed of pigs, wild boars, and rabbits [1]. HEV3 is widespread, whereas HEV4 is mainly found in Asian countries, such as China and Japan [3], although it was recently detected in Europe [4]. HEV genotypes 5 and 6 are known to infect wild boars, but no human infections have been reported to date. Finally, genotypes 7 and 8 were recently identified in camels and could infect humans occasionally [1, 5].

HEV is a small (27–34 nm) single-stranded, positive-sense RNA virus with an icosahedral capsid [1]. The HEV genome, which is approximately 7.2 kb long, is capped at the 5′ end and poly-adenylated at the 3′ end. It contains three or four open reading frames (ORFs) depending on the HEV genotype [6]. ORF1 encodes a nonstructural protein of approximately 1,700 amino acids containing the RNA-dependent RNA polymerase [1]. In genotype 1 strains, ORF4, the translation of which is promoted by endoplasmic reticulum stress, stimulates RNA polymerase activity [6]. ORF2 encodes the 660-amino acid capsid protein, which holds the immunogenic epitopes [1]. ORF2 protein is associated with infectious particles but is also massively secreted as free glycoproteins by infected cells [7]. The soluble form of ORF2 protein, which is the majority of ORF2 protein detected in patient serum, could inhibit antibody-mediated neutralization and participate in immune evasion [8]. Finally, ORF3 encodes a small phosphoprotein, which is 113 residues in genotype 3 and 114 residues in other genotypes, and is involved in virus egress [1]. While HEV virion is naked in the environment and feces, it was found be associated with a lipid membrane in the blood and urine of infected patients and in culture supernatants [9]. Therefore, like the hepatitis A virus, HEV is considered to be a quasi-enveloped virus [1].

Although most HEV3 and HEV4 infections cause self-limiting hepatitis in immunocompetent subjects, they can progress to chronic hepatitis in immunosuppressed patients such as solid organ transplant recipients [10, 11], stem cell transplant patients [12, 13], human immunodeficiency virus-infected patients with low CD4 T-cell count [14], and patients receiving chemotherapy or immunotherapy [15]. In this comprehensive review, we summarize current knowledge regarding HEV epidemiology, diagnosis, clinical manifestations, and treatments in transplant recipients.

Disease Epidemiology: Transmission

HEV prevalence in transplant recipients is not easy to determine because of the significant variability of HEV prevalence within countries and the different serological assays used. HEV seroprevalence in solid organ transplant recipients obtained from endemic and non-endemic areas is between 8.3% and 43%, depending on the HEV endemicity in the areas studied and the eating habits of populations [16–18]. The prevalence and incidence of replicative HEV infection, attested by HEV RNA detection in blood, range from 0% to 3.2% and 1–3.2/100 person-years, respectively [16].

HEV is mainly transmitted enterically. The discovery that porcine HEV also infects humans revealed that hepatitis E is a zoonosis [19]. Therefore, HEV 3 and 4 contaminations are principally linked to direct contact with the animal reservoir, which mainly consists of domestic pigs, wild boars, rabbits, and deer, or the consumption of undercooked meat or offal [20]. In addition to zoonotic transmission, blood products have been identified as a potential source of HEV [21]. Only a minority of the recipients of contaminated blood products develops symptomatic hepatitis E. Nevertheless, when administered to immunosuppressed patients, the risk of developing chronic HEV infection increased [22]. The risk of blood-derived HEV infection correlates with the incidence of HEV RNA among blood donors according to the country (Table 1). Moreover, recent data suggests that HEV viremia could be prolonged by more than 2 months in healthy blood donors, thereby increasing the risk of transmission through multiple donations [23]. Transplant patients often receive red blood cell transfusions during the perioperative period or fresh plasma administered in desensitization protocols. In solid organ transplant recipients, several cases of transfusion-transmitted HEV infection have been already published [21, 24]. The minimal total infectious dose of HEV virions through transfusion seems to be approximately 1×10^4 international unit (IU) [21]. More recently, plasma exchange, including with pathogen-reduced plasma, has been associated with HEV infection in kidney transplant patients [25]. Consequently, countries with a high HEV prevalence are now evaluating the general screening for HEV RNA in blood products so that they can provide HEV-negative products for organ transplant recipients [21].

Rare cases of graft-transmitted HEV infections have been reported for genotype 3 in kidney and liver recipients [26, 27]. For genotype 4, multiple HEV transmissions that have affected five transplant recipients have been recently described from the same donor with HEV viremia [28]. The incubation period ranged between 34 and 67 days. In this case, all the HEV infections evolved to chronic hepatitis [28].

No reactivation of HEV infection was noted after solid organ transplantation for anti-HEV IgG-positive patients [16]. Moreover, low anti-HEV antibodies (<7 IU/ml) did not seem to protect solid organ transplant recipients from HEV reinfection or from the development of chronic infection [29]. Similarly, in rhesus macaques, a mean anti-HEV IgG level of 7.1 IU/ml only led to partial protection against reinfections. This leads to the notion that serum IgG level is correlated with

Table 1 HEV RNA detection in healthy blood donors

Country	Genotypes	Methods	Donations	Results	References
Spain	HEV3	Individual testing	9,998	1/3,333	Sauleda et al. [80]
Spain	HEV3	Individual testing	11,313	1/2,828	Rivero-Juarez et al. [81]
Austria	HEV3	Pools of 96	58,915	1/8,416	Fischer et al. [82]
Poland	HEV3	Individual testing	12,664	1/1,266	Grabarczyk et al. [83]
The Netherlands	HEV3	Pools of 96/192	59,474	1/1,440	Hogema et al. [84]
Denmark	HEV3	Individual testing	25,637	1/2,331	Harritshøj et al. [85]
Sweden	HEV3	Pools of 96	95,835	1/7,986	Baylis et al. [86]
Germany	HEV3	Pools of 24	18,737	1/815	Westhölter et al. [87]
France	HEV3	Pools of 96	53,234	1/2,218	Gallian et al. [88]
Italy	HEV3	Individual testing	198	1/198	Marcantonio et al. [89]
Ireland	HEV3	Individual testing	24,985	1/5,000	O'Riordan et al. [90]
England	HEV3	Pools of 24	1,838,747	1/3,831	Harvala et al. [91]
Scotland	HEV3	Pools of 24	94,302	1/2,481	Thom et al. [92]
United States	HEV3	Individual testing	18,829	1/9,500	Stramer et al. [93]
United States	HEV3	Pools of 96	128,020	1/42,673	Roth et al. [94]
Australia	HEV3	Pools of 6	74,131	1/74,131	Hoad et al. [95]
Thailand	HEV3	Pools of 6	30,115	1/1,158	Intharasongkroh et al. [96]
China	HEV4	Individual testing	5,345	1/356	Wen et al. [97]
China	HEV4	Individual testing	10,741	1/2,500	Ren et al. [98]
Japan	HEV3	Pools of 50 or 500	620,140	1/15,075	Minagi et al. [99]

HEV hepatitis E virus

the degree of protection and that HEV reinfection can occur despite preexistent humoral immunity [30].

In bone marrow transplant recipients, few data are available concerning HEV infection epidemiology. In the Netherlands, a retrospective cross-sectional study showed that the incidence of HEV infection in allogeneic hematopoietic stem cell transplant recipients was 2.4% (8/328) [31]. Reactivation of HEV infection in anti-HEV IgG-positive patients seems to be anecdotal after bone marrow transplantation [32, 33]. The mode of transmission of HEV has not been well studied in this population. However, one case of transmission via a hematopoietic stem cell donor has been reported [13].

Most chronic HEV infections that have been reported are genotype 3-infected immunocompromised patients [10]. Recently, 12 cases of genotype 4 chronic infections were also published [11, 28, 34]. Moreover, one case of chronic HEV genotype 7 infection was reported in a liver transplant recipient from the Middle East who consumed camel-derived food products including milk and meat [5].

More recently, rat hepatitis E virus has been implicated in chronic hepatitis in liver transplant patients, which required antiviral treatment [35]. Therefore, *Orthohepevirus C* could also be a source of zoonotic hepatitis in immunosuppressed patients. Further studies are needed to define the pathogenicity of rat strains in humans and the mechanisms that lead to the zoonotic capacities of *Orthohepevirus*.

Clinical Impact on Transplant: Both Solid Organ and Bone Marrow/Stem Cell

Clinical Manifestations

In immunocompetent subjects, hepatitis E infections are mostly asymptomatic or self-limiting illnesses [1]. The main symptoms associated with acute hepatitis E are jaundice, weakness, and weight loss. In immunocompromised subjects, two-thirds of the infected HEV patients are also asymptomatic [36]. Fatigue and diarrhea are the main symptoms observed at diagnosis, while jaundice is rare [36]. Fulminant hepatic failure is exceptional in the case of acute hepatitis E in transplant recipients [37]. The increase in liver enzyme levels is usually low in transplanted patients, sometimes less than twofold the upper limit of the normal value, and can mimic drug-induced liver injury. Consequently, the diagnosis of HEV infection may be delayed in some cases.

Chronic Infections

Solid organ transplant patients are the immunocompromised patients among whom chronic HEV infections are most often reported. Typically, patients with resolving hepatitis eliminate the virus within the first month following infection. Without any modification of the immunosuppressive regimen, no case of spontaneous HEV clearance has been reported beyond 3 months after infection [38]. Therefore, patients with persistent HEV replication beyond 3 months are considered to be chronically infected [38]. Approximately two-thirds of HEV-infected solid organ transplant recipients develop chronic HEV infection [36, 39]. Rapid progression of liver fibrosis can result in cirrhosis in ~10% of chronically infected transplant patients within a few years [10, 36, 40]. The histological aspect of HEV hepatitis is nonspecific, with inflammatory infiltrates within both portal tracts and the lobules [10, 40, 41]. Mixed lymphocytic and neutrophil polymorph cholangitis are also reported [41]. Strong immunosuppression is associated with an increased risk of chronic development, specifically, an impaired adaptive T-cell immune response [42]. Chronic infections were associated with the use of tacrolimus-based

immunosuppressive therapy, rather than cyclosporine A [36]. In vitro studies have shown that mTOR inhibitors and calcineurin inhibitors promote HEV replication [43]. Conversely, mycophenolic acid inhibits HEV replication in vitro, with additional antiviral potency when associated with ribavirin [44]. Nevertheless, clinical studies do not strongly support such effects, probably due to the significant level of T-cell inhibition induced by mycophenolic acid [36]. In fact, low lymphocyte subset counts have been clearly associated with increased chronic development [10, 39]. Similarly, low liver enzyme levels during infection and a shorter delay after transplantation or after the last treated acute rejection episode have been associated with chronic hepatitis E, which highlights the deleterious impact of a decreased immune response [10, 36]. Finally, greater quasispecies heterogeneity has been associated with persistent HEV infection whereby the emergence of viruses with an enhanced ability to persist is promoted [45].

Few data are available concerning the course of HEV infection in HSCT recipients. Most infected patients are asymptomatic but usually show a high increase in liver enzyme levels [12]. Liver histological analyses show active hepatitis with T-cell infiltrates which can be associated with severe fibrosis [12, 31]. The rate of progression to chronicity is ~60%, similar to that of solid organ transplant recipients [31]. In general, patients cleared HEV during the cessation of immunosuppressive therapy introduced to treat or prevent graft versus host disease [31].

Extrahepatic Manifestations

HEV can cause extrahepatic manifestations, as suggested by its ability to replicate in a large number of cell types in vitro, including neuronal-derived cells and kidney cells [1]. The most common extrahepatic manifestations reported in both immunocompetent and immunocompromised HEV-infected patients are neurological disorders, mostly Guillain-Barré syndrome, [46] neurologic amyotrophy, and encephalitis [47, 48]. Therefore, 5–11% of patients with Guillain-Barré syndrome have signs of very early HEV infection [46, 49]. In patients with acute hepatitis E, a high frequency of neuropathic pain suggesting small fiber neuropathy and painless sensory disorders has been recently reported [50]. All neurological manifestations were more frequent in immunocompetent subjects compared to immunocompromised patients, suggesting pathophysiological mechanisms involving the immune system [50]. Moreover, in some patients, HEV variants characterized in cerebrospinal fluid differed from those found in serum, suggesting the appearance of neurotropic variants [48].

Acute hepatitis E has been associated with decreased kidney function in both liver and kidney transplant recipients [51]. In solid organ transplant patients, HEV infection is associated with a high frequency of cryoglobulinemia, ranging from ~35% at the acute phase to ~50% in chronically infected patients [52]. Most cases of cryoglobulinemia are asymptomatic and disappear after viral clearance, but some cases of HEV-induced cryoglobulinemia are associated with membranoproliferative glomerulonephritis or rheumatologic symptoms [51–53]. Other specific

nephropathies have been identified during HEV infection, including membranous nephropathy and relapsing IgA nephropathy after kidney transplantation [51, 54]. The mechanisms responsible for renal damage have been poorly studied; HEV RNA and antigen have been detected in the urine of HEV-infected transplant patients [9].

Diagnosis Including Clinical Evaluation and Available/Emerging Diagnostics

Serological Tests

Despite a significant genetic variability, only one serotype is described for HEV. Therefore, anti-HEV antibodies cross-react between genotypes [30]. Acute HEV infection can be diagnosed by detecting specific anti-HEV antibodies by various commercial enzyme immunoassays. For acute infection in solid organ transplant recipients, anti-HEV IgM assays have a sensitivity of approximately 85% but a high specificity of >99% [55]. In the same phase, the sensitivity of anti-HEV IgG antibodies is only 45% and usually indicates a past infection. The detection limits of commercial assays for anti-IgG vary between 0.25 and 2.5 IU per ml. Although it does not improve the diagnostic performance of serological tests at the acute phase of infection, the quantification of anti-HEV IgG could be useful to assess the risk of reinfection in immunocompromised patients in high endemic areas [18]. For bone marrow transplant patients, the sensitivity of serological tests seems to be lower, which argues for direct HEV RNA detection [56].

Antigen Detection

The secreted form of ORF2 capsid protein can also be detected in the serum and urine of immunocompromised patients during acute hepatitis E [9]. The sensitivity and specificity of commercial assays in serum are, respectively, 94% and 100%, and seem to be similar in urine [9, 57]. Considering that this is more sensitive than anti-HEV IgM testing, it could be an alternative to RNA detection in laboratories without molecular diagnostic devices. It is interesting to note that a high level of serum HEV antigen during acute hepatitis E has been associated with an increased risk in the development of chronic HEV infection in solid organ transplant recipients [9]. Further studies are needed to clarify the role of soluble ORF2 protein in chronically infected patients and its importance in patient follow-up.

Molecular Diagnosis

Molecular detection of HEV RNA is the gold standard for active hepatitis E diagnosis. DNA primers are targeted in regions that are highly conserved across genotypes, generally ORF3 [58]. The performance of RT-PCR tests varies widely,

and assays that include the WHO international standard should be used [58, 59]. With recent commercial testing, the lower limits of detection are approximately 7–80 IU/ml [59]. The serum and stool samples of immunocompromised patients should be tested for HEV RNA to confirm the diagnosis of hepatitis E and to assess the progression of HEV infection and the response to antiviral therapy. However, the presence of PCR inhibitors in a feces-derived matrix can make detecting HEV RNA difficult, and the extraction protocol should be strictly validated in each laboratory [60].

HEV genotypes and subgenotypes can be determined by sequencing a part of the ORF2 region. This could be a useful tool for epidemiological monitoring and to identify the source of infection. Some mutations in the ORF1 region (RNA-dependent RNA polymerase) have been associated with a decrease in susceptibility to ribavirin in vitro [61, 62]. Nevertheless, their detection in clinical practice does not seem to have any impact on the therapeutic response or the management of antiviral therapy [63, 64].

Natural History and Diagnostic Algorithm

After contamination, 2–4 weeks of incubation are usually described during which HEV RNA and antigens can be detected in blood samples. Subsequently, HEV RNA persists in blood and feces for ~4 and ~6 weeks, respectively, in immunocompetent patients [1]. Capsid antigens could persist longer than detectable viremia in immunosuppressed patients. Anti-HEV IgM appears during the symptomatic period, mainly revealed by elevated liver enzyme levels. It then persists for several months [1]. In general, specific IgG develops secondarily but persists several years. The persistence of HEV RNA in blood and stool for more than 3 months characterizes a chronic HEV infection [38]. The diagnostic algorithm for the suspicion of HEV infection in immunocompromised patients is summarized in Fig. 1.

Management Including Emerging Treatment Modalities and Options for Prophylaxis/Prevention

Immunosuppressive Regimen Management

No data is available concerning the optimal management of acute HEV infection. In chronically infected patients, reducing immunosuppressive therapies that target T-cells is the first therapeutic option [39]. In fact, tacrolimus levels and steroid doses were lower in solid organ transplant recipients who eliminate the HEV compared to those who remained viremic [39]. Approximately, one-third of patients achieve viral clearance after immunosuppressive drug doses are reduced, especially calcineurin and mTOR inhibitors [39]. Effective antiviral therapy is needed for patients who remain viremic and for those whose immunosuppressants cannot be reduced.

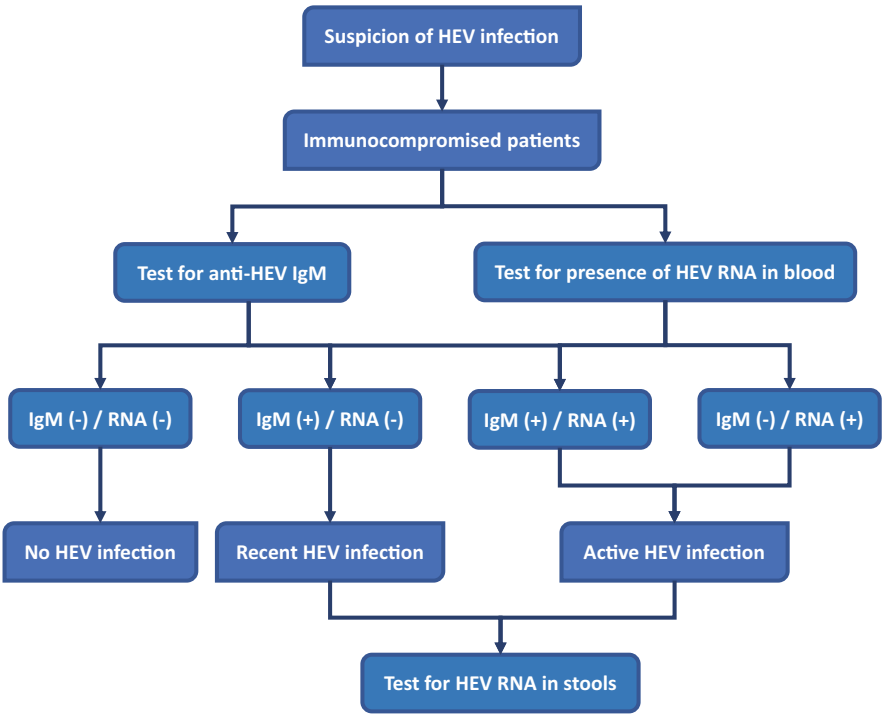


Fig. 1 Diagnostic algorithm for HEV infection in immunocompromised patients. Abbreviations: HEV, hepatitis E virus

Ribavirin Monotherapy

Ribavirin, a guanosine analogue, has shown anti-HEV activity both in vivo and in vitro [65]. Its pleiotropic antiviral activity against HEV is not fully understood but has been associated with cellular GTP pool depletion and mutagenesis [65, 66]. Several studies have demonstrated the efficacy of oral ribavirin alone in the treatment of chronic hepatitis E [67, 68]. Overall sustained virological response (SVR) rates range from 63% to 78% after 3–6 months of monotherapy. Nevertheless, no statistically significant difference has been noticed between patients who were treated for 3 months or less and those who received ribavirin for more than 3 months [67]. Consequently, a 12-week long ribavirin treatment is currently recommended as first-line drug therapy for immunosuppressed patients with persisting HEV replication (see Fig. 2 for treatment algorithm) [18]. In chronically HEV-infected transplant recipients, a fall in HEV RNA of >0.5 $\log_{10}$IU/ml at day 7 of treatment predicted SVR with a positive predictive value of 88% [69]. However, ribavirin trough levels that were checked at day 7 or at steady state had no impact on SVR [69]. Relapses after ribavirin cessation have also been associated with lower liver enzyme levels or low lymphocyte counts at the

initiation of treatment, which probably indicates deeper immunosuppression and a lower T-cell immune response [64, 67]. Persistent HEV shedding in stools at the end of ribavirin therapy leads to systematic relapse [64]. Therefore, patients on antiviral therapy should be tested for HEV RNA in both serum and stools before ribavirin withdrawal. In the case of persistent HEV excretion in feces, ribavirin should be prolonged for 12 additional weeks [64]. Finally, HEV clearance can be established 12 weeks after ribavirin cessation if HEV RNA in both blood and stools remains undetectable [70].

Ribavirin treatment can be associated with side effects, mostly dose-dependent anemia [67]. Ribavirin doses should be carefully adapted to kidney function [71].

Cases of ribavirin treatment failures despite multiple retreatments have been reported. A G1634R mutation has been identified in the RNA-dependent RNA polymerase in patients who relapse [61]. This mutation does not provide ribavirin resistance in vitro but increases the replicative capacity of HEV [62]. Nevertheless, in clinical practice, the presence of the G1634R mutation before treatment had no impact on the early virological response, SVR, or virological response after retreatment [63, 64]. Other mutations have also been recently described including Y1320H, K1383N, Y1587F, V1479I, and D1384G [62, 66]. Their impact on ribavirin treatment response in vivo remains speculative.

In stem cell transplant recipients, only few case series have been published on the treatment of chronically HEV-infected patients. A 3-month course of ribavirin showed good efficacy in this setting, enabling continuation of cytotoxic treatments for underlying malignancies [56].

Other Antivirals

Despite only moderate in vitro anti-HEV activity [72], pegylated interferon has been successfully used to treat some chronically infected liver transplants [73, 74]. Nevertheless, interferon is usually considered contraindicated in other organ transplant patients because of the increased risk of acute rejection due to the immunostimulatory effect [75].

Some in vitro studies show an antiviral effect of sofosbuvir against HEV [76]. Sofosbuvir, a nucleotide analogue, is a direct-acting anti-hepatitis C virus drug that targets HCV RNA-dependent RNA polymerase. Data suggests that a combination of sofosbuvir and ribavirin provides an additive antiviral effect against HEV replication in vitro [76]. Nevertheless, pharmacological data suggest that an inhibitory concentration of sofosbuvir for HEV is tenfold that for the hepatitis C virus (HCV), theoretically requiring major dose adjustment [77]. Finally, no convincing in vivo data support the use of sofosbuvir, as highlighted by several treatment failures [78].

Some chronically HEV-infected immunosuppressed patients do not achieve SVR even after repeated ribavirin treatment. For these patients, further research is warranted to find new antivirals to cure HEV infection.

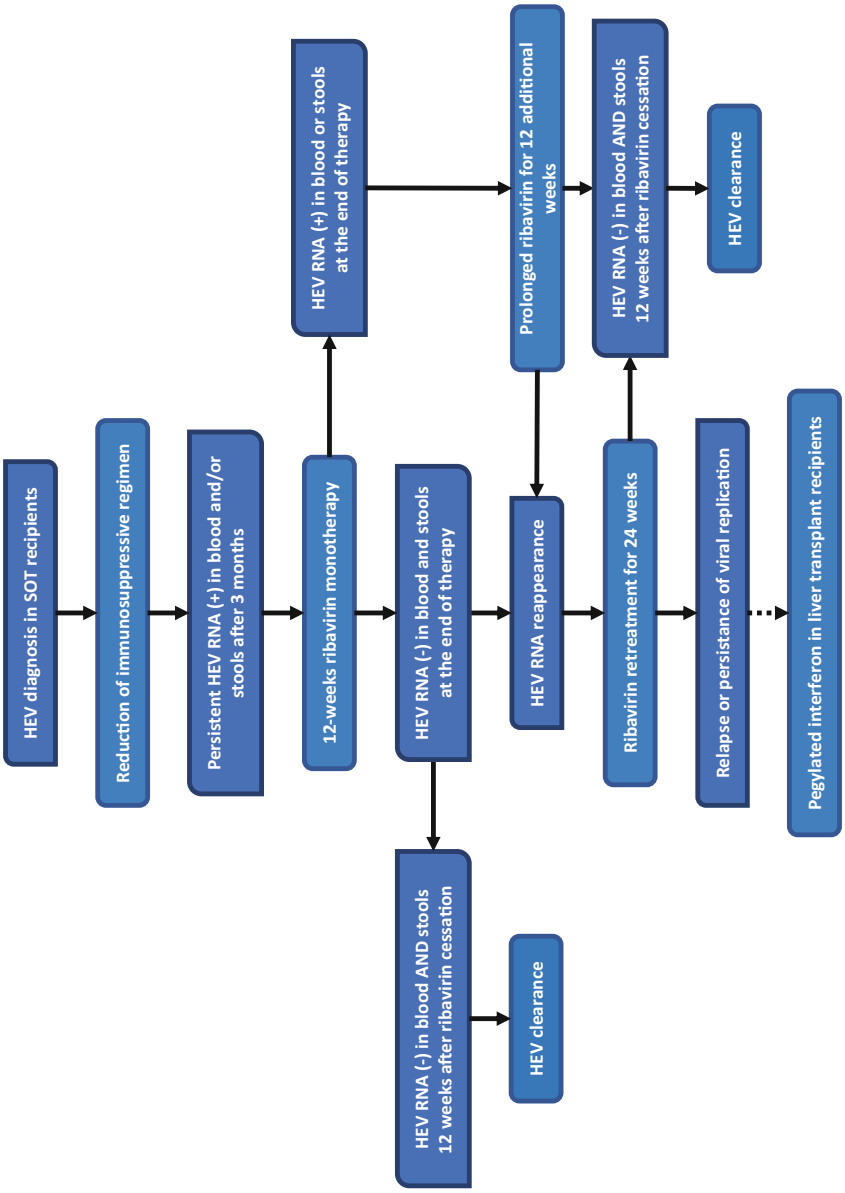


Fig. 2 Management of chronic HEV infection in solid organ transplant recipients. Abbreviations: HEV, hepatitis E virus; SOT, solid organ transplant

Prevention

HEV transmission could be prevented by avoiding the consumption of undercooked meat, game products, or offal in populations at risk for chronic infection. One effective vaccine candidate has been developed but is only licensed in China [79]. Its efficacy has not been evaluated in immunosuppressed patients with a risk of a decreased immune response to a vaccine.

Conclusion

HEV has been recognized as a major causative agent of acute viral hepatitis worldwide. In immunocompromised patients, especially transplant recipients, HEV causes acute and chronic infection with a high risk of rapidly progressive liver fibrosis. Ribavirin monotherapy has shown good efficacy in the treatment of chronic HEV infection. However, cases of HEV infection that are refractory to ribavirin have emerged, requiring the development of new antiviral therapies.

Key Points

- The hepatitis E virus is responsible for acute and chronic hepatitis in solid organ transplant recipients.
- Approximately two-thirds of HEV-infected transplant patients develop chronic infections.
- A 12-week ribavirin treatment is recommended as first-line drug therapy for immunosuppressed patients with persisting HEV replication.

References

1. Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, et al. Hepatitis E virus infection. Nat Rev Dis Primers. 2017;3:17086.
2. Smith DB, Simmonds P. Classification and genomic diversity of enterically transmitted hepatitis viruses. Cold Spring Harb Perspect Med. 2018;8(9):pii: a031880.
3. Kamar N, Pischke S. Acute and Persistent hepatitis E virus genotype 3 and 4 infection: clinical features, pathogenesis, and treatment. Cold Spring Harb Perspect Med. 2018.
4. Hakze-van der Honing RW, van Coillie E, Antonis AFG, van der Poel WHM. First isolation of hepatitis E virus genotype 4 in Europe through swine surveillance in the Netherlands and Belgium. PLoS One. 2011;6(8):e22673.
5. Lee G-H, Tan B-H, Teo EC-Y, Lim S-G, Dan Y-Y, Wee A, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. Gastroenterology. 2016;150(2):355–357.e3.
6. Nair VP, Anang S, Subramani C, Madhvi A, Bakshi K, Srivastava A, et al. Endoplasmic reticulum stress induced synthesis of a novel viral factor mediates efficient replication of genotype-1 hepatitis E virus. PLoS Pathog. 2016;12(4):e1005521.

7. Montpellier C, Wychowski C, Sayed IM, Meunier J-C, Saliou J-M, Ankavay M, et al. Hepatitis E virus lifecycle and identification of 3 forms of the ORF2 capsid protein. Gastroenterology. 2018;154(1):211–223.e8.
8. Yin X, Ying D, Lhomme S, Tang Z, Walker CM, Xia N, et al. Origin, antigenicity, and function of a secreted form of ORF2 in hepatitis E virus infection. Proc Natl Acad Sci. 2018;115(18): 4773–8.
9. Marion O, Capelli N, Lhomme S, Dubois M, Pucelle M, Abravanel F, et al. Hepatitis E virus genotype 3 and capsid protein in the blood and urine of immunocompromised patients. J Infect. 2019;78(3):232–240.
10. Kamar N, Selves J, Mansuy J-M, Ouezzani L, Péron J-M, Guitard J, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med. 2008;358(8):811–7.
11. Sridhar S, Chan JFW, Yap DYH, Teng JLL, Huang C, Yip CCY, et al. Genotype 4 hepatitis E virus is a cause of chronic hepatitis in renal transplant recipients in Hong Kong. J Viral Hepat. 2018;25(2):209–13.
12. Tavitian S, Péron J-M, Huynh A, Mansuy J-M, Ysebaert L, Huguet F, et al. Hepatitis E virus excretion can be prolonged in patients with hematological malignancies. J Clin Virol. 2010;49(2):141–4.
13. Koenecke C, Pischke S, Beutel G, Ritter U, Ganser A, Wedemeyer H, et al. Hepatitis E virus infection in a hematopoietic stem cell donor. Bone Marrow Transplant. 2014;49(1):159–60.
14. Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. N Engl J Med. 2009;361(10):1025–7.
15. Ollier L, Tieulie N, Sanderson F, Heudier P, Giordanengo V, Fuzibet J-G, et al. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. Ann Intern Med. 2009;150(6):430–1.
16. Legrand-Abravanel F, Kamar N, Sandres-Saune K, Lhomme S, Mansuy J-M, Muscari F, et al. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. Emerg Infect Dis. 2011;17(1):30–7.
17. Moal V, Legris T, Motte A, Vacher-Coponat H, Fages L, Jourde-Chiche N, et al. Systematic serological testing for hepatitis E virus in kidney transplant recipients. J Clin Microbiol. 2015;53(5):1523–30.
18. European Association for the Study of the Liver. EASL clinical practice guidelines on hepatitis E virus infection. J Hepatol. 2018;68:1256–71.
19. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA. 1997;94(18):9860–5.
20. Pavio N, Meng X-J, Doceul V. Zoonotic origin of hepatitis E. Curr Opin Virol. 2015;10:34–41.
21. Izopet J, Lhomme S, Chapuy-Regaud S, Mansuy J-M, Kamar N, Abravanel F. HEV and transfusion-recipient risk. Transfus Clin Biol. 2017;24(3):176–81.
22. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet. 2014;384(9956):1766–73.
23. Hogema BM, Molier M, Sjerps M, de Waal M, van Swieten P, van de Laar T, et al. Incidence and duration of hepatitis E virus infection in Dutch blood donors. Transfusion (Paris). 2016;56(3):722–8.
24. Westhölter D, Hiller J, Denzer U, Polywka S, Ayuk F, Rybczynski M, et al. HEV-positive blood donations represent a relevant infection risk for immunosuppressed recipients. J Hepatol. 2018;69(1):36–42.
25. Mallet V, Sberro-Soussan R, Roque-Afonso A-M, Vallet-Pichard A, Deau B, Portal A, et al. Transmission of hepatitis E virus with plasma exchange in kidney transplant recipients: a retrospective cohort study. Transplantation. 2018;102(8):1351–7.
26. Pourbaix A, Ouali N, Soussan P, Roque Afonso AM, Péraldi M-N, Rondeau E, et al. Evidence of hepatitis E virus transmission by renal graft. Transpl Infect Dis. 2017;19:e12624
27. Schlosser B, Stein A, Neuhaus R, Pahl S, Ramez B, Krüger DH, et al. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. J Hepatol. 2012;56(2):500–2.

28. Sridhar S, Cheng VCC, Wong S-C, Yip CCY, Wu S, Lo AWI, et al. Donor-derived genotype 4 hepatitis E virus infection, Hong Kong, China, 2018. Emerg Infect Dis. 2019;25(3):425–33.
29. Abravanel F, Lhomme S, Chapuy-Regaud S, Mansuy J-M, Muscari F, Sallusto F, et al. Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. J Infect Dis. 2014;209(12):1900–6.
30. Huang W, Zhang H, Harrison TJ, Lang S, Huang G, Wang Y. Cross-protection of hepatitis E virus genotypes 1 and 4 in rhesus macaques. J Med Virol. 2008;80(5):824–32.
31. Versluis J, Pas SD, Agteresch HJ, de Man RA, Maaskant J, Schipper MEI, et al. Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation. Blood. 2013;122(6):1079–86.
32. le Coutre P, Meisel H, Hofmann J, Röcken C, Vuong GL, Neuburger S, et al. Reactivation of hepatitis E infection in a patient with acute lymphoblastic leukaemia after allogeneic stem cell transplantation. Gut. 2009;58(5):699–702.
33. Abravanel F, Mansuy J-M, Huynh A, Kamar N, Alric L, Peron J-M, et al. Low risk of hepatitis E virus reactivation after haematopoietic stem cell transplantation. J Clin Virol. 2012;54(2):152–5.
34. Wang Y, Chen G, Pan Q, Zhao J. Chronic hepatitis E in a renal transplant recipient: the first report of genotype 4 hepatitis E virus caused chronic infection in organ recipient. Gastroenterology. 2018;154(4):1199–201.
35. Sridhar S, Yip CCY, Wu S, Cai J, Zhang AJ-X, Leung K-H, et al. Rat hepatitis E virus as cause of persistent hepatitis after liver transplant. Emerg Infect Dis. 2018;24(12):2241–50.
36. Kamar N, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. Gastroenterology. 2011;140(5):1481–9.
37. Tenorio González E, Robles Díaz M, Sanjuan Jiménez R, González Grande R, Olmedo Martín RV, Rodrigo López JM, et al. Retransplant due to fulminant hepatic failure from hepatitis E virus: a case report. Transplant Proc. 2018;50(2):685–6.
38. Kamar N, Rostaing L, Legrand-Abravanel F, Izopet J. How should hepatitis E virus infection be defined in organ-transplant recipients? Am J Transplant. 2013;13(7):1935–6.
39. Kamar N, Abravanel F, Selves J, Garrouste C, Esposito L, Lavayssière L, et al. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. Transplantation. 2010;89(3):353–60.
40. Kamar N, Mansuy J-M, Cointault O, Selves J, Abravanel F, Danjoux M, et al. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. Am J Transplant. 2008;8(8):1744–8.
41. Malcolm P, Dalton H, Hussaini HS, Mathew J. The histology of acute autochthonous hepatitis E virus infection. Histopathology. 2007;51(2):190–4.
42. Suneetha PV, Pischke S, Schlaphoff V, Grabowski J, Fytili P, Gronert A, et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. Hepatology. 2012;55(3):695–708.
43. Zhou X, Wang Y, Metselaar HJ, Janssen HLA, Peppelenbosch MP, Pan Q. Rapamycin and everolimus facilitate hepatitis E virus replication: revealing a basal defense mechanism of PI3K-PKB-mTOR pathway. J Hepatol. 2014;61(4):746–54.
44. Wang Y, Zhou X, Debing Y, Chen K, Van Der Laan LJW, Neyts J, et al. Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. Gastroenterology. 2014;146(7):1775–83.
45. Lhomme S, Abravanel F, Dubois M, Sandres-Saune K, Rostaing L, Kamar N, et al. Hepatitis E virus quasispecies and the outcome of acute hepatitis E in solid-organ transplant patients. J Virol. 2012;86(18):10006–14.
46. van den Berg B, van der Eijk AA, Pas SD, Hunter JG, Madden RG, Tio-Gillen AP, et al. Guillain-Barré syndrome associated with preceding hepatitis E virus infection. Neurology. 2014;82(6):491–7.
47. van Eijk JJJ, Dalton HR, Ripellino P, Madden RG, Jones C, Fritz M, et al. Clinical phenotype and outcome of hepatitis E virus-associated neuralgic amyotrophy. Neurology. 2017;89:909–17.

48. Kamar N, Bendall RP, Peron JM, Cintas P, Prudhomme L, Mansuy JM, et al. Hepatitis E virus and neurologic disorders. Emerg Infect Dis. 2011;17(2):173–9.
49. Stevens O, Claeys KG, Poesen K, Saegeman V, Van Damme P. Diagnostic challenges and clinical characteristics of hepatitis E virus-associated Guillain-Barré syndrome. JAMA Neurol. 2017;74(1):26–33.
50. Abravanel F, Pique J, Couturier E, Nicot F, Dimeglio C, Lhomme S, et al. Acute hepatitis E in French patients and neurological manifestations. J Infect. 2018;77(3):220–6.
51. Kamar N, Weclawiak H, Guilbeau-Frugier C, Legrand-Abravanel F, Cointault O, Ribes D, et al. Hepatitis E virus and the kidney in solid-organ transplant patients. Transplantation. 2012;93(6):617–23.
52. Marion O, Abravanel F, Bello AD, Esposito L, Lhomme S, Puissant-Lubrano B, et al. Hepatitis E virus-associated cryoglobulinemia in solid-organ-transplant recipients. Liver Int. 2018;38:2178–89.
53. Pischke S, Behrendt P, Manns MP, Wedemeyer H. HEV-associated cryoglobulinaemia and extrahepatic manifestations of hepatitis E. Lancet Infect Dis. 2014;14(8):678–9.
54. Taton B, Moreau K, Lepreux S, Bachelet T, Trimoulet P, De Ledinghen V, et al. Hepatitis E virus infection as a new probable cause of de novo membranous nephropathy after kidney transplantation. Transpl Infect Dis. 2013;15(6):E211–5.
55. Abravanel F, Chapuy-Regaud S, Lhomme S, Miedougé M, Peron J-M, Alric L, et al. Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients. J Clin Virol. 2013;58(4):624–8.
56. Tavitian S, Peron J-M, Huguet F, Kamar N, Abravanel F, Beyne-Rauzy O, et al. Ribavirin for chronic hepatitis prevention among patients with hematologic malignancies. Emerg Infect Dis. 2015;21(8):1466–9.
57. Trémeaux P, Lhomme S, Chapuy-Regaud S, Peron J-M, Alric L, Kamar N, et al. Performance of an antigen assay for diagnosing acute hepatitis E virus genotype 3 infection. J Clin Virol. 2016;79:1–5.
58. Abravanel F, Sandres-Saune K, Lhomme S, Dubois M, Mansuy J-M, Izopet J. Genotype 3 diversity and quantification of hepatitis E virus RNA. J Clin Microbiol. 2012;50(3):897–902.
59. Abravanel F, Chapuy-Regaud S, Lhomme S, Dubois M, Peron J-M, Alric L, et al. Performance of two commercial assays for detecting hepatitis E virus RNA in acute or chronic infections. J Clin Microbiol. 2013;51(6):1913–6.
60. Abravanel F, Lacipière A, Lhomme S, Dubois M, Minier L, Peron J-M, et al. Performance of a commercial assay for detecting and quantifying HEV RNA in faeces. J Clin Virol. 2018;109:1–5.
61. Debing Y, Gisa A, Dallmeier K, Pischke S, Bremer B, Manns M, et al. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. Gastroenterology. 2014;147(5):1008–1011.e7; quiz e15–16.
62. Debing Y, Ramière C, Dallmeier K, Piorkowski G, Trabaud M-A, Lebossé F, et al. Hepatitis E virus mutations associated with ribavirin treatment failure result in altered viral fitness and ribavirin sensitivity. J Hepatol. 2016;65:499–508.
63. Lhomme S, Kamar N, Nicot F, Ducos J, Bismuth M, Garrigue V, et al. Mutation in the Hepatitis E virus polymerase and outcome of ribavirin therapy. Antimicrob Agents Chemother. 2015;60(3):1608–14.
64. Marion O, Lhomme S, Bello AD, Abravanel F, Esposito L, Hébral AL, et al. Monitoring hepatitis E virus fecal shedding to optimize ribavirin treatment duration in chronically infected transplant patients. J Hepatol [Internet]. 2018. Disponible sur: https://www.journal-of-hepatology.eu/article/S0168-8278(18)32385-7/abstract
65. Debing Y, Emerson SU, Wang Y, Pan Q, Balzarini J, Dallmeier K, et al. Ribavirin inhibits in vitro Hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with alpha interferon. Antimicrob Agents Chemother. 2014;58(1):267–73.
66. Todt D, Gisa A, Radonic A, Nitsche A, Behrendt P, Suneetha PV, et al. In vivo evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. Gut. 2016;65(10):1733–43.

67. Kamar N, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N Engl J Med. 2014;370(12):1111–20.
68. Pischke S, Hardtke S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, et al. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. Liver Int. 2013;33(5): 722–6.
69. Kamar N, Lhomme S, Abravanel F, Cointault O, Esposito L, Cardeau-Desangles I, et al. An early viral response predicts the virological response to ribavirin in hepatitis E virus organ transplant patients. Transplantation. 2015;99(10):2124–31.
70. Marion O, Lhomme S, Abravanel F, Izopet J, Kamar N. Should 12- or 24-week post-ribavirin follow-up be considered to define sustained virological response in transplant patients treated for chronic hepatitis E virus infection? Transpl Infect Dis. 2019;21:e13065.
71. Kamar N, Chatelut E, Manolis E, Lafont T, Izopet J, Rostaing L. Ribavirin pharmacokinetics in renal and liver transplant patients: evidence that it depends on renal function. Am J Kidney Dis. 2004;43(1):140–6.
72. Todt D, François C, Anggakusuma, Behrendt P, Engelmann M, Knegendorf L, et al. Antiviral activities of different interferon types and subtypes against hepatitis E virus replication. Antimicrob Agents Chemother. 2016;60(4):2132–9.
73. Kamar N, Rostaing L, Abravanel F, Garrouste C, Esposito L, Cardeau-Desangles I, et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. Clin Infect Dis. 2010;50(5):e30–3.
74. Haagsma EB, Riezebos-Brilman A, van den Berg AP, Porte RJ, Niesters HGM. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. Liver Transpl. 2010;16(4):474–7.
75. Rostaing L, Izopet J, Baron E, Duffaut M, Puel J, Durand D. Treatment of chronic hepatitis C with recombinant interferon alpha in kidney transplant recipients. Transplantation. 1995;59(10):1426–31.
76. Dao Thi VL, Debing Y, Wu X, Rice CM, Neyts J, Moradpour D, et al. Sofosbuvir inhibits hepatitis E virus replication in vitro and results in an additive effect when combined with ribavirin. Gastroenterology. 2016;150(1):82–85.e4.
77. Wang W, Hakim MS, Nair VP, de Ruiter PE, Huang F, Sprengers D, et al. Distinct antiviral potency of Sofosbuvir against hepatitis C and E viruses. Gastroenterology. 2016;151(6): 1251–3.
78. Kamar N, Pan Q. No clear evidence for an effect of Sofosbuvir against hepatitis E virus in organ transplant patients. Hepatology. 2019;69:1846–7.
79. Zhang J, Zhang X-F, Huang S-J, Wu T, Hu Y-M, Wang Z-Z, et al. Long-term efficacy of a hepatitis E vaccine. N Engl J Med. 2015;372(10):914–22.
80. Sauleda S, et al. Seroprevalence of hepatitis E virus (HEV) and detection of HEV RNA with a transcription-mediated amplification assay in blood donors from Catalonia (Spain). Transfusion (Paris). 2015;55:972–979.
81. Rivero-Juarez A, et al. Hepatitis E virus in Spanish donors and the necessity for screening. J. Viral Hepat. 2019. https://doi.org/10.1111/jvh.13064.
82. Fischer C, et al. Seroprevalence and incidence of hepatitis E in blood donors in Upper Austria. PLoS ONE. 2015;10.
83. Grabarczyk P, et al. Molecular and serological infection marker screening in blood donors indicates high endemicity of hepatitis E virus in Poland. Transfusion (Paris). 2018;58:1245–1253.
84. Hogema BM, et al. Incidence and duration of hepatitis E virus infection in Dutch blood donors. Transfusion (Paris). 2016;56:722–728.
85. Harritshøj LH, et al. Low transfusion transmission of hepatitis E among 25,637 single-donation, nucleic acid-tested blood donors. Transfusion (Paris). 2016;56:2225–2232.
86. Baylis SA, Gärtner T, Nick S, Ovemyr J, Blümel J Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. Vox Sang. 2012;103:89–90.
87. Westhölter D, et al. HEV-positive blood donations represent a relevant infection risk for immunosuppressed recipients. J. Hepatol. 2018;69:36–42.

88. Gallian P, et al. Hepatitis E virus infections in blood donors, France. Emerg. Infect. Dis. 2014;20:1914–1917.
89. Marcantonio C, et al. Incidence of hepatitis E virus infection among blood donors in a high endemic area of Central Italy. J. Viral Hepat. 2019;26:506–512.
90. O'Riordan J, et al. Hepatitis E virus infection in the Irish blood donor population. Transfusion (Paris). 2016;56:2868–2876.
91. Harvala H, et al. Hepatitis E virus in blood donors in England, 2016 to 2017: from selective to universal screening. Euro Surveill. Bull. Eur. Sur Mal. Transm. Eur. Commun. Dis. Bull. 2019;24.
92. Thom K, et al. Hepatitis E virus (HEV) in Scotland: evidence of recent increase in viral circulation in humans. Eurosurveillance. 2018;23.
93. Stramer SL, et al. Hepatitis E virus: seroprevalence and frequency of viral RNA detection among US blood donors. Transfusion (Paris). 2016;56:481–488.
94. Roth NJ, et al. Low hepatitis E virus RNA prevalence in a large-scale survey of United States source plasma donors. Transfusion (Paris). 2017;57:2958–2964.
95. Hoad VC, et al. Hepatitis E virus RNA in Australian blood donors: prevalence and risk assessment. Vox Sang. 2017;112:614–621.
96. Intharasongkroh D, et al. Hepatitis E virus infection in Thai blood donors. Transfusion (Paris). 2019;59:1035–1043.
97. Wen G-P, et al. Long-term HEV carriers without antibody seroconversion among eligible immunocompetent blood donors. Emerg. Microbes Infect. 2018;**7**:125.
98. Ren F, et al. Hepatitis E virus seroprevalence and molecular study among blood donors in China. Transfusion (Paris) 2014;54:910–917.
99. Minagi T, et al. Hepatitis E virus in donor plasma collected in Japan. Vox Sang. 2016;111:242–246.

Hepatitis B and Hepatitis D Infections in the Transplant Setting

38

Brian Kim and Norah A. Terrault

Contents

Introduction 928
Diagnostic Evaluation of Organ Recipients and Donors 929
Evaluation of Stage of Liver Disease 931
HBV Reactivation and Implications for Transplant Recipients 932
Natural History of Post-Transplant Recipients with Chronic Hepatitis B 933
Liver Transplant Recipients 933
Other Solid Organ Transplant Recipients 933
Hematopoietic Stem Cell Transplant Recipients 934
Anti-HBc-Positive, HBsAg-Negative Transplant Recipients 934
Management of Hepatitis B in the Transplant Setting 934
Overview of HBV Therapies 935
Vaccination to Prevent De Novo HBV in the Transplant Setting 936
Prevention of Recurrent HBV in Liver Transplant Recipients 936
Prevention of De Novo Infection in Recipients of Anti-HBc-Positive Donors 938
Managing Anti-HBc-Positive Transplant Recipients (HBsAg-Negative) 940
Management of Recipients of HBsAg-Positive Donors 941
Managing Transplant Recipients with Chronic Hepatitis B 942
Hepatitis D Virus 944
Liver Transplant Recipients 944
Conclusion 944
Key Points 945
Cross-References 945
References 945

B. Kim · N. A. Terrault (✉)
Department of Medicine, Division of Gastrointestinal and Liver Diseases, Keck School of Medicine of University of Southern California, Los Angeles, CA, USA
e-mail: Brian.Kim2@med.usc.edu; terrault@usc.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_37

Introduction

Hepatitis B virus infection (HBV) is one of the most significant causes of liver-related morbidity and mortality across the world, accounting for more than 620,000 deaths annually, predominantly from complications of cirrhosis and hepatocellular carcinoma (HCC) [1]. An estimated 248 million individuals are positive for hepatitis B surface antigen (HBsAg) worldwide with higher prevalence found in regions of Africa and Asia [2]. Nearly one-third of the global population or two billion people have serologic evidence of past or present HBV infection [3]. An estimated 5% of patients with chronic hepatitis B (CHB) have coinfection with hepatitis D virus (HDV) with higher prevalence found in the Mediterranean Basin, Middle East, and Central and Northern Asia [4, 5]. The mode of transmission of HDV is similar to that of HBV but can only occur in the presence of HBsAg. In the United States, the majority of individuals with CHB are foreign-born, reflecting the endemicity rates of HBsAg positivity in their countries of origin [6]. However, new HBV infections are on the rise among Americans, in part, related to the opioid epidemic and spread of HBV among those who inject drugs [7]. As a result, all organ transplant recipients and donors require evaluation of current or prior HBV infection.

Approximately 5% of all liver transplants (LT) are performed for patients with complications of CHB in the United States [8, 9]. Accurate data on the frequency of HDV coinfection are lacking. Among LT recipients with HBV, HCC is the most common indication for LT, with decompensated cirrhosis and acute liver failure (ALF) being less common [8, 9]. Among other solid organ transplants (SOT), HBsAg-positive recipients account for only 1–2% of recipients [10], with kidney transplant (KT) recipients being the best studied among non-liver transplant recipients.

HBV and HDV can have a significant impact on patients undergoing transplantation. In the setting of immunosuppression, the natural history of HBV and HDV can be accelerated and carry significant morbidity and mortality. Even for those who have cleared HBsAg, a risk for reactivation remains in the context of immunosuppression. Clinical awareness is key to identifying those patients at risk for hepatic complications associated with HBV and HDV and for providing appropriate prophylactic and therapeutic interventions to prevent liver-related complications.

The natural course of hepatitis B infection is characterized by the fluctuations in the levels of viral replication, measured by HBV DNA, and the host response, indicated by levels of alanine transaminase (ALT). HBV is not inherently cytopathic to hepatocytes (except in very unusual circumstances) but induces innate and adaptive immune responses in the host [11, 12]. A robust immune response yields resolution of infection (defined by HBsAg loss), but an inadequate immune response leads to chronicity and sets the stage for ongoing liver inflammation and injury.

While effective anti-HBV therapies are available, they are suppressive and not curative. Achievement of HBsAg loss either spontaneously or with treatment is the

best means of minimizing the risk of CHB complications. Unfortunately, in patients with CHB, the likelihood of spontaneous and treatment-associated clearance of HBsAg is very low – approximately 1% per year [13, 14]. New drug therapies, currently in early phase development, are increasingly focused on HBsAg loss as the endpoint of therapy [15]. However, even patients who achieve HBsAg seroconversion remains at risk for HBV complications due to the persistence of covalently closed circular DNA (cccDNA), a stable minichromosome inside the hepatocyte nucleus [16]. If host immune control is compromised, as occurs in the setting of transplantation, there is risk for reactivation of HBV.

The goals of therapy in the transplant setting are similar to those in the non-transplant setting – to prevent liver-related complications. Longitudinal assessment of HBV replication is essential to ensure treatment is provided when needed [17]. Antiviral therapy reduces the risk of clinical liver disease progression, can reverse symptoms of decompensation, improves indices of liver function (including model for end-stage liver disease score), and reduces HCC incidence [18, 19]. Most importantly for patients listed for LT, achieving a low or undetectable HBV DNA level prior to LT is associated with lower risk of HBV recurrence post-LT [20].

Diagnostic Evaluation of Organ Recipients and Donors

All transplant candidates and donors undergo serologic testing for HBV. Testing establishes whether there is evidence of current versus prior infection and current immune status (Table 1).

Table 1 Interpretation of screening tests for HBV infection in transplant candidates

Screening test results			
HBsAg	Anti-HBc	Anti-HBs	Interpretation
+	+	–	Chronic HBV
+	–	–	Very recent vaccine administration
–	+	+	Resolved HBV infection, naturally immune
–	–	+	Vaccine-induced immunity
–	–	–	Uninfected, not immune
–	+	–	(1) Resolved HBV infection, with loss of anti-HBs[a] (2) False positive anti-HBc (3) Recovering from acute HBV infection (4) Passive transmission from recent blood products (especially with IVIG)

HBV hepatitis B virus, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibody to hepatitis B core antigen, *anti-HBs* antibody to hepatitis B surface antigen, *IVIG* intravenous immunoglobulin
[a]Note HBV DNA may be intermittently detected at low levels (referred to as occult HBV infection)

- HBsAg is the serologic marker of acute and chronic HBV infection. HBsAg usually appears in the serum 1–10 weeks after acute exposure to HBV, and its persistence in the serum for more than 6 months generally defines CHB. Transient HBsAg detection can occur after vaccination [21].
- Hepatitis B surface antibody (anti-HBs) is the marker of immunity. Anti-HBs can develop in response to HBV exposure (natural immunity) or through vaccination (vaccine immunity).
- Antibody to hepatitis B core antigen (anti-HBc) indicates prior exposure to HBV and remains positive even if anti-HBs may wane. After an acute HBV exposure, a window period may exist during which time the HBsAg is declining and anti-HBs is rising. During this period, both HBsAg and anti-HBc may be undetectable, and IgM anti-HBc and HBV DNA are used to diagnose acute HBV in this window period. The presence of isolated anti-HBc in the absence of HBsAg and anti-HBs can be seen in several settings, but it most frequently represents past exposure to HBV with resolved or resolving infection. Rarely, an isolated anti-HBc can represent occult infection with detectable HBV DNA [22]. Transient detection of anti-HBc can also be seen in patients who have recently received blood products, especially intravenous immunoglobulin infusion [23].

For transplant candidates with CHB, as evidenced by a positive serum HBsAg, HBV DNA level should be obtained to determine the need for HBV antiviral therapy and, if antiviral therapy is initiated, response to therapy. For those patients on the waiting list who do not meet the criteria for antiviral therapy, serial monitoring of ALT and HBV DNA levels every 3–6 months is recommended [17]. HDV antibody testing should be performed for those with positive HBsAg, regardless of HBV DNA level. If anti-HDV antibody is positive, HDV RNA testing should be measured to assess for active chronic HDV infection.

For those transplant candidates who are HBsAg-negative and anti-HBc-positive, no further testing is needed unless ALT levels are elevated. HBV DNA will help to rule out occult HBV infection. In this setting, if HBV DNA is detected, levels are typically low (<100 IU/mL) [24] and may not warrant treatment unless the patient is immunosuppressed. HBsAg-negative and anti-HBc-positive patients (with or without antibody) are not at risk of HBV-related complications unless HBV reactivation occurs (see below), and antiviral therapy can be used to prevent reactivation in the setting of immunosuppression.

The current Organ Procurement Transplant Network (OPTN) policy in the United States requires that all deceased organ donors are tested for HBsAg and anti-HBc (Table 2). OPTN policy also requires all potential living donors to be tested for HBsAg, anti-HBs, and anti-HBc [25]. Additional testing, including donor IgM anti-HBc or HBV DNA using sensitive nucleic acid test (NAT), may be performed by the individual organ procurement organizations to further guide decisions about accepting these potential organs and should be done in high-risk donors [26]. Rare HBV transmission and de novo hepatitis B infection (DNB) have been described in recipients who receive organs from donors with negative anti-HBc, HBsAg, and

Table 2 Interpretation of screening tests in donors

Screening test results			Interpretation of donor's HBV status	Donor status for transplantation
HBsAg	Anti-HBc IgG/IgM	HBV DNA		
–	–	–	No HBV	Acceptable
+	+	+/–	Chronic HBV	Reject – though rarely centers may use in life-threatening situations
–	IgG+	+	Occult HBV	Reject – though rarely centers may use in life-threatening situations
–	IgG+	–	Prior HBV exposure or false positive (if DNA negative)	Acceptable, with high-risk consent
–	IgM+	+	Acute HBV	Reject – though rarely centers may use in life-threatening situations
–	IgG-/ IgM-	+	If PHS high-risk donors, may be early acute infection	Reject – though rarely centers may use in life-threatening situations

HBsAg hepatitis B surface antigen, *anti-HBc* antibody to hepatitis B core antigen, *HBV* hepatitis B virus, *PHS* public health service

HBV DNA but with high-risk behavior, including injection drug use [27]. The recent opioid crisis and increasing number of deaths due to overdose have resulted in an increase in donors with Public Health Services high-risk profiles from 8.9% in 2010 to 26.3% in 2017. Given this statistic, all donors should be tested for HBV DNA by NAT.

In the United States, 4.8% of donors from 2010 to 2017 were from anti-HBc-positive donors, while 0.1% of donors were from HBsAg-positive donors [28]. Donors who are HBsAg-positive are high risk for transmission of HBV and are rarely used for transplantation. Donors who are anti-HBc-positive or HBV DNA-positive (but HBsAg-negative) may transmit HBV but with the risk varying by organ type and HBV serologic status of the recipient. These donors may be used in recipients in urgent need of transplantation after detailed review of the risks [29].

Evaluation of Stage of Liver Disease

Assessment of hepatic fibrosis severity and liver function is essential in HBsAg-positive non-liver transplant candidates, to both exclude the need for dual transplantation as well the need for antiviral therapy. Advanced hepatic fibrosis and/or hepatic decompensation may preclude a safe non-liver transplant and lead to consideration of dual organ transplantation. The presence of cirrhosis is an indication for antiviral therapy regardless of HBV DNA and ALT levels [17].

Historically, a liver biopsy was used to determine fibrosis stage, but noninvasive options are available, allowing most patients to avoid a liver biopsy. Measurement of

liver stiffness using transient elastography or magnetic resonance elastography is the primary mode to noninvasively assess fibrosis. However, limitations of these noninvasive assessments in certain patient populations are important to recognize. As an example, elevated central venous pressure and passive hepatic congestion that occur in end-stage renal disease and heart failure may lead to an overestimation of fibrosis stage by elastography measures [30]. Nevertheless, elastography measurement can be useful even in this population given that a low stiffness score, with its high negative predictive value for cirrhosis, may effectively rule out the presence of cirrhosis [31]. Serum blood tests, such as Fibrosis-4 (FIB-4) and AST to Platelet Ratio Index (APRI), combined with liver stiffness measurements can increase the accuracy of excluding cirrhosis [32]. If the noninvasive tests indicate the presence of advanced fibrosis (stage F3 or F4), a liver biopsy is recommended to assess further. If done via the transjugular route, the hepatic venous pressure gradient can also be measured concurrently, providing information on whether portal hypertension is present.

HBV Reactivation and Implications for Transplant Recipients

Reactivation of HBV is defined as one of the following: (1) $\geq$2-log$_{10}$ increase in HBV DNA level, (2) detection of HBV DNA in a patient with previously undetectable DNA, or (3) reverse HBsAg seroconversion (reappearance of HBsAg in previously HBsAg-negative patient) [17]. HBV reactivation can be followed by an ALT flare, defined as the abrupt increase in ALT to $\geq$100 IU/L and $\geq$3 times baseline level. Flares are preceded by increasing HBV DNA levels. In response to the increased levels of HBV DNA and antigen expression, the immune response is enhanced resulting in hepatocellular injury [33].

The clinical outcome of HBV reactivation with flare in the setting of immunosuppression can range from asymptomatic to severe hepatitis. Severe hepatitis can potentially lead to ALF, characterized by severe hepatic dysfunction with coagulopathy and encephalopathy. According to the United States Acute Liver Failure Study Group, of the 156 cases of HBV-associated ALF from 1998 to 2015, 28 cases (18%) occurred in the setting of immunosuppression use. Immunosuppression-induced ALF from HBV was associated with lower 21-day transplant-free survival [34]. Even in those patients that avoid the development of ALF, the presence of active CHB portends poorer long-term outcomes in the setting of post-transplant immunosuppression with increased rates of histologic progression, development of cirrhosis, and diagnosis of HCC [35, 36].

Thus, clinicians should be aware of two distinct, “at-risk” populations of transplant recipients. First are the HBsAg-positive recipients, whose chronic infection can be exacerbated by immunosuppression and lead to a rapid progression of hepatitis. Second, are recipients with resolved HBV infection (HBsAg-negative, anti-HBc-positive, $\pm$anti-HBs) who may experience reactivation of HBV

in the setting of transplant-related immunosuppression. Additionally, donors with prior exposure to HBV may potentially transmit the virus to uninfected recipients, leading to DNB.

Natural History of Post-Transplant Recipients with Chronic Hepatitis B

Liver Transplant Recipients

Historically, patients with CHB undergoing LT had worse long-term survival than patients without HBV. For LT recipients, recurrence of HBV was frequent and associated with 5-year survival of only 50% as compared to 80% seen in those transplanted for other types of liver diseases [37, 38]. Factors associated with recurrence were high HBV DNA levels and presence of hepatitis B envelope antigen, whereas as those with ALF and HDV coinfection had lower rates of recurrence [39]. A unique variant of recurrent HBV called severe fibrosing cholestatic hepatitis (FCH) occurred in less than 5% of transplant recipients but was associated with rapidly progressive hepatitis leading to graft loss. Histologically, FCH is characterized by cholestasis, ballooned hepatocytes, a paucity of inflammatory cells, and pericellular fibrosis [40]; HBV DNA levels are very high (both in serum and in liver) leading to the conclusions that the injury is related to the direct cytopathic effects of HBV, rather than injury from the immune response [41]. In the current era, FCH is rarely seen, as safe and effective antivirals can effectively prevent and treat recurrent HBV infection in LT recipients.

Other Solid Organ Transplant Recipients

The outcomes of non-liver SOT recipients with CHB are best studied in KT recipients. Prior to the availability of safe and effective antivirals, KT recipients positive for HBsAg had higher rates of mortality and graft failure than recipients negative for HBsAg [36]. Liver-related complications were the primary cause of death [42, 43]. However, with routine use of antiviral therapy after KT, this risk has been mitigated, and excellent outcomes have been observed [44, 45]. Frequent HBV reactivation has been reported after heart transplantation, but with antiviral therapy, the outcomes for HBsAg-positive recipients have been excellent without increases in HBV-related morbidity and mortality [46].

SOT recipients with CHB are at risk for HCC. In a review of 178,367 non-liver SOT recipients, the reported HCC incidence was 6.5 per 100,000 person-years with a hazard ratio of 9.7 (95% CI 2.8–33) for those with HBsAg positivity [47]. Standard criteria for HCC surveillance apply to SOT recipients with CHB including all patients with cirrhosis and those in high-risk subgroups such as HDV coinfection (Table 3). Surveillance for HCC with ultrasound (with or without serum alpha-fetoprotein levels) every 6 months is recommended.

Table 3 Indications for hepatocellular carcinoma surveillance in transplant recipients with chronic HBV[a]

Who to screen?
• All HBsAg-positive persons with cirrhosis • HBsAg-positive adults without cirrhosis if: 1. Asian or black men over 40 years of age 2. Asian women over 50 years of age 3. First-degree family member with a history of HCC 4. HDV coinfection
How to screen?
• Surveillance should be performed with ultrasound with or without serum AFP levels, every 6 months

HBV hepatitis B virus, *HBsAg* hepatitis B surface antigen, *HCC* hepatocellular carcinoma, *HDV* hepatitis D virus, *AFP* alpha-fetoprotein
[a]Excludes recipients with HCC pre-transplant

Hematopoietic Stem Cell Transplant Recipients

A systemic review of 42 studies described a more aggressive behavior of HBV in the setting of hematologic malignancy and hematopoietic stem cell transplant (HSCT) [48]. The use of cytotoxic chemotherapy enhances the immunosuppressive state in these patients, and patients with CHB are at high risk for HBV reactivation [49]. Prophylactic antiviral therapy significantly reduces rates of HBV reactivation in patients with hematologic malignancies [50] and is recommended for all HBsAg-positive HSCT recipients [51].

Anti-HBc-Positive, HBsAg-Negative Transplant Recipients

While HBsAg-positive transplant recipients are the minority of SOT recipients, a larger proportion of transplant recipients will have evidence of resolved HBV infection (HBsAg negative, anti-HBc positive ± anti-HBs). Such recipients have a potential risk of HBV reactivation post-transplant, under the effects of immunosuppression [52, 53]. However, reactivation after transplant is rare in the reports of liver, kidney, and heart transplants published to date, and when managed well, outcomes post-transplant appear to be similar to recipients without prior HBV exposure [52–54]. In contrast, reactivation in HSCT recipients is more frequent and associated with risk of liver complications, and thus antiviral prophylaxis is recommended in anti-HBc-positive HSCT recipients [51].

Management of Hepatitis B in the Transplant Setting

Given the potential impact of HBV on patient outcomes after transplant, management strategies should focus on (1) prevention of HBV recurrence in LT recipients; (2) reducing risk of HBV disease progression post-transplant in those with CHB; and

(3) prevention of HBV transmission from donor to recipient. These goals can be achieved through the combination of prudent vaccination, observation, and pharmacologic therapy. Additionally, prevention of DNB infection in the pre- and post-transplant settings through comprehensive vaccination programs is essential.

Overview of HBV Therapies

First-line antiviral therapy for treatment of active HBV includes the oral nucleoside analogue, entecavir, and nucleotide analogues, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) (Table 4). These nucleos(t)ide analogues (NA) have excellent tolerability and achieve high rates of viral suppression with low risk of viral resistance. Older NAs including lamivudine, adefovir, and telbivudine are not preferred owing to their higher risk of resistance. In patients with renal dysfunction or bone disease, entecavir or TAF is recommended over TDF given the risk of decline in bone density and renal function with chronic TDF use [17]. TAF is more stable in plasma than TDF and delivers the active metabolite to hepatocytes more effectively at a lower dose. In phase 3 studies, TAF caused significantly less decline in renal function and bone density as compared to TDF at 48 weeks of treatment [55, 56]. This is especially relevant in transplant recipients, where concurrent use of calcineurin inhibitors increases risk for renal dysfunction post-transplant, and chronic steroids use decreases bone density. In patients with prior lamivudine exposure, TDF or TAF should be used as there is greater risk of entecavir resistance in these patients (up to 50% after 5 years) [57]. Resistance is very rare with TDF and TAF, though recently, a quadruple mutation that conferred high-level tenofovir resistance was described [58]. In any adherent patient with virologic breakthrough on antiviral therapy (defined by >1 $\log_{10}$ increase in HBV DNA or >100 IU/mL if

Table 4 Antiviral therapies for transplant recipients with chronic HBV

	Entecavir	TDF	TAF
Dose	0.5 mg once daily	300 mg once daily	25 mg once daily
Generic available	Yes	Yes	No
Dose adjustments (mL/min/1.73 m^2)	if CrCl<50	if CrCl<50	if CrCl<15
Co-existing conditions			
Renal disease	+	-	+
Osteoporosis	+	-	+
HIV infection	-[a]	+	+
HBV-related factors			
Prior lamivudine exposure	-	+	+
Cirrhosis	+	+	+

HBV hepatitis B virus, *TDF* tenofovir disoproxil fumarate, *TAF* tenofovir alafenamide, *CrCl* creatinine clearance, *HIV* human immunodeficiency virus, *ART* antiretroviral therapy

[a]Unless on fully suppressive ART

previously undetectable), resistance testing should be considered and a change in antivirals made. For patients on entecavir with virologic breakthrough, the NA therapy should be switched to TDF or TAF. If tenofovir resistance is noted, the NA therapy should be switched to entecavir. In case of multidrug resistance, the combination of TDF or TAF with entecavir can be used [17].

Pegylated interferon is also considered a first-line therapy for HBV and is the only available therapy for HDV [17]. However, its use in transplant recipients with CHB is not recommended since well-tolerated, safe, and highly effective oral therapies are available. Moreover, pegylated interferon is associated with bone marrow suppression and risk of graft rejection [59, 60]. However, for transplant recipients with severe or progressive liver disease due to HDV infection, pegylated interferon may still need to be considered.

Vaccination to Prevent De Novo HBV in the Transplant Setting

For those with serologies negative for HBsAg, anti-HBc, and anti-HBs, vaccination is indicated. In patients with positive, isolated anti-HBc with negative HBsAg and anti-HBs, the serologies may indicate occult HBV, prior exposure to HBV with low, undetectable anti-HBs titer, or false-positive anti-HBc test. To avoid a missed opportunity to protect susceptible individuals (i.e., those with false-positive anti-HBc test), any patient with absent anti-HBs should be vaccinated [17]. In immunocompetent patients, the protection is generally considered life-long, and postvaccination serologic testing is not routinely recommended. However, in immunocompromised patients, as in patients with end-stage diseases awaiting transplant, measurement of anti-HBs titer after vaccination may be important to determine protection [61]. Annual anti-HBs titer is recommended in immunocompromised patients or patients undergoing hemodialysis. If titers fall below 10 IU/L, booster vaccination is recommended. Whenever possible, the vaccination series should be given prior to transplant since the vaccine response is less effective after transplant [62, 63]. In patients with cirrhosis, hemodialysis patients, and post-transplant patients, strategies to increase vaccine responsiveness include the use of high dose or four (versus three doses) series [64]. The recently approved HEPLISAV-B vaccine, which includes a Toll-like receptor-9 as adjuvant and is given as 2 doses 1 month apart, has shown superior response rates compared to three-dose Engerix® in healthy adults and those with diabetes so may offer advantages in patients with end-stage disease awaiting transplantation [65].

Prevention of Recurrent HBV in Liver Transplant Recipients

Guidelines recommend antiviral therapy for all HBsAg-positive patients with cirrhosis, regardless of HBV DNA or ALT levels; thus, all LT patients on the waiting list should be on antiviral therapy (Fig. 1) [17].

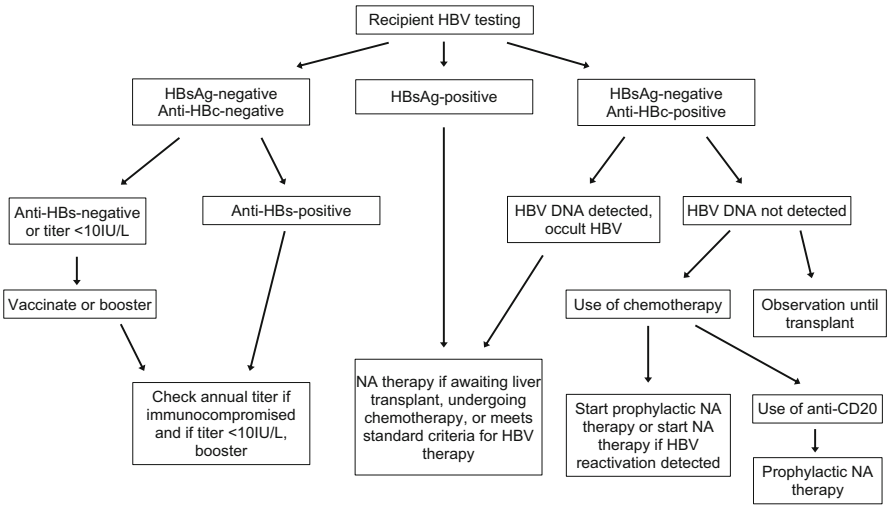


Fig. 1 Management of recipients prior to organ transplantation. Abbreviations: *HBV* hepatitis B virus, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibody to hepatitis B core antigen, *anti-HBs* antibody to hepatitis B surface antigen, *NA* nucleos(t)ide analogue

In HBsAg-positive LT recipients, reinfection and recurrence of HBV are frequent unless prophylactic therapy is provided. Reinfection is defined by the reappearance of HBsAg in the recipient after LT and is almost always followed by recurrence of HBV liver disease if untreated. Prophylactic strategies include treatment with NA with and without hepatitis B immune globulin (HBIG). HBIG is a polyclonal preparation of anti-HBs from plasma donors that binds and neutralizes circulating virions, reducing liver graft reinfection. Prior to the availability of NA, the use of post-LT HBIG prophylaxis alone dramatically improved outcomes of recipients undergoing transplant for HBV complications [66, 67]. The combination of NA and HBIG post-LT has further reduced HBV recurrence rates to less than 10% [68]. HBIG helps to prevent reinfection of the graft, while NA prevents HBV disease and recurrence through viral suppression. While many LT centers utilize HBIG in the early post-LT setting, oral NA alone appears to be equally effective with low rates of recurrent HBV disease with active viral suppression [69, 70] There is currently no consensus on the dose and duration of HBIG therapy after LT. It is a reasonable strategy to give daily HBIG in the immediate post-LT setting for 5–7 days and transition to NA therapy alone afterward [71]. Long-term HBIG with NA can be considered if the patient has higher than average risk for HBV recurrence including the presence of drug-resistant HBV, high DNA at time of LT, and lack of access to NAs with high barrier to resistance (entecavir, TDF, and TAF) [72] (Table 5). HBIG should not be used alone without NA given higher rates of HBV recurrence. Post-LT, NA should be continued indefinitely.

Special consideration for long-term HBIG can also be made for patients with HDV or human immunodeficiency virus (HIV) coinfection with HBV. Oral NA

Table 5 Factors influencing the choice of antiviral prophylaxis after liver transplantation in HBsAg-positive recipients

Indefinite NA alone or with short-term HBIG	Indefinite NA with long-term HBIG
Absence of drug-resistant HBV	Drug-resistant HBV
Undetectable to low HBV DNA at time of transplantation	High HBV DNA at time of transplantation
Lack of access to HBIG	Lack of access to high-potency NA (entecavir, TDF, or TAF)
HBsAg-positive liver graft	HIV coinfection
	HDV coinfection

HBsAg hepatitis B surface antigen, *NA* nucleos(t)ide analogue, *HBIG* hepatitis B immune globulin, *HBV* hepatitis B virus, *TDF* tenofovir disoproxil fumarate, *TAF*, *HIV* human immunodeficiency virus, *HDV* hepatitis D virus

has no direct efficacy against HDV. Given that HDV requires HBsAg for infection, post-LT combination therapy with NA and long-term HBIG to optimize HBsAg clearance may be ideal. In patients with HIV and HBV coinfection, low-level viremia without detectable HBsAg was detected in approximately 50% of recipients despite combination NA and HBIG therapy, suggesting that long-term HBIG may have an added role in this population to reduce HBV reinfection [73].

Prevention of De Novo Infection in Recipients of Anti-HBc-Positive Donors

Donors with anti-HBc (with or without anti-HBs) pose the highest risk of HBV transmission to liver graft recipients [74]. Recipients of non-liver SOT are at low risk, and thus, prophylactic strategies are primarily determined by the type of transplant recipient. However, further modification of risk is dependent upon the HBV status of the recipients. As a general strategy, use of these organs in recipients who already have markers of HBV exposure is preferred (HBsAg- or anti-HBc-positive) as it simplifies post-transplant management [26]. However, given the current organ shortage, this option is not always available, and recipients who are HBsAg- and anti-HBc-negative are often given these organs, placing them at risk for DNB.

Liver Transplantation

The majority of data on use of anti-HBc-positive donors derive from experience in LT. Without prophylaxis, the risk for DNB, defined by the appearance of HBsAg or detectable HBV DNA, can be substantial but varies with the immune status of the recipient [75]. In a review of 90 cases of DNB in 194 liver recipients, the risk of transmission was highest in anti-HBs-negative recipients (76.6%), lowest in anti-HBs-positive, anti-HBc-positive recipients with natural immunity (0%), and modest (18.2%) in patients with vaccine-induced immunity (HBsAg-negative, anti-HBs-positive, and anti-HBc-negative) [76].

Combination antiviral therapy with NA and HBIG has been used and associated with reduction in the DNB rate from 58% to 11% in HBV nonimmune recipients. In patients with vaccine immunity, the rate dropped from 18% to 2%. Prophylaxis did not further reduce the risk of transmission in recipients with natural immunity (3–4%) [73]. Subsequent studies have shown HBIG alone did not confer a significant benefit, and NA alone is effective in lowering DNB rates [76, 77]. High-potency NA (entecavir, TDF, or TAF) is recommended in this setting over lower-potency NA (lamivudine, adefovir, or telbivudine). In a retrospective study of 964 LT recipients in Hong Kong, anti-HBc-positive grafts did not adversely impact long-term outcomes. Of the HBsAg-negative recipients that received anti-HBc-positive organ, the risk of DNB was overall low at 2.8%. However, all DNB occurred in the group receiving lamivudine prophylaxis, while none occurred in the entecavir prophylaxis group [78]. Lamivudine may be an acceptable choice where cost influences decision-making.

Nonimmune LT recipients without prior exposure to HBV (anti-HBs-negative, anti-HBc-negative) and recipients with vaccine immunity (anti-HBs-positive, anti-HBc-negative), who receive an isolated anti-HBc-positive liver graft, should receive indefinite NA therapy alone without HBIG [17]. NA therapy is not recommended if the recipient has natural immunity to HBV (anti-HBs-positive, anti-HBc-positive), but monitoring with HBV DNA and/or HBsAg every 3 months for the first year and during periods of intensified immunosuppression, as with treatment of acute graft rejection, is recommended (Fig. 2). Additionally, if abnormal liver tests develop post-LT, testing of HBV serologies and HBV DNA should be considered.

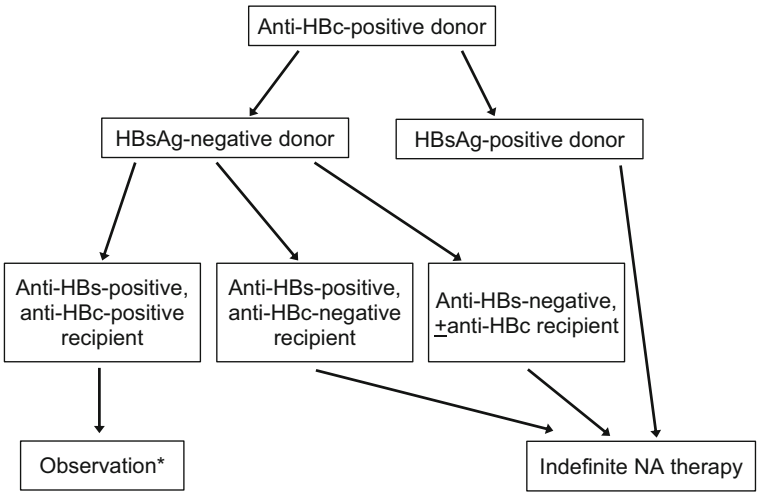


Fig. 2 Algorithm for the use of liver grafts from anti-HBc-positive donors in recipients with and without chronic HBV. ∗Monitor with HBsAg or HBV DNA every 3 months for the first year and during any period of intensified immunosuppression (i.e., treatment of acute rejection). Abbreviations: *anti-HBc* antibody to hepatitis B core antigen, *HBV* hepatitis B virus, *HBsAg* hepatitis B surface antigen, *anti-HBs* antibody to hepatitis B surface antigen, *NA* nucleos(t)ide analogue

Non-liver Transplantation

The reported risk of DNB from isolated anti-HBc-positive donor in non-LT recipients is much lower than compared to LT recipients, about 0.3% in KT [79]. As in LT recipients, the risk of DNB depends on the recipients' HBV immune status. In non-liver transplant recipients who are immune (vaccine or natural immunity), the DNB risk is negligible, and no antiviral prophylaxis is required [80, 81]. Close monitoring with HBV DNA and/or HBsAg should be performed every 3 months for the first year or when immunosuppression is most intensive such as with treatment of acute graft rejection. In nonimmune recipients (anti-HBs-negative, ±anti-HBc), DNB risk is presumably due to the presence of HBV DNA in the plasma of the donor organ. Thus, if donor serum HBV DNA is undetectable, no antiviral therapy is warranted. If donor serum HBV DNA is positive or unknown, prophylaxis with either NA or HBIG in the early post-transplant period and for up to 6–12 months should be considered (Fig. 3) [82].

Managing Anti-HBc-Positive Transplant Recipients (HBsAg-Negative)

Presence of anti-HBc indicates prior exposure to HBV; false-positive tests can occur, albeit very infrequently. Such recipients may or may not have anti-HBs, but if anti-

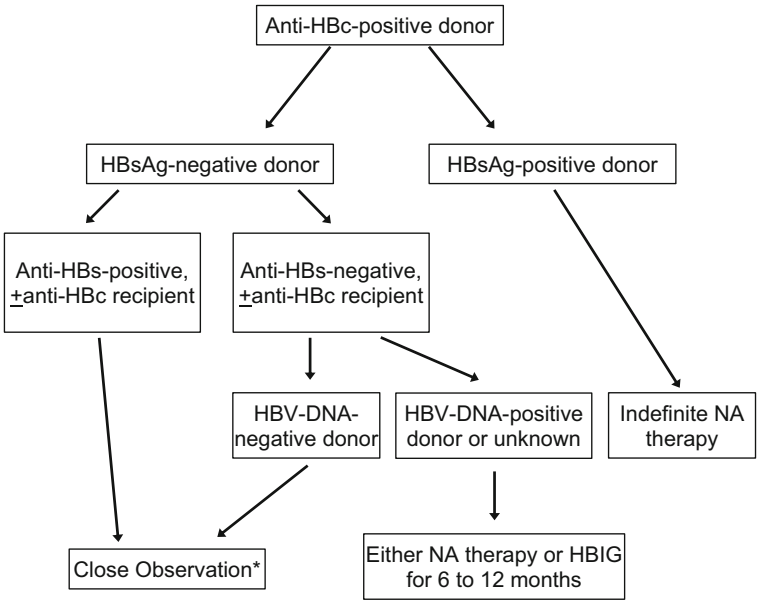


Fig. 3 Algorithm for the use of non-liver grafts from anti-HBc-positive donors in recipients without chronic HBV. ∗Monitor with HBsAg or HBV DNA every 3 months for the first year and during any period of intensified immunosuppression (i.e., treatment of acute rejection). Abbreviations: *anti-HBc* antibody to hepatitis B core antigen, *HBV* hepatitis B virus, *HBsAg* hepatitis B surface antigen, *anti-HBs* antibody to hepatitis B surface antigen, *NA* nucleos(t)ide analogue, *HBIG* hepatitis B immune globulin

HBs is present, this confirms prior exposure and is less likely to be associated with occult HBV infection, defined as presence of viremia in HBsAg-negative patients with prior HBV exposure. HBV DNA should be obtained pre-transplant to identify the rare patient with occult CHB, and if HBV DNA is positive, these patients should be treated similarly to patients with CHB.

The majority of transplant recipients with positive anti-HBc will have undetectable HBV DNA pre-transplant but are at variable risk for HBV reactivation post-transplant. In HBsAg-negative, anti-HBc-positive liver recipients receiving HBV-negative grafts, HBV replication has been demonstrated on liver biopsy, but none of the LT recipients became HBsAg-positive post-transplant [54]. Presumably, the source of HBV in these patients is from extrahepatic reservoirs but is not of clinical consequence. Thus, HBV prophylaxis and routine monitoring of HBV reactivation are not required in HBsAg-negative but anti-HBc-positive LT patients.

In non-liver transplants, the risk of post-transplant HBV reactivation in anti-HBc-positive donors is estimated to be at most 5% [52, 83]. Here, the source of HBV is cccDNA from the recipient's liver that reactivates under the effects of immunosuppression. Routine HBV antiviral therapy is generally not indicated. However, the risk is increased during intense immunosuppression. Rituximab, a monoclonal antibody that targets the B-cell marker CD20, is often used in KT recipients for desensitization and is associated with high rates of HBV reactivation [84]. In these patients, prophylactic, antiviral therapy with entecavir, TDF, or TAF is recommended. Cessation of therapy can be considered after 6 months of therapy with high-dose immunosuppression or 12 months after anti-CD20 therapy [85]. Repeat HBsAg and HBV DNA testing is not required in patients managed with antiviral prophylaxis unless there are concerns regarding medication adherence.

Reactivation occurs due to the loss of protective anti-HBs (if present at baseline) followed by a rise in HBV DNA and reverse HBsAg seroconversion. Therefore, in patients who do not start antiviral prophylaxis, close monitoring for HBV reactivation can be performed with HBV DNA and HBsAg every 3 months for the first year or in the context of intensification of immunosuppression as in treatment of acute graft rejection. If a patient has reverse HBsAg seroconversion or HBV DNA progressively rises, a high-potency NA (entecavir, TDF, or TAF) should be started [82]. Additionally, evaluating for HBV reactivation should be considered if unexplained ALT elevations develop.

Management of Recipients of HBsAg-Positive Donors

The risk of DNB is high in nonimmune, HBsAg-negative recipients who receive organs from HBsAg-positive donors [86]. Therefore, risks and benefits of accepting such organs should be carefully weighed, especially in nonurgent transplants. For transplant candidates who already have CHB, the risk associated with a HBsAg-positive donor is negligible for non-liver organ recipients but may be significant for LT recipients without appropriate prevention.

Liver Transplantation

Livers from HBsAg-positive donors have been donated to recipients with CHB. Comparison of recipients with CHB who received livers from HBsAg-positive and HBsAg-negative donors have shown no difference in postoperative morbidity and mortality. All recipients received oral NA postoperatively. HBsAg persisted after transplant in all recipients who received HBsAg-positive donor livers despite HBIG use [87, 88]. This is not surprising since the liver had established HBV infection at the time of transplantation, so HBIG has no role. Thus, CHB recipients who receive liver grafts from HBsAg-positive donors should be treated with indefinite NA alone after LT (Fig. 2). To minimize any risk for virologic breakthrough, the NA with high barrier to resistance should be used. Moreover, since prior NA exposure history may not be available for the donor, it is prudent to use TDF or TAF and not entecavir as the latter has lower efficacy if there has been prior lamivudine exposure [57].

Care should be taken during liver procurement to ensure that the donor liver does not have significant fibrosis that might impact long-term post-LT graft survival. Both the recipient and donor need to be tested for HDV coinfection. HDV coinfection is a contraindication to the use of organs from HBsAg-positive donors due to the lack of safe and effective therapies for HDV after LT. As HBsAg universally persists after HBsAg-positive liver donation despite prophylaxis, HDV can establish in the liver graft and can cause active necroinflammation, potentially leading to graft failure despite the control of the HBV with NA [89].

Non-liver Transplantation

Successful transplants of kidneys from HBsAg-positive donors to HBV immune (anti-HBs-positive) recipients have been described. Although potential transmission risk is present, the combination of HBIG and oral antiviral therapy have been effective in preventing HBV-related complications with similar graft and patient survival. In a study of 129 KT recipients with anti-HBs titer above 100 IU/L, no difference in patient survival, graft survival, and HBV transmission was seen between the HBsAg-positive and HBsAg-negative donor groups, even in the absence of HBV prophylaxis, suggesting that high-titer anti-HBs may lower DNB in this cohort [90]. Thus, organs from HBsAg-positive donors can be considered in patients who are immune to HBV. All patients should receive indefinite NA therapy with high barrier to resistance (entecavir, TDF, or TAF) post-transplant [26]. Monitoring with HBV DNA and HBsAg should be performed every 3 months in the first year and 6 months indefinitely thereafter, plus as indicated clinically (Fig. 3) [26, 82].

Managing Transplant Recipients with Chronic Hepatitis B

Post-transplant recipients with CHB include those with pre-transplant HBV infection and those who acquire HBV post-transplant from donor or other sources. All HBsAg-positive patients who are the waiting list should be evaluated to determine the need for pre-transplant antiviral treatment (Fig. 1). As highlighted above, all LT

candidates should be on antiviral therapy. For other transplants, the decision to treat while on the waiting list is based on levels of HBV DNA and the stage of liver fibrosis (Table 6). Entecavir, TDF, and TAF are preferred NAs. Surveillance for HCC should also be initiated in individuals with cirrhosis, positive family history for HCC, HDV coinfection, or prolonged duration of infection (ethnicity and age based) (Table 3) [17].

Post-transplant, all non-liver recipients with pre-transplant CHB should be on antiviral therapy with NA to prevent HBV reactivation and/or progression, at least for the first year and potentially indefinitely. As mentioned previously, patients with CHB on post-transplant immunosuppression are at risk for accelerated progression of their liver disease. The primary goal of patients with CHB post-transplant is sustained virologic suppression to prevent HBV-related hepatitis and progression to cirrhosis and/or HCC. The ideal duration of therapy has not been established, and since most post-transplant recipients receive life-long immunosuppression, indefinite antiviral therapy provides the surest means of preventing HBV-related complications. As in the pre-transplant setting, entecavir, TDF, and TAF are preferred NAs. Entecavir and TAF are the preferred NAs in patients at risk for renal insufficiency. Dose adjustment may be required for all of the NAs, based on the patient's creatinine clearance (Table 4).

For patients who acquire DNB during or after transplant, progression of liver disease can be rapid in the face of concurrent immunosuppression. These patients need NA therapy to be started as soon as the diagnosis is made and continued on antiviral therapy indefinitely to minimize risk of future liver-related complications.

Table 6 Indications for antiviral therapy in transplant candidates and recipients with chronic HBV

Type of transplant	Indications for treatment	When to start pre-transplant	When to stop post-transplant	Comments
Liver	All patients, due to underlying cirrhosis	Immediately upon recognition of cirrhosis	Treatment is indefinite	Monitor HBV DNA every 3 months to ensure response and no evidence of virologic breakthrough
Non-Liver	Presence of cirrhosis	Immediately upon recognition of cirrhosis	Treatment is indefinite	
	HBV DNA quantifiable	Pre-transplant	Consider long-term treatment	
	HBV DNA < LLOQ	At time of transplant	At least 1-year post-transplant; can consider long-term therapy HSC: indefinite treatment	If treatment is stopped, life-long monitoring for HBV reactivation is necessary with HBV DNA tests every 3-6 months

HBV hepatitis B virus, *LLOQ* lower limit of quantification, *HSCT* hematopoietic stem cell transplant

Hepatitis D Virus

Hepatitis D virus is a defective RNA virus that requires the presence of HBV for its lifecycle. Consequently, HDV infection can only be found in HBsAg-positive patients. All patients with CHB, undergoing consideration for SOT or HSCT, should have screening performed for HDV infection with antibody testing for HDV [17]. If anti-HDV is positive, confirmation with HDV RNA testing should be performed. In patients with HDV coinfection, HBV DNA may be low or undetectable [91]. In HDV-infected patients, the annual rates of progression to cirrhosis and HCC are 4% and 2.8%, respectively [92]. There appears to be a 2.2-fold increase in hepatic decompensation and 3.2-fold increase in the development of HCC as compared to those with HBV infection alone [93].

Liver Transplant Recipients

Despite increased risk of pre-transplant decompensation and HCC development, overall outcomes after LT for HDV coinfection are generally better than in patients with HBV infection alone [39]. This is likely due to HDV suppression of HBV replication, leading to lower rates of HBV reinfection after LT [94]. If both HDV and HBV reinfection does occur after LT, clinical hepatitis is often seen with elevated ALT [95]. In a study involving serial liver biopsies of patients with HDV and HBV reinfection after LT, four of nine patients progressed to cirrhosis during a mean follow-up period of 20 months without antiviral therapy [96].

Antiviral therapy with NA does not have any efficacy against HDV infection. Interferon alfa is the only currently approved treatment for HDV but is generally poorly tolerated in the pre- and post-transplant settings and typically requires years of treatment due to the high risk of relapse [97]. Thus, the goal of therapy in the transplant setting is to prevent reinfection of a liver graft after transplant. Post-LT combination therapy with NA and long-term HBIG to optimize HBsAg clearance is recommended to reduce the risk of reinfection with HBV and HDV (Table 4).

Currently there are no published cases of donor-derived HDV transmission in non-liver transplants. OPTN does not currently recommend routine donor HDV serologic testing. Nevertheless, care and consideration for HDV coinfection should be made in donors with positive HBsAg. When possible, additional donor testing (anti-HDV and, if positive, HDV RNA) should be considered.

Conclusion

Hepatitis B virus and hepatitis D virus infections can have significant impact on transplant recipients. The evaluation and management of these patients begin well before the transplant event itself. Serologic testing of recipients and potential donors is crucial to risk-stratify patients for HBV- and HDV-related complications. With the

combination of appropriate vaccination, close observation, and pharmacologic therapy, significant morbidity and mortality can be avoided.

Key Points

- De novo hepatitis B infection, reactivation of hepatitis B during immunosuppression, and donor transmission of hepatitis B are all potential complications that can occur in the transplant setting.
- If managed appropriately, patients with chronic hepatitis B infections can have similar post-transplant outcomes to those without chronic hepatitis B infection.
- All donors and recipients with chronic hepatitis B infection should have serologies checked for hepatitis D virus infection.
- With the opioid epidemic and shortage of available organs, there will be a continued rise in use of HBV-exposed donor organs, but risk-based antiviral prophylaxis can achieve excellent outcomes in these transplants.
- Treatment with high-potency nucleos(t)ide analogues is indicated for all chronic hepatitis B-infected patients with cirrhosis who are on the liver transplant waiting list and for all transplant recipients on immunosuppression.
- Depending on the type of transplant, donor immune status, and degree of immunosuppression, prophylactic treatment with antiviral therapy may be warranted in cases where either the donor or recipient has evidence of prior hepatitis B exposure without active infection.

Cross-References

- ▶ Introduction to Immunosuppression, Tolerance, and Infection Risk
- ▶ Pretransplant Screening for Infection Exposure: Approach to the Patients with Epidemiologic and Geographic Risk Factors
- ▶ Solid Organ Transplantation in HIV Positive Patients

References

1. Goldstein ST, Zhou F, Hadler SC, et al. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol. 2005;34:1329–39.
2. Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet. 2015;386:1546–55.
3. Liaw YF. Natural history of chronic hepatitis B virus infection and long-term outcome under treatment. Liver Int. 2009;29(Suppl 1):100–7.
4. Abbas Z, Jafri W, Raza S. Hepatitis D: scenario in the Asia-Pacific region. World J Gastroenterol. 2010;16:554–62.
5. Rizzetto M, Hepatitis D. Virus: introduction and epidemiology. Cold Spring Harb Perspect Med. 2015;5:a021576.

6. Ghany MG, Perrillo R, Li R, et al. Characteristics of adults in the hepatitis B research network in North America reflect their country of origin and hepatitis B virus genotype. Clin Gastroenterol Hepatol. 2015;13:183–92.
7. Harris AM, Iqbal K, Schillie S, et al. Increases in acute hepatitis B virus infections – Kentucky, Tennessee, and West Virginia, 2006–2013. MMWR Morb Mortal Wkly Rep. 2016;65:47–50.
8. Flemming JA, Kim WR, Brosgart CL, et al. Reduction in liver transplant wait-listing in the era of direct-acting antiviral therapy. Hepatology. 2017;65:804–12.
9. Kim WR, Terrault NA, Pedersen RA, et al. Trends in waiting list registration for liver transplantation for viral hepatitis in the United States. Gastroenterology. 2009;137:1680–6.
10. Chopra B, Sureshkumar KK. Outcomes of kidney transplantation in patients exposed to hepatitis B virus: analysis by phase of infection. Transplant Proc. 2017;49:278–80.
11. Maini MK, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. J Hepatol. 2016;64:S60–70.
12. Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. J Hepatol. 2016;64:S71–83.
13. Zhou K, Contag C, Whitaker E, et al. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. Lancet Gastroenterol Hepatol. 2019;4:227–38.
14. Yeo YH, Ho HJ, Yang HI, et al. Factors associated with rates of HBsAg seroclearance in adults with chronic HBV infection: a systematic review and meta-analysis. Gastroenterology. 2019;156:635–46.. e9
15. Xia Y, Liang TJ. Development of direct-acting antiviral and host-targeting agents for treatment of hepatitis B virus infection. Gastroenterology. 2019;156:311–24.
16. Ji M, Hu K. Recent advances in the study of hepatitis B virus covalently closed circular DNA. Virol Sin. 2017;32:454–64.
17. Terrault NA, Lok AS, McMahon BJ, et al. Update on prevention, diagnosis, and treatment and of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67:1560–99.
18. Peng CY, Chien RN, Liaw YF. Hepatitis B virus-related decompensated liver cirrhosis: benefits of antiviral therapy. J Hepatol. 2012;57:442–50.
19. Singal AK, Salameh H, Kuo YF, et al. Meta-analysis: the impact of oral anti-viral agents on the incidence of hepatocellular carcinoma in chronic hepatitis B. Aliment Pharmacol Ther. 2013;38:98–106.
20. Marzano A, Gaia S, Ghisetti V, et al. Viral load at the time of liver transplantation and risk of hepatitis B virus recurrence. Liver Transpl. 2005;11:402–9.
21. Mohan D, Railey M, Al Rukhaimi M. Vaccination and transient hepatitis B surface antigenemia. NDT Plus. 2011;4:190–1.
22. Raimondo G, Pollicino T, Cacciola I, et al. Occult hepatitis B virus infection. J Hepatol. 2007;46:160–70.
23. Benwell N, Boan P, Raby E, et al. False positive hepatitis B virus core and surface antibodies due to intravenous immunoglobulin. Intern Med J. 2017;47:119–20.
24. Seed CR, Allain JP, Lozano M, et al. International forum on occult hepatitis B infection and transfusion safety. Vox Sang. 2019;114(4):397–406.
25. Organ Procurement and Transplant Network (OPTN). Policies. 2019. Available from: https://optn.transplant.hrsa.gov/media/1200/optn_policies.pdf. Effective Date March 1, 2019. Dated Accessed 9 Mar 2019. 2019.
26. Huprikar S, Danziger-Isakov L, Ahn J, et al. Solid organ transplantation from hepatitis B virus-positive donors: consensus guidelines for recipient management. Am J Transplant. 2015;15:1162–72.
27. Bixler D, Annambholta P, Abara WE, et al. Hepatitis B and C virus infections transmitted through organ transplantation investigated by CDC, United States, 2014–2017. Am J Transplant. 2019;19(9):2570–82.
28. Abara WE, Collier MG, Moorman A, et al. Characteristics of deceased solid organ donors and screening results for hepatitis B, C, and human immunodeficiency viruses – United States, 2010–2017. MMWR Morb Mortal Wkly Rep. 2019;68:61–6.

29. Malinis M, Boucher HW, Practice ASTIDCo. Screening of donor and candidate prior to solid organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13548.
30. Millonig G, Friedrich S, Adolf S, et al. Liver stiffness is directly influenced by central venous pressure. J Hepatol. 2010;52:206–10.
31. Del Poggio P, Colombo S. Is transient elastography a useful tool for screening liver disease? World J Gastroenterol. 2009;15:1409–14.
32. Liang XE, Zhong C, Huang L, et al. Optimization of hepatitis B cirrhosis detection by stepwise application of transient elastography and routine biomarkers. J Gastroenterol Hepatol. 2017;32:459–65.
33. Liaw YF, Pao CC, Chu CM. Changes of serum HBV-DNA in relation to serum transaminase level during acute exacerbation in patients with chronic type B hepatitis. Liver. 1988;8:231–5.
34. Karvellas CJ, Cardoso FS, Gottfried M, et al. HBV-associated acute liver failure after immunosuppression and risk of death. Clin Gastroenterol Hepatol. 2017;15:113–22.
35. Fornairon S, Pol S, Legendre C, et al. The long-term virologic and pathologic impact of renal transplantation on chronic hepatitis B virus infection. Transplantation. 1996;62:297–9.
36. Fabrizi F, Martin P, Dixit V, et al. HBsAg seropositive status and survival after renal transplantation: meta-analysis of observational studies. Am J Transplant. 2005;5:2913–21.
37. Starzl TE, Demetris AJ, Van Thiel D. Liver transplantation (2). N Engl J Med. 1989;321:1092–9.
38. Todo S, Demetris AJ, Van Thiel D, et al. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. Hepatology. 1991;13:619–26.
39. Samuel D, Muller R, Alexander G, et al. Liver transplantation in European patients with the hepatitis B surface antigen. N Engl J Med. 1993;329:1842–7.
40. Davies S, Portmann B, O'Grady J, et al. Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. Hepatology. 1991;13:150–7.
41. Mason A, Wick M, White H, et al. Increased hepatocyte expression of hepatitis B virus transcription in patients with features of fibrosing cholestatic hepatitis. Gastroenterology. 1993;105:237–44.
42. Mathurin P, Mouquet C, Poynard T, et al. Impact of hepatitis B and C virus on kidney transplantation outcome. Hepatology. 1999;29:257–63.
43. Lee J, Cho JH, Lee JS, et al. Pretransplant hepatitis B viral infection increases risk of death after kidney transplantation: a multicenter cohort study in Korea. Medicine (Baltimore). 2016;95:e3671.
44. Yap DY, Tang CS, Yung S, et al. Long-term outcome of renal transplant recipients with chronic hepatitis B infection-impact of antiviral treatments. Transplantation. 2010;90:325–30.
45. Harmanci O, Ilin S, Ocal S, et al. The effect of hepatitis B virus on graft and overall survival in kidney transplant patients. Exp Clin Transplant. 2015;13(Suppl 3):36–40.
46. Chen YC, Chuang MK, Chou NK, et al. Twenty-four year single-center experience of hepatitis B virus infection in heart transplantation. Transplant Proc. 2012;44:910–2.
47. Hoffmann CJ, Subramanian AK, Cameron AM, et al. Incidence and risk factors for hepatocellular carcinoma after solid organ transplantation. Transplantation. 2008;86:784–90.
48. Gentile G, Andreoni M, Antonelli G, et al. Screening, monitoring, prevention, prophylaxis and therapy for hepatitis B virus reactivation in patients with haematologic malignancies and patients who underwent haematologic stem cell transplantation: a systematic review. Clin Microbiol Infect. 2017;23:916–23.
49. Lau GK, Leung YH, Fong DY, et al. High hepatitis B virus (HBV) DNA viral load as the most important risk factor for HBV reactivation in patients positive for HBV surface antigen undergoing autologous hematopoietic cell transplantation. Blood. 2002;99:2324–30.
50. Lau GK, Yiu HH, Fong DY, et al. Early is superior to deferred preemptive lamivudine therapy for hepatitis B patients undergoing chemotherapy. Gastroenterology. 2003;125:1742–9.
51. Sarmati L, Andreoni M, Antonelli G, et al. Recommendations for screening, monitoring, prevention, prophylaxis and therapy of hepatitis B virus reactivation in patients with

haematologic malignancies and patients who underwent haematologic stem cell transplantation-a position paper. Clin Microbiol Infect. 2017;23:935–40.
52. Knoll A, Pietrzyk M, Loss M, et al. Solid-organ transplantation in HBsAg-negative patients with antibodies to HBV core antigen: low risk of HBV reactivation. Transplantation. 2005;79:1631–3.
53. Querido S, Weigert A, Adragao T, et al. Risk of hepatitis B reactivation in hepatitis B surface antigen seronegative and core antibody seropositive kidney transplant recipients. Transpl Infect Dis. 2019;21:e13009.
54. Abdelmalek M, Pasha T, Zein N, et al. Subclinical reactivation of hepatitis B virus in liver transplant recipients with past exposure. Liver Transpl. 2003;9:1253–7.
55. Buti M, Gane E, Seto WK, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of patients with HBeAg-negative chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol. 2016;1:196–206.
56. Chan HL, Fung S, Seto WK, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of HBeAg-positive chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol. 2016;1:185–95.
57. Tenney DJ, Rose RE, Baldick CJ, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. Hepatology. 2009;49:1503–14.
58. Park ES, Lee AR, Kim DH, et al. Identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients. J Hepatol. 2019;70(6):1093–102.
59. Magnone M, Holley J, Shapiro R, et al. Interferon-a-induced acute renal allograft rejection. Transplantation. 1995;59:1068–70.
60. Baid S, Tolkoff-Rubin N, Saidman S, et al. Acute humoral rejection in hepatitis C-infected renal transplant recipients receiving antiviral therapy. Am J Transplant. 2003;3:74–8.
61. Rangel MC, Coronado VG, Euler GL, et al. Vaccine recommendations for patients on chronic dialysis. The Advisory Committee on Immunization Practices and the American Academy of Pediatrics. Semin Dial. 2000;13:101–7.
62. Feuerhake A, Muller R, Lauchart W, et al. HBV-vaccination in recipients of kidney allografts. Vaccine. 1984;2:255–6.
63. Carey W, Pimentel R, Westveer MK, et al. Failure of hepatitis B immunization in liver transplant recipients: results of a prospective trial. Am J Gastroenterol. 1990;85:1590–2.
64. Rubin LG, Levin MJ, Ljungman P, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58:e44–100.
65. Schillie S, Harris A, Link-Gelles R, et al. Recommendations of the Advisory Committee on Immunization Practices for use of a hepatitis B vaccine with a novel adjuvant. MMWR Morb Mortal Wkly Rep. 2018;67:455–8.
66. Terrault NA, Zhou S, Combs C, et al. Prophylaxis in liver transplant recipients using a fixed dosing schedule of hepatitis B immunoglobulin. Hepatology. 1996;24:1327–33.
67. Samuel D, Bismuth A, Mathieu D, et al. Passive immunoprophylaxis after liver transplantation in HBsAg-positive patients. Lancet. 1991;337:813–5.
68. Khemichian S, Hsieh MJ, Zhang SR, et al. Nucleoside-nucleotide analog combination therapy is effective in preventing recurrent hepatitis B after liver transplantation. Dig Dis Sci. 2015;60:2807–12.
69. Fung J, Wong T, Chok K, et al. Long term outcomes of entecavir monotherapy for chronic hepatitis B after liver transplantation: results up to 8 years. Hepatology. 2017;66 (4):1036–44.
70. Gane EJ, Patterson S, Strasser SI, et al. Combination of lamivudine and adefovir without hepatitis B immune globulin is safe and effective prophylaxis against hepatitis B virus recurrence in hepatitis B surface antigen-positive liver transplant candidates. Liver Transpl. 2013;19:268–74.
71. Radhakrishnan K, Chi A, Quan DJ, et al. Short course of postoperative hepatitis B immunoglobulin plus antivirals prevents reinfection of liver transplant recipients. Transplantation. 2017;101:2079–82.

72. Fox AN, Terrault NA. Individualizing hepatitis B infection prophylaxis in liver transplant recipients. J Hepatol. 2011;55:507–9.
73. Coffin CS, Stock PG, Dove LM, et al. Virologic and clinical outcomes of hepatitis B virus infection in HIV-HBV coinfected transplant recipients. Am J Transplant. 2010;10:1268–75.
74. Wachs M, Amend W, Ascher N, et al. The risk of transmission of hepatitis B from HBsAg(−), HBcAb(+), HBIgM(−) organ donors. Transplantation. 1995;59:230–4.
75. Skagen CL, Jou JH, Said A. Risk of de novo hepatitis in liver recipients from hepatitis-B core antibody-positive grafts – a systematic analysis. Clin Transpl. 2011;25:E243–9.
76. Yen RD, Bonatti H, Mendez J, et al. Case report of lamivudine-resistant hepatitis B virus infection post liver transplantation from a hepatitis B core antibody donor. Am J Transplant. 2006;6:1077–83.
77. Cholongitas E, Papatheodoridis GV, Burroughs AK. Liver grafts from anti-hepatitis B core positive donors: a systematic review. J Hepatol. 2010;52:272–9.
78. Wong TC, Fung JY, Yu-Shi Cui T, et al. Liver transplantation using hepatitis B core positive grafts with antiviral monotherapy prophylaxis. J Hepatol. 2019;70(6):1114–22.
79. Mahboobi N, Tabatabaei SV, Blum HE, et al. Renal grafts from anti-hepatitis B core-positive donors: a quantitative review of the literature. Transpl Infect Dis. 2012;14:445–51.
80. Chung RT, Feng S, Delmonico FL. Approach to the management of allograft recipients following the detection of hepatitis B virus in the prospective organ donor. Am J Transplant. 2001;1:185–91.
81. Dhillon GS, Levitt J, Mallidi H, et al. Impact of hepatitis B core antibody positive donors in lung and heart-lung transplantation: an analysis of the United Network for Organ Sharing Database. Transplantation. 2009;88:842–6.
82. Levitsky J, Doucette K, Practice ASTIDCo. Viral hepatitis in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):147–68.
83. Blanpain C, Knoop C, Delforge ML, et al. Reactivation of hepatitis B after transplantation in patients with pre-existing anti-hepatitis B surface antigen antibodies: report on three cases and review of the literature. Transplantation. 1998;66:883–6.
84. Lee J, Park JY, Huh KH, et al. Rituximab and hepatitis B reactivation in HBsAg-negative/anti-HBc-positive kidney transplant recipients. Nephrol Dial Transplant. 2017;32:906.
85. Reddy KR, Beavers KL, Hammond SP, et al. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. Gastroenterology. 2015;148:215–9; quiz e16-7
86. Wei HK, Loong CC, King KL, et al. HBsAg(+) donor as a kidney transplantation deceased donor. Transplant Proc. 2008;40:2097–9.
87. Jeng LB, Thorat A, Yang HR, et al. Successful use of hepatitis B surface antigen-positive liver grafts – an effective source for donor organs in endemic areas: a single-center experience. Ann Transplant. 2015;20:103–11.
88. Yu S, Yu J, Zhang W, et al. Safe use of liver grafts from hepatitis B surface antigen positive donors in liver transplantation. J Hepatol. 2014;61:809–15.
89. Franchello A, Ghisetti V, Marzano A, et al. Transplantation of hepatitis B surface antigen-positive livers into hepatitis B virus-positive recipients and the role of hepatitis delta coinfection. Liver Transpl. 2005;11:922–8.
90. Chancharoenthana W, Townamchai N, Pongpirul K, et al. The outcomes of kidney transplantation in hepatitis B surface antigen (HBsAg)-negative recipients receiving graft from HBsAg-positive donors: a retrospective, propensity score-matched study. Am J Transplant. 2014;14:2814–20.
91. Raimondo G, Brunetto MR, Pontisso P, et al. Longitudinal evaluation reveals a complex spectrum of virological profiles in hepatitis B virus/hepatitis C virus-coinfected patients. Hepatology. 2006;43:100–7.
92. Romeo R, Del Ninno E, Rumi M, et al. A 28-year study of the course of Hepatitis Delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. Gastroenterology. 2009;136:1629–38.
93. Fattovich G, Giustina G, Christensen E, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). Gut. 2000;46:420–6.

94. Krogsgaard K, Kryger P, Aldershvile J, et al. Delta-infection and suppression of hepatitis B virus replication in chronic HBsAg carriers. Hepatology. 1987;7:42–5.
95. Samuel D, Zignego A, Reynes M, et al. Long-term clinical and virological outcome after liver transplantation for cirrhosis caused by chronic delta hepatitis. Hepatology. 1995;21:333–9.
96. David E, Rahier J, Pucci A, et al. Recurrence of hepatitis D (delta) in liver transplants: histopathological aspects. Gastroenterology. 1993;104:1122–8.
97. Heidrich B, Yurdaydin C, Kabacam G, et al. Late HDV RNA relapse after peginterferon alpha-based therapy of chronic hepatitis delta. Hepatology. 2014;60:87–97.

Emerging Management of Hepatitis C in Transplant

39

Lorena Puchades and Marina Berenguer

Contents

Introduction 952
Pretransplant HCV Management 952
Treatment Efficacy in Patients with Decompensated Cirrhosis 952
Functional and Clinical Evolution Following DAAs 954
Potential Harms of Treating Patients on the Waiting List 955
DAA Benefits in Patients with HCC on the Waiting List for Liver Transplant 956
Impact of DAA on LT Waiting Lists 956
Posttransplant HCV Management 957
DAA Regimens After Liver Transplantation 957
Impact of DAA on Posttransplant Outcomes of HCV-Positive Recipients 958
Impact of DAA on the More Extensive Use of HCV-Positive Donors 960
Use of Viremic Donor Organs for Transplant into HCV-Negative Recipients 961
Conclusion 963
Key Points 963
References 964

L. Puchades
Department of Gastroenterology, Hepatology Unit and Instituto de Investigación La Fe, Hospital Universitari i Politècnic La Fe, Valencia, Spain

Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Valencia, Spain

M. Berenguer (✉)
Department of Gastroenterology, Hepatology Unit and Instituto de Investigación La Fe, Hospital Universitari i Politècnic La Fe, Valencia, Spain

Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Valencia, Spain

School of Medicine, University of Valencia, Valencia, Spain
e-mail: marina.berenguer@uv.es

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_38

Introduction

Chronic hepatitis C virus (HCV) infection affects about 71 million people worldwide, two-thirds living in Europe, South Asia, and North Africa. Seven countries (China, Pakistan, India, Egypt, Russia, USA, and Nigeria) account for half of the global population of HCV infected persons [1]. Each year, Hepatitis C is responsible for approximately 399,000 deaths worldwide, mostly from cirrhosis and liver cancer [1]. HCV-related advanced liver disease and HCV-related hepatocellular carcinoma (HCC) are leading indications for liver transplantation (LT) worldwide representing 20% of all LT performed in Europe and the United States (USA) [2]. Recently, the proportion of HCV-induced cirrhosis requiring liver transplant has begun to decline in the United States, as more people gain access to high effective antiviral agents.

Sustained virological response (SVR), defined as undetectable HCV-RNA 12 months posttherapy [3, 4], represents the cure of the infection. In the interferon (IFN) era, treatment was rarely feasible in LT candidates due to their advanced liver insufficiency, and in the posttransplant setting results were modest with high toxicity [5–7]. For practical purposes, three time-periods can be identified based on HCV-treatments availability: prior to 2010 when only IFN-based regimens were available (IFN-era), from 2010 to 2013 when the first generation of direct-acting antivirals (DAAs) – boceprevir and telaprevir – two nonstructural protease inhibitors (PIs) combined with IFN and ribavirin were available (PI-IFN era) and after 2013 when all-oral DAAs combinations were approved (DAA era). Currently, pangenotypic regimes are available and represent the standard-of-care recommended by general guidelines [4]. IFN-free regimens based on these specific drug combinations are characterized by shorter treatment duration, typically 8–12 weeks. They have SVR rates greater than 97%, both in transplanted populations and nontransplant groups, pangenotypic activity, are exclusively oral administration, and generally possess an excellent safety and tolerability profile, with limited drug-drug interactions [4].

The present review provides an overview of chronic HCV infection current management and focuses on the use of DAA treatment in the field of transplant, both in the pretransplant and the posttransplant period.

Pretransplant HCV Management

Treatment Efficacy in Patients with Decompensated Cirrhosis

Several studies have investigated the efficacy of DAAs in patients with different degrees of hepatic decompensation, generally based on Child-Turcotte-Pugh (CTP) classification's classes A, B, and C (Table 1). Based on these studies, it has become clear that: (i) protease inhibitors should not be used in HCV-patients with decompensated cirrhosis (CTP-B or C); (ii) patients with decompensated cirrhosis, particularly those with CTP class C achieve lower rates of SVR; and (iii) ribavirin

Table 1 Efficacy of Direct Antiviral Agents (DAAs) in patients with decompensated cirrhosis

	SOLAR-1 [8]	SOLAR-2 [9]	ASTRAL-4 [10]	UK-EAP [11][a]		HEPA-C Registry [12]	
Patients, n	108	160	267	467		175	
DAA therapy	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Velpatasvir +/− RBV	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Daclatasvir +/− RBV	Sofosbuvir-based regimens[b] +/− RBV	Sofosbuvir-free regimens[c] +/− RBV
Treatment duration	12 or 24 weeks	12 or 24 weeks	12 or 24 weeks	12 weeks		12 or 24 weeks	
Genotypes (1 to 6)	1 or 4	1 or 4	All genotypes	All genotypes		All genotypes	
Degree of hepatic decompensation (Child-Turcotte-Pugh-CTP-class)	CTP-B (59 patients) CTP-C (49 patients)	CTP-B (78 patients) CTP-C (82 patients)	CTP-B	CTP-A (112 patients) CTP-B (309 patients) CTP-C (46 patients)		CTP-B (309 patients) CTP-C (46 patients)	
SVR-12 rates (%)	87–89% (CTP-B) 86–87% (CTP-C)	87–96% (CTP-B) 78–85% (CTP-C)	83–94% (CTP-B)	82% (CTP-B and C)		78% (CTP-B and C)	

[a]United Kingdom expanded access programme
[b]Sofosbuvir-Simeprevir; Sofosbuvir-Daclatasvir or Sofosbuvir-Ledipasvir
[c]Ombitasvir-Paritaprevir/ritonavir with or without Dasabuvir

(RBV) is required in some of these patients to increase efficacy [8–12]. In brief, rates of viral eradication decrease as liver function worse with SVR rates ≥90% in patients with CTP class A cirrhosis, 80–90% in patients with CTP class B cirrhosis, and only 60–80% in those with CTP Class C cirrhosis.

Functional and Clinical Evolution Following DAAs

Viral eradication results in histological and clinical improvement [13], also in patients with advanced decompensated disease, with reversal of disease severity as assessed by the model for end-stage liver disease (MELD) and Child-Turcotte-Pugh scores, and/or decreased portal hypertensive complications, eventually leading to "re-compensation" and LT avoidance. Two European studies, each one including more than 100 patients, found a significant reduction in portal hypertension following successful antiviral therapy with an overall medium reduction of hepatic venous pressure gradient (HVPG) between 2.2 and 2.6 mmHg after SVR [14, 15]. HVPG reduction though was less likely among patients with more advanced liver dysfunction (CTP B class) and clinically significant portal hypertension (HVPG ≥10 mmHg) persisted in many patients despite HCV eradication indicating a persisting risk of morbidity from portal hypertensive complications. Similarly, several studies reported liver function improvement after SVR with DAA therapy [8–10, 16, 17], quantified as reductions in MELD and CTP scores from baseline to posttreatment (Table 2); a significative reduction in rates of serious decompensation events, sepsis, or hospitalizations beyond 6 months following viral clearance was also reported [18]. Moreover, the impact of HCV eradication on the waiting list for LT was investigated in a large European study which included 103 consecutive listed patients without HCC. Delisting was feasible in about one out of five patients after a mean follow up of 60 weeks [19]. Extended follow-up to 2 years after delisting showed that DAA treatments result in long-term improvement of liver synthetic

Table 2 Liver function improvement following DAA therapy with sofosbuvir-based therapies in HCV-patients with decompensated cirrhosis

	SOLAR-1 [8, 16]	SOLAR-2 [9, 16]	ASTRAL-4 [10, 16]	GS-US-334-0125 [16, 17]
DAA therapy	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Velpatasvir +/− RBV	Sofosbuvir + RBV
Patients, n	$n = 622$			
Degree of hepatic decompensation (child-Turcotte-Pugh-CTP-class)	CTP-B (502 patients) CTP-C (120 patients)			
Follow-up from DAA-start (weeks)	36 weeks			
Re-compensation[a] rates (%)	32% (CTP-B at baseline) 12% (CTP-C at baseline)			

[a]Reduction of CPT score to class A

function with a low risk of liver-related complications requiring relisting. Two years after delisting, a new HCC was diagnosed in only 4.5% (2/44) of patients previously removed from the LT waitlist [20].

Potential Harms of Treating Patients on the Waiting List

Efficacy, safety, and tolerability of DAA treatments worsen as the severity of liver disease increases. This reduction in antiviral therapy success is due to an increment in the number of nonvirologic failures (death from underlying disease progression) and virologic failures (those related to the development of long-term drug resistances which may difficult retreatment options following LT) [21]. Although retreatment options are currently available for patients who fail previous DAA therapies (glecaprevir/pibrentasvir and sofosbuvir/velpatasvir/voxilaprevir), their use is limited in patients with decompensated cirrhosis as both combinations include a protease inhibitor (NS3 PI). Furthermore, many patients in the waiting list have concomitant renal insufficiency, and the best DAA therapies in terms of security for patients with advanced liver disease, sofosbuvir-based [21], should still be used with caution in patients with severe renal impairment (estimated glomerular filtration rate [eGFR] <30 ml/min/1.73 m^2) or end-stage renal disease in hemodialysis because of residual concern about cumulative toxicity concerns regarding sofosbuvir and its renal excreted metabolite GS-331007 [22, 23]. Results from a recently ended phase 2b clinical trial, which evaluated two sofosbuvir combinations (sofosbuvir 400 mg + RBV 200 mg during 24 weeks; and ledipasvir + sofosbuvir during 12 weeks) in HCV infected patients with severe renal insufficiency, concluded that no dose adjustments can be suggested in this population, and that its use remains beyond license recommendations (https://www.ClinicalTrial.gov: NCT01958281).

Another potential limitation to treat waitlisted patients is the eventual lack of significant clinical improvements despite SVR. The "point of no return" that defines a limited hepatic repair capacity in the sickest patients is still unknown [21]. Furthermore, improvement in MELD scores after viral clearance may not reach a sufficient cut-off for delisting, leaving patients with a lower MELD score than before therapy with therefore lower LT access, according to the current organ allocation system [24]. This is referred as the "MELD limbo or purgatory" which is more likely to affect patients with high MELD scores as the magnitude of improvement in liver function from baseline ("delta-MELD") needs to be significantly higher to achieve real clinical benefits [19]. Particularly, *Belli and colleagues* showed that in patients with a MELD score >15, a delta MELD greater than 4 was required to result in meaningful improvement in the medium-long term [19]. Thus, both major Liver Transplant Societies (The European Liver and Intestine Transplant Association -ELITA- and the International Liver Transplant Society -ILTS-) recommend DAA therapy for waitlisted patients with HCV-decompensated cirrhosis and MELD score lower than 20 while in patients with higher MELD score the decision to treat should be individualized after carefully weighing the risk of a MELD purgatory effect related to an expected prolonged waiting time [21, 25]. In reality, the drive to treat HCV ahead of transplant is also balanced against the wait times for liver allocation in

that patient's region, and their willingness to also take a HCV-viremic donor. In many cases in the United States, the time taken to allocate a viremic organ is significantly less, hence waiting to initiate DAA treatment until after transplant, as a means of facilitating more rapid transplant, may be preferred.

Objective tools to assess whether the benefits of treating patients with HCV-decompensated cirrhosis outweigh the previously mention risks have been recently investigated. A study from Spain [26] which included 43 patients with cirrhosis treated with new DAAs explored baseline liver volume obtained through imaging assessment and adjusted by body surface area, as a noninvasive instrument to predict improvement of liver function over time after viral eradication. In addition, a clinically based tool to predict liver repair capacity following DAA therapy was recently published, consisting in a predictive score (BE3A score) based on 5 clinical and analytical variables; so that a body mass index <25 kg/m^2, serum levels of albumin >3.5 g/dL, and alanine aminotransferase (ALT) >60 IU/L together with absence of encephalopathy and/or ascites predict a greater probability of achieving re-compensation (reduction of CPT score to class A) and thus future benefit from DAA treatment before LT [16].

DAA Benefits in Patients with HCC on the Waiting List for Liver Transplant

Although initial studies suggested a potential increase in the risk of aggressive tumor recurrence and/or development of de novo HCC in patients treated with DAA therapy [27, 28], recent and more properly designed studies have demonstrated that these therapies are not harmful for LT candidates with HCC. Results from the French ANRS collaborative study group on HCC, prospectively including more than 6000 patients from three different cohorts, did not show a higher risk of HCC recurrence after DAA therapy than that historically observed in HCV-patients not treated with DAA combinations [29]. Concordantly, in a retrospective single center study of 149 waitlisted patients for HCV-related HCC in the United States from 2014 to 2016, DAA therapy had no impact on rates of HCC recurrence after local-regional treatment and was associated with a reduction in waitlist dropout due to death or tumor progression and with a trend towards increased likelihood of survival [30]. Several recent very large studies as well as meta-analyses have confirmed that both de novo and recurrent HCC are significantly reduced following SVR. Finally, in a large recent North American study which included 62,354 patients who initiated antiviral therapy in the Veterans Affairs national healthcare system from 1 Jan 1999 to 31 Dec 2015, viral eradication was associated to an overall HCC risk reduction of 70%, which confirms DAA therapies' benefits in terms of HCV-related HCC [31].

Impact of DAA on LT Waiting Lists

The main consequence of these therapies has been the significant reduction in new registrations for HCV-indications (Table 3) [2, 32–34]. This reduction has been more

Table 3 Impact of Direct -Acting Antivirals (DAA) on LT waiting lists (WL)

	Spanish single-center study (La Fe Hospital) [32]	Spanish National Transplant Organization Registry (ONT) [33]	Scientific Registry of Transplant Recipients (SRTR) [34]	European Liver Transplant Registry (ELTR) [2]
Liver transplant (LT) database, region	Valencia (Spain)	Spain	United States	Europe
Patients, n	2379 LT recipients	30,653 waitlisted for LT	47,591 waitlisted for LT	60,527 LT recipients
Study period, year	1996–2016	1997–2017	2003–2015	2007–2017
Reduction of HCV new registrations in the WL or transplants[a], %	−16% (overall) +21% (HCC)	N/A[b]	−8% (overall) −32% (decompensated cirrhosis) +34% (HCC)	−5% (overall) −60% (decompensated cirrhosis) −40% (HCC)
Reduction in waitlistings for HCV, %	−16% (2014–2016)	−10% (2014–2017)	−13% (2012–2016)	−5% (2007–2017)

[a]In the DAA era (after 2013) as compared to the Interferon era (prior 2010)
[b]Non applicable

significant in patients with HCV-decompensated cirrhosis than in those with HCV-related-HCC [2, 32–34]. This fact has been postulated to be related to (i) an increased residual risk for HCC versus decompensation in HCV-patients after viral eradication, (ii) aging of LT candidates with the consideration of old candidates with HCV-decompensated cirrhosis to be worse candidates than those with HCV-related-HCC, and (iii) possibility of delisting for clinical improvement following SVR in HCV-patients with decompensated cirrhosis unlike patients with HCC who cannot avoid LT indication in spite of decompensation reversal [35]. On the other hand, as the proportion of LT procedures for HCV-related diseases decline, the rate of LT indications for nonalcoholic fatty liver disease (NAFLD) and for alcohol-associated liver disease increases thus maintaining the number of LT performed in Europe and USA, stables over time [2, 34, 35].

Posttransplant HCV Management

DAA Regimens After Liver Transplantation

In LT recipients for HCV-related disease, HCV recurrence is nearly universal [36]. In the posttransplant setting, the natural history of HCV infection is accelerated compared to the pretransplant phase mostly due to immunosuppression. The proportion of HCV recipients that evolve to cirrhosis within 5 years after LT is approximately

15–30% [37, 38] with a subset of subjects developing a rare but severe form of recurrence, which leads to liver failure and death in only few months, so-called fibrosing cholestatic hepatitis (FCH) [37, 38]. Such events are now considered rare with efficacious early posttransplant administration of DAA therapy.

Treatment of HCV recurrence in LT recipients has been traditionally challenging because of the poor efficacy, high toxicity, and risk of rejection associated to IFN-based regimens [39]. A variety of oral DAA combinations have shown very high success rates and excellent safety profiles in patients with HCV without cirrhosis or with compensated cirrhosis post-LT (Table 4) [8, 9, 40–45]. Excellent SVR rates have also been reported in subjects with FCH, the most uncommon and life-threatening form of HCV recurrence (Table 5) [8, 9, 46, 47]. However, similarly to what has been published in nontransplanted subjects, worst results are observed in HCV recipients with decompensated graft cirrhosis and portal hypertension (Table 5) [8, 9, 46, 47].

In terms of timing of therapy post-LT, most experts recommend starting therapy as early as possible once the complications from the LT surgery have subsided, to improve tolerability and reduce drug-drug interactions. Indeed, an exquisite evaluation of drug-drug interactions is needed in all patients, but particularly in transplant recipients given the potential effect on immunosuppression trough levels. Strict monitoring of drug levels with adjustments as needed is required both during and after antiviral therapy [21, 25].

Impact of DAA on Posttransplant Outcomes of HCV-Positive Recipients

The advent of newer DAA therapies has dramatically changed post-LT outcomes of HCV recipients. In the large European study based on the ELTR database, authors showed a significant improvement of 3-year survival in patients transplanted for HCV-decompensated cirrhosis, from 65.1% in the IFN-era to 76.9% in the DAA era, results that are now equal to those achieved by LT recipients with chronic hepatitis B virus (HBV) infection [2]. Concordant results were observed in the recent USA-Scientific Registry of Transplant Recipients study where 52,526 LT recipients were included. In the DAA era, the 3-year graft survival was found to have increased four-fold more for HCV-positive recipients than for non-HCV recipients, with both groups of patients currently obtaining similar outcomes following LT [48].

Early studies in the modern DAA era assessed recurrent HCV infection after LT. SOLAR-1 was a randomized, multicenter trial, run out of the United States, with 223 liver transplant recipients with genotype 1 and 4, across all stages of fibrosis and cirrhosis. SVR12 rates using ledipasvir, sofosbuvir, and ribavirin for 12 or 24 weeks were 96–98%. SOLAR-2 trial had a similar design in an international setting and resulted in SVR12 rates ranging from 87% to 100% depending on the genotype and degree of liver fibrosis. More contemporaneous regimens, including the pan-genotypic DAA cocktails, have equally proven effective. Glecaprevir/pibrentasvir was evaluated in the MAGELLAN-2 trial, where 80 liver recipients

Table 4 Efficacy of Direct Antiviral Agents (DAAs) after liver transplantation in patients without cirrhosis or with compensated cirrhosis

	Kwo et al. [40]	Charlton et al. [8] (SOLAR-1)	Manns et al. [9] (SOLAR-2)	Coilly et al. [41] (ANRS CUPILT)	Gutiérrez et al. [42]	Reau et al. [43] (MAGELLAN-2)	Agarwal et al. [44]	Cardona-González et al. [45]
Patients, n	34	162	168	137	61	80	79	1
DAA therapy	3D combination[a] + RBV	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Daclatasvir +/-RBV	Sofosbuvir-Simeprevir +/− RBV	Glecaprevir-Pibrentasvir	Sofosbuvir-Velpatasvir	Sofosbuvir-Velpatasvir-Voxilaprevir + RBV
Treatment duration	24 weeks	12 or 24 weeks	12 or 24 weeks	12 or 24 weeks	12 weeks	12 weeks	12 weeks	16 weeks
Genotypes (1 to 6)	1	1 or 4	1 or 4	All genotypes	1	All genotypes	1,2,3,4	3
% of cirrhosis (F4 fibrosis stage) **or severe fibrosis** (F3 fibrosis stage)	0%, F4 (All F0, F1 or F2)[b]	30%, F4	40%, F4	34%, F4	30%, F3/F4	0%, F4 15%, F3	18% F4	0% F4 (F0 patient)[b]
Overall SVR-12 rates (%)	97%	97%[c]	97%[c]	99%	93%	98%	96%	100%

[a] *Dasabuvir with Ombitasvir-Paritaprevir/ritonavir*

[b] Moderate fibrosis (F2 fibrosis stage) and low fibrosis (F0-F1 fibrosis stage)

[c] No significant differences between 12 versus 24 weeks nor between patients without cirrhosis versus with compensated cirrhosis nor between with versus without RBV

Table 5 Efficacy of Direct Antiviral Agents (DAAs) in liver transplant recipients with decompensated cirrhosis (CTP, Child-Turcotte-Pugh (**class B or C**)) or FCH, Fibrosing cholestatic hepatitis)

	Charlton et al. [8] (SOLAR-1)	Manns et al. [9] (SOLAR-2)	Forns et al. [46]	Leroy et al. [47]	
Patients, n	67	58	104	23	
DAA therapy	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-Ledipasvir +/− RBV	Sofosbuvir-RBV +/− PegIFN	Sofosbuvir-RBV +/− PegIFN ($n = 8$)	Sofosbuvir-Daclatasvir +/−RBV ($n = 15$)
Treatment duration	12 or 24 weeks	12 or 24 weeks	24 weeks	24 weeks	
Genotypes, 1 to 6	1 or 4	1 or 4	1,2,3,4	1 or 4	
Degree of hepatic decompensation (Child- Turcotte-Pugh-CTP-class) **and/or FCH**	CTP-B ($n = 52$) CTP-C ($n = 9$) FCH ($n = 6$)	CTP-B ($n = 46$) CTP-C ($n = 7$) FCH ($n = 5$)	CTP-A, B or C ($n = 52$) FCH ($n = 52$)	All with FCH	
SVR-12 rates (%)	85–88% (CTP-B) 60–75% (CTP-C) 100% (FCH)	95–100% (CTP-B) 50%–80% (CTP-C) 100% (FCH)	43% (CTP-A, B or C) 73% (FCH)	88%	100%

and 20 kidney recipients were treated. 98% achieved SVR12, with excellent tolerability and generally low rates of rejection [49–51].

Impact of DAA on the More Extensive Use of HCV-Positive Donors

According to estimates, there are around 110 million people worldwide with positive anti-HCV antibodies and around 80 million with active infection (detectable HCV-RNA) which correspond to a prevalence of HCV among organ donors between 1.4% and 5.5% with a wide variability depending on the geographical area [25, 52, 53].

Grafts from HCV-positive patients have been classically used in HCV-positive LT recipients with detectable HCV-RNA with good outcomes [54–57]. However, in the last 5 years, the high efficacy and safety of DAA therapies both in immunocompetent and in organ transplant recipients, including kidney, cardiac, and pulmonary recipients [58–67], has lead health authorities to encourage the use of HCV-positive donors, in HCV-negative patients, in order to increase their transplant options by reducing their time on the waiting list [21, 25, 68–70]. The transmission of HCV infection to the recipient from any HCV-positive graft is not universal as it mainly

depends on the detection of active viral replication in the donor. Depending on the geographical area and the recent use of DAA, the percentage of those viremic among serologically positive individuals may vary substantially. If the donor is viremic (positive HCV-RNA), transmission of HCV infection is the rule. Conversely, the risk of transmission of a nonviremic HCV donor (negative HCV-RNA) is very low, although not null, in cases of very low levels of viremia or in the case of window period of infection (very recent infection) [57]. From a clinical point of view, the expected transmission of HCV is manageable with new DAA used early after transplantation before liver injury has taken place. Caution remains within most Societies, with guidelines still suggesting caution when using HCV-viremic donors. In particular, limiting the use viremic organs in HCV-negative recipients, particularly if the latter had previously been successfully treated with DAA, so as not to require a second treatment course. Also transplant centers should be especially careful about using viremic donors for transplant in the absence of guaranteed access to DAA therapy post operatively, given a desire to start treatment as early after transplantation as possible to avoid fibrosis cholestatic hepatitis risk [21, 25, 68–70]. Finally, liver donors with extensive fibrosis (F3-4) should be declined preferentially using those with no or mild fibrosis. It is still debatable whether HCV-positive F2 donor grafts should be accepted for transplantation. Interestingly, liver grafts from HCV positive donors may differ in terms of quality with those coming from young donors, possibly in the setting of drug overdose and recent infection being typically of high quality whereas those from older donors, generally infected for years and decades, showing significant inflammation and fibrosis, and thus not qualifying for liver donation.

Use of Viremic Donor Organs for Transplant into HCV-Negative Recipients

Seropositive organ donors can be safely used for transplant into HCV negative organ recipients with no additional risk of developing HCV. In areas of the globe where drug overdose, particularly intravenous, results in a high proportion of the suitable donors, there remains a low but not zero risk of window period HCV infection.

Kidney transplantation was the first clinical area to highlight the potential for using HCV viremic donors and transplanting them into HCV negative recipients (D+/R−). The THINKER trial reported their experience with 20 patients, who were transplanted with donors who had HCV genotype 1. Each recipient was treated with elbasvir/grazoprevir for 12 weeks. While each recipient was found to be viremic by day 3 posttransplant, and DAA therapy was then initiated, all patients achieved SVR12, and a 12-month follow-up report noted HCV cure, excellent renal function, and a good quality of life off dialysis for all concerned. The EXPANDER trial similarly enrolled 10 kidney transplant recipients, although attempted a “prophylactic” strategy, also using elbasvir/grazoprevir, although dosing began preoperatively

on the day of the transplant. A number of other centers have since described experience with D+/R− kidney transplantation, all with comparable success.

In the interest of being rational with DAA use, some centers have also begun to explore variations in treatment duration. One United States report, of the DAPPerR study, describes the immediate day zero initiation of sofosbuvir/velpatasvir. In the initial group, patients received their first dose of drug 6 h prior to kidney implantation and then a single postoperative dose. Transmission of HCV occurred in 3/10 patients. A second phase of the study enrolled a further 40 patients, with additional doses on day 2 and 3. Three patients became HCV-viremic, yet all were successfully treated with regular length of DAA treatment afterwards. The study demonstrated that shorter courses of DAA therapy might be possible, if initiated very early on after a D+/R− transplant [71–74]. The exact ideal length of therapy remains unknown.

For heart transplants, undergoing a D+/R− transplant offers a rapid pathway towards a lifesaving organ. An early report from the United States demonstrated high SVR12 rates with 70 HCV D+/R− donors in 2016 and 2019. Waiting times for transplantation in the initial phase fell to a median of just 3 days postconsent (although this likely reflects organ availability and lack of competition for HCV-viremic hearts at the time). HCV viremia developed in 96% of patients and yet 100% achieved SVR12 ([70, 75]. Treatment was "delayed," initiated after discharge from hospital, yet SVR was still achieved and no fibrosing cholestatic hepatitis or significant graft damage (e.g., accelerated transplant coronary angiopathy) was noted.

Additional thoracic transplant experience was observed in HCV D+/R− heart and lung transplants performed in Boston. The DONATE HCV study included 8 heart and 36 lung transplant recipients, who each received only 4 weeks of sofosbuvir/velpatasvir, initiated within a few hours of transplantation. The primary outcome was a mixture of both SVR12 and graft survival at 6 months. 35/35 (100%) of patients met the primary endpoint with undetectable viremia. Although increased rates of rejection were noted in the lung transplant group compared to a non-HCV cohort, potential confounding limited evaluation of whether rejection was more significant or not.

Cost remains a significant barrier to D+/R− transplantation. In North America, a course of DAA therapy is typically many thousands of US$ per month. By contrast, however, the additional cost of waiting on dialysis is usually more over the space of a 2-year period, thereby making D+/R− transplants financially viable in many circumstances. Such models have not been put forward outside of a US context. Having a center with capable transplant pharmacy support to manage drug interactions that come with pan-genotypic regimens is important. Similarly, being aware of rare but important complications is key. In recipients who elect to wait for DAA treatment, there remains a small risk of rapidly progressive fibrosing cholestasis. Similarly, inadvertent secondary infections to caregivers should be discussed and mitigation techniques espoused. Finally, nonhepatic complications, such as membranous or immune deposition nephritis, or cryoglobulinemia, have been rarely described.

In summary, direct acting antivirals agents have allowed exploration of safe and effective HCV therapy after transplantation with HCV-viremic donor organs. Regardless of the organ involved, numerous studies have now demonstrated viable pathways to more rapid transplant, although more work is required to delineate the best time to begin DAA therapy (immediately before, during or after transplant), and the duration of therapy required. It should be noted, however, that most of these studies are in North America, where the epidemiology of the opiate epidemic has driven up the number of otherwise suitable donors who are actively infected with HCV. It is not clear whether the advantages offered in these trials can be extrapolated to other parts of the world.

Conclusion

The availability of newer and highly effective DAAs therapies has facilitated the management of HCV infection in the field of solid organ transplantation, leading to viral eradication in a classically considered "difficult to treat" population. In the pretransplant setting, functional and clinical improvement following DAAs treatment has been reported in patients with HCV-decompensated cirrhosis allowing 1 out of 4 patients to avoid LT. However, a small group of patients with HCV-decompensated cirrhosis will not derive clinical benefit despite viral eradication, indicating a limited hepatic repair capacity, a situation which is more common in the sickest patients where the risks and potential benefits of therapy in the waiting list need to be carefully weighted. Similarly, HCV-viremic patients on the kidney, heart, and lung waiting lists can generally be moved to transplant with confidence, knowing posttransplant SVR12 and cure rates are now outstanding, comparable to presurgical. Equally relevant are the DAA benefits in subjects awaiting LT for HCV-related HCC with a decrease in waitlist drop out due to death or tumor progression. In the posttransplant setting, DAA treatments have excellent efficacy and low toxicity and have dramatically improved outcomes of HCV recipients. Moreover, the new therapies have opened the door to a more extensive use of HCV positive donors, including into HCV-negative recipients, allowing an important expansion of the donor pool with subsequent benefits both at an individual and community level.

Key Points

- Hepatitis C infection
- Direct-acting antivirals
- Impact on the Waiting list
- Donor pool expansion

Acknowledgments The funding organization Instituto de Salud Carlos III supported the research of both Lorena Puchades (Grant number: CM17/00006) and Marina Berenguer (Grant number PI19/01360).

References

1. Hepatitis C. Geneva: World Health Organisation. Available in: http://www.who.int/mediacentre/factsheets/fs164/en/. Last checked 5 Apr 2019.
2. Belli LS, Perricone G, Adam R, Cortesi PA, Strazzabosco M, Facchetti R, Karam V, Salizzoni M, Andujar RL, Fondevila C, De Simone P, Morelli C, Fabregat-Prous J, Samuel D, Agarwaal K, Moreno Gonzales E, Charco R, Zieniewicz K, De Carlis L, Duvoux C; all the contributing centers (www.eltr.org) and the European Liver and Intestine Transplant Association (ELITA). Impact of DAAs on liver transplantation: major effects on the evolution of indications and results. An ELITA study based on the ELTR registry. J Hepatol. 2018;69(4):810–817. https://doi.org/10.1016/j.jhep.2018.06.010. [PMID: 29940268; Epub 2018 June 27].
3. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nat Rev Gastroenterol Hepatol. 2013;10(9):553–62. https://doi.org/10.1038/nrgastro.2013.107. [PMID: 23817321; Epub 2013 July 2].
4. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol. 2017;66(1):153–194. https://doi.org/10.1016/j.jhep.2016.09.001. [PMID: 27667367; Epub 2016 Sep 22].
5. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358(9286):958–65. [PMID: 11583749]
6. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347(13):975–82. https://doi.org/10.1056/NEJMoa020047. [PMID: 12324553]
7. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM, PEGASYS International Study Group. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140(5):346–55. [PMID: 14996676]
8. Charlton M, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS Jr, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N, SOLAR-1 Investigators. Ledipasvir and sofosbuvir plus ribavirin for treatment of HCV infection in patients with advanced liver disease. Gastroenterology. 2015;149(3):649–59. https://doi.org/10.1053/j.gastro.2015.05.010. [PMID: 25985734; Epub 2015 May 15]
9. Manns M, Samuel D, Gane EJ, Mutimer D, McCaughan G, Buti M, Prieto M, Calleja JL, Peck-Radosavljevic M, Müllhaupt B, Agarwal K, Angus P, Yoshida EM, Colombo M, Rizzetto M, Dvory-Sobol H, Denning J, Arterburn S, Pang PS, Brainard D, McHutchison JG, Dufour JF, Van Vlierberghe H, van Hoek B, Forns X, SOLAR-2 investigators. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. Lancet Infect Dis. 2016;16(6):685–97. https://doi.org/10.1016/S1473-3099(16)00052-9. [PMID: 26907736; Epub 2016 Feb 18]
10. Curry MP, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, Reddy KR, Lawitz E, Flamm SL, Schiano T, Teperman L, Fontana R, Schiff E, Fried M, Doehle B, An D, McNally J, Osinusi A, Brainard DM, McHutchison JG, Brown RS Jr, Charlton M, ASTRAL-4 Investigators. Sofosbuvir and velpatasvir for hcv in patients with decompensated cirrhosis. N Engl

J Med. 2015;373(27):2618–28. https://doi.org/10.1056/NEJMoa1512614. [PMID: 26569658; Epub 2015 Nov 16]
11. Foster GR, Irving WL, Cheung MC, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ, Brown A, Gelson WT, MacDonald DC, Agarwal K, HCV Research, UK. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol. 2016;64(6):1224–31. https://doi.org/10.1016/j.jhep.2016.01.029. [PMID: 26829205; Epub 2016 Jan 30].
12. Fernández Carrillo C, Lens S, Llop E, Pascasio JM, Crespo J, Arenas J, Fernández I, Baliellas C, Carrión JA, de la Mata M, Buti M, Castells L, Albillos A, Romero M, Turnes J, Pons C, Moreno-Planas JM, Moreno-Palomares JJ, Fernández-Rodriguez C, García-Samaniego J, Prieto M, Fernández Bermejo M, Salmerón J, Badia E, Salcedo M, Herrero JI, Granados R, Blé M, Mariño Z, Calleja JL. Treatment of hepatitis C virus infection in patients with cirrhosis and predictive value of model for end-stage liver disease: analysis of data from the Hepa-C registry. Hepatology. 2017;65(6):1810–22. https://doi.org/10.1002/hep.29097. [PMID: 28170112; Epub 2017 Apr 28]
13. Simmons B, Saleem J, Heath K, Cooke GS, Hill A. Long-term treatment outcomes of patients infected with hepatitis C virus: a systematic review and meta-analysis of the survival benefit of achieving a sustained virological response. Clin Infect Dis. 2015;61(5):730–40. https://doi.org/10.1093/cid/civ396. [PMID: 25987643; PMCID: PMC4530725; Epub 2015 May 17]
14. Mandorfer M, Kozbial K, Schwabl P, Freissmuth C, Schwarzer R, Stern R, Chromy D, Stättermayer AF, Reiberger T, Beinhardt S, Sieghart W, Trauner M, Hofer H, Ferlitsch A, Ferenci P, Peck-Radosavljevic M. Sustained virologic response to interferon-free therapies ameliorates HCV-induced portal hypertension. J Hepatol. 2016;65(4):692–9. https://doi.org/10.1016/j.jhep.2016.05.027. [PMID: 27242316; Epub 2016 May 27]
15. Lens S, Alvarado-Tapias E, Mariño Z, Londoño MC, LLop E, Martinez J, Fortea JI, Ibañez L, Ariza X, Baiges A, Gallego A, Bañares R, Puente A, Albillos A, Calleja JL, Torras X, Hernández-Gea V, Bosch J, Villanueva C, Forns X, García-Pagán JC. Effects of all-oral antiviral therapy on HVPG and systemic hemodynamics in patients with hepatitis C virus-associated cirrhosis. Gastroenterology. 2017;153(5):1273–1283.e1. https://doi.org/10.1053/j.gastro.2017.07.016. [PMID: 28734831; Epub 2017 July 20]
16. El-Sherif O, Jiang ZG, Tapper EB, Huang KC, Zhong A, Osinusi A, Charlton M, Manns M, Afdhal NH, Mukamal K, McHutchison J, Brainard DM, Terrault N, Curry MP. Baseline factors associated with improvements in decompensated cirrhosis after direct-acting antiviral therapy for hepatitis C virus infection. Gastroenterology. 2018;154(8):2111–2121.e8. https://doi.org/10.1053/j.gastro.2018.03.022. [PMID: 29535028; Epub 2018 Mar 11]
17. Afdhal N, Everson GT, Calleja JL, McCaughan GW, Bosch J, Brainard DM, McHutchison JG, De-Oertel S, An D, Charlton M, Reddy KR, Asselah T, Gane E, Curry MP, Forns X. Effect of viral suppression on hepatic venous pressure gradient in hepatitis C with cirrhosis and portal hypertension. J Viral Hepat. 2017;24(10):823–31. https://doi.org/10.1111/jvh.12706. [PMID: 28295923; Epub 2017 Apr 10]
18. Cheung MCM, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ, Brown A, Gelson WTH, MacDonald DC, Agarwal K, Foster GR, Irving WL, HCV Research UK. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol. 2016;65(4):741–7. https://doi.org/10.1016/j.jhep.2016.06.019. [PMID: 27388925; Epub 2016 July 5]
19. Belli LS, Berenguer M, Cortesi PA, Strazzabosco M, Rockenschaub SR, Martini S, Morelli C, Donato F, Volpes R, Pageaux GP, Coilly A, Fagiuoli S, Amaddeo G, Perricone G, Vinaixa C, Berlakovich G, Facchetti R, Polak W, Muiesan P, Duvoux C, European Liver and Intestine Association (ELITA). Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: a European study. J Hepatol. 2016;65(3):524–31. https://doi.org/10.1016/j.jhep.2016.05.010. [PMID: 27212241; Epub 2016 May 17]
20. Perricone G, Duvoux C, Berenguer M, Cortesi PA, Vinaixa C, Facchetti R, Mazzarelli C, Rockenschaub SR, Martini S, Morelli C, Monico S, Volpes R, Pageaux GP, Fagiuoli S, Belli LS, European Liver and Intestine Transplant Association (ELITA). Delisting HCV-infected liver

transplant candidates who improved after viral eradication: outcome 2 years after delisting. Liver Int. 2018;38(12):2170–7. https://doi.org/10.1111/liv.13878. [PMID: 29750389; Epub 2018 May 25]
21. Terrault NA, Berenguer M, Strasser SI, Gadano A, Lilly L, Samuel D, Kwo PY, Agarwal K, Curry MP, Fagiuoli S, Fung JYY, Gane E, Brown KA, Burra P, Charlton M, Pessoa MG, McCaughan GW. International liver transplantation society consensus statement on hepatitis C management in liver transplant recipients. Transplantation. 2017;101(5):956–67. https://doi.org/10.1097/TP.0000000000001704. [PMID: 28437388]
22. Saxena V, Koraishy FM, Sise ME, Lim JK, Schmidt M, Chung RT, Liapakis A, Nelson DR, Fried MW, Terrault NA, HCV-TARGET. Safety and efficacy of sofosbuvir-containing regimens in hepatitis C-infected patients with impaired renal function. Liver Int. 2016;36(6):807–16. https://doi.org/10.1111/liv.13102. [PMID: 26923436; Epub 2016 Mar 24]
23. Puchades Renau L, Berenguer M. Introduction to hepatitis C virus infection: overview and history of hepatitis C virus therapies. Hemodial Int. 2018;22(Suppl 1):S8–S21. https://doi.org/10.1111/hdi.12647. [PMID: 29694724]
24. Vinaixa C, Strasser S, Berenguer M. Disease reversibility in patients with post-hepatitis C cirrhosis: is the point of no return the same before and after liver transplantation? A review. Transplantation. 2017;101(5):916–23. https://doi.org/10.1097/TP.0000000000001633. [PMID: 28060241]
25. Belli LS, Duvoux C, Berenguer M, Berg T, Coilly A, Colle I, Fagiuoli S, Khoo S, Pageaux GP, Puoti M, Samuel D, Strazzabosco M. ELITA consensus statements on the use of DAAs in liver transplant candidates and recipients. J Hepatol. 2017;67(3):585–602. https://doi.org/10.1016/j.jhep.2017.03.006. [PMID: 28323126; Epub 2017 Mar 18]
26. Di Maira T, Torregrosa A, Navarro V, Sánchez D, Fornés V, Berenguer M. Liver volume as a predictor of functional improvement post-DAA treatment. Transplantation. 2018;102(2):e74–81. https://doi.org/10.1097/TP.0000000000001990. [PMID: 29087972]
27. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol. 2016;65(4):719–26. https://doi.org/10.1016/j.jhep.2016.04.008. [PMID: 27084592; Epub 2016 Apr 13]
28. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016;65(4):727–33. https://doi.org/10.1016/j.jhep.2016.06.015. [PMID: 27349488; Epub 2016 June 24]
29. ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT cohorts). Electronic address: stanislas.pol@aphp.fr/. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. J Hepatol. 2016;65(4):734–740. https://doi.org/10.1016/j.jhep.2016.05.045. [PMID: 27288051; Epub 2016 June 7].
30. Huang AC, Mehta N, Dodge JL, Yao FY, Terrault NA. Direct-acting antivirals do not increase the risk of hepatocellular carcinoma recurrence after local-regional therapy or liver transplant waitlist dropout. Hepatology. 2018;68(2):449–61. https://doi.org/10.1002/hep.29855. [PMID: 29476694; PMCID: PMC6097892 [Available on 2019-08-01]; Epub 2018 May 16]
31. Su F, Ioannou GN. The impact of direct-acting antiviral therapy for hepatitis C on hepatocellular carcinoma risk. Curr Hepatol Rep. 2018;17(4):377–84. https://doi.org/10.1007/s11901-018-0424-8. [PMID: 30923667; PMCID: PMC6433385 [Available on 2019-12-01]; Epub 2018 Sept 20]
32. Sáez-González E, Vinaixa C, San Juan F, Hontangas V, Benlloch S, Aguilera V, Rubín A, García M, Prieto M, López-Andujar R, Berenguer M. Impact of hepatitis C virus (HCV) antiviral treatment on the need for liver transplantation (LT). Liver Int. 2018;38(6):1022–7. https://doi.org/10.1111/liv.13618. [PMID: 29105320; Epub 2017 Dec 2]

33. Berenguer M, de la Rosa RG, Domínguez-Gil B. Significant impact of new oral therapies against HCV on the waiting list for liver transplantation in Spain. J Hepatol. 2018;69(4):966–8. https://doi.org/10.1016/j.jhep.2018.06.011. [PMID: 30227917; Epub 2018 July 7]
34. Flemming JA, Kim WR, Brosgart CL, Terrault NA. Reduction in liver transplant wait-listing in the era of direct-acting antiviral therapy. Hepatology. 2017;65(3):804–12. https://doi.org/10.1002/hep.28923. [PMID: 28012259; PMCID: PMC5333888; Epub 2016 Dec 24]
35. Terrault NA, Pageaux GP. A changing landscape of liver transplantation: King HCV is dethroned, ALD and NAFLD take over! J Hepatol. 2018;69(4):767–8. https://doi.org/10.1016/j.jhep.2018.07.020. [PMID: 30104027; Epub 2018 Aug 10]
36. Wright TL, Donegan E, Hsu HH, Ferrell L, Lake JR, Kim M, Combs C, Fennessy S, Roberts JP, Ascher NL, et al. Recurrent and acquired hepatitis C viral infection in liver transplant recipients. Gastroenterology. 1992;103(1):317–22. [PMID: 1377143]
37. Garcia-Retortillo M, Forns X, Feliu A, Moitinho E, Costa J, Navasa M, Rimola A, Rodes J. Hepatitis C virus kinetics during and immediately after liver transplantation. Hepatology. 2002;35(3):680–7. https://doi.org/10.1053/jhep.2002.31773. [PMID: 11870384]
38. Berenguer M, Schuppan D. Progression of liver fibrosis in post-transplant hepatitis C: mechanisms, assessment and treatment. J Hepatol. 2013;58(5):1028–41. https://doi.org/10.1016/j.jhep.2012.12.014. [PMID: 23262248; Epub 2012 Dec 20]
39. Selzner N, Guindi M, Renner EL, Berenguer M. Immune-mediated complications of the graft in interferon-treated hepatitis C positive liver transplant recipients. J Hepatol. 2011;55(1):207–17. https://doi.org/10.1016/j.jhep.2010.11.012. [PMID: 21145865; Epub 2010 Nov 29]
40. Kwo PY, Mantry PS, Coakley E, Te HS, Vargas HE, Brown R Jr, Gordon F, Levitsky J, Terrault NA, Burton JR Jr, Xie W, Setze C, Badri P, Pilot-Matias T, Vilchez RA, Forns X. An interferon-free antiviral regimen for HCV after liver transplantation. N Engl J Med. 2014;371(25):2375–82. https://doi.org/10.1056/NEJMoa1408921. [PMID: 25386767; Epub 2014 Nov 11]
41. Coilly A, Fougerou-Leurent C, de Ledinghen V, Houssel-Debry P, Duvoux C, Di Martino V, Radenne S, Kamar N, D'Alteroche L, Leroy V, Canva V, Lebray P, Moreno C, Dumortier J, Silvain C, Besch C, Perre P, Botta-Fridlund D, Anty R, Francoz C, Abergel A, Debette-Gratien M, Conti F, Habersetzer F, Rohel A, Rossignol E, Danjou H, Roque-Afonso AM, Samuel D, Duclos-Vallée JC, Pageaux GP, ANRS C023 CUPILT study group. Multicentre experience using daclatasvir and sofosbuvir to treat hepatitis C recurrence – The ANRS CUPILT study. J Hepatol. 2016;65(4):711–8. https://doi.org/10.1016/j.jhep.2016.05.039. [PMID: 27262758; Epub 2016 June 1]
42. Gutierrez JA, Carrion AF, Avalos D, O'Brien C, Martin P, Bhamidimarri KR, Peyton A. Sofosbuvir and simeprevir for treatment of hepatitis C virus infection in liver transplant recipients. Liver Transpl. 2015;21(6):823–30. https://doi.org/10.1002/lt.24126. [PMID: 25825070]
43. Reau N, Kwo PY, Rhee S, Brown RS Jr, Agarwal K, Angus P, Gane E, Kao JH, Mantry PS, Mutimer D, Reddy KR, Tran TT, Hu YB, Gulati A, Krishnan P, Dumas EO, Porcalla A, Shulman NS, Liu W, Samanta S, Trinh R, Forns X. Glecaprevir/pibrentasvir treatment in liver or kidney transplant patients with hepatitis C virus infection. Hepatology. 2018;68(4):1298–307. https://doi.org/10.1002/hep.30046. [PMID: 29672891; PMCID: PMC6220874; Epub 2018 July 25]
44. Agarwal K, Castells L, Müllhaupt B, Rosenberg WMC, McNabb B, Arterburn S, Camus G, McNally J, Stamm LM, Brainard DM, Mani Subramanian G, Mariño Z, Dufour JF, Forns X. Sofosbuvir/velpatasvir for 12 weeks in genotype 1-4 HCV-infected liver transplant recipients. J Hepatol. 2018;69(3):603–7. https://doi.org/10.1016/j.jhep.2018.05.039. [PMID: 29886154; Epub 2018 June 8]
45. Cardona-Gonzalez MG, Goldman JD, Narayan L, Brainard DM, Kowdley KV. Sofosbuvir, velpatasvir, and voxilaprevir for treatment of recurrent hepatitis C virus infection after liver transplantation. Hepatol Commun. 2018;2(12):1446–50. https://doi.org/10.1002/hep4.1280. [PMID: 30556034; PMCID: PMC6287482; eCollection 2018 Dec]
46. Forns X, Charlton M, Denning J, McHutchison JG, Symonds WT, Brainard D, Brandt-Sarif T, Chang P, Kivett V, Castells L, Prieto M, Fontana RJ, Baumert TF, Coilly A, Londoño MC,

Habersetzer F. Sofosbuvir compassionate use program for patients with severe recurrent hepatitis C after liver transplantation. Hepatology. 2015;61(5):1485–94. https://doi.org/10.1002/hep.27681. [PMID: 25557906; Epub 2015 Mar 20]
47. Leroy V, Dumortier J, Coilly A, Sebagh M, Fougerou-Leurent C, Radenne S, Botta D, Durand F, Silvain C, Lebray P, Houssel-Debry P, Kamar N, D'Alteroche L, Petrov-Sanchez V, Diallo A, Pageaux GP, Duclos-Vallee JC, Agence Nationale de Recherches sur le SIDA et les Hépatites Virales CO Compassionate Use of Protease Inhibitors in Viral C in Liver Transplantation Study Group. Efficacy of sofosbuvir and daclatasvir in patients with fibrosing cholestatic hepatitis C after liver transplantation. Clin Gastroenterol Hepatol. 2015;13(11):1993–2001.e1-2. https://doi.org/10.1016/j.cgh.2015.05.030. [PMID: 26044317; Epub 2015 June 1]
48. Cotter TG, Paul S, Sandıkçı B, Couri T, Bodzin AS, Little EC, Sundaram V, Charlton M. Improved graft survival after liver transplantation for recipients with hepatitis C virus in the direct-acting antiviral era. Liver Transpl. 2019;25(4):598–609. https://doi.org/10.1002/lt.25424. [PMID: 30716208]
49. Manns M, Samuel D, Gane EJ, et al. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. Lancet Infect Dis. 2016;16(6):685–97.
50. Agarwal K, Castells L, Mullhaupt B, et al. Sofosbuvir/velpatasvir for 12 weeks in genotype 1-4 HCV-infected liver transplant recipients. J Hepatol. 2018;69(3):603–7.
51. Reau N, Kwo PY, Rhee S, et al. Glecaprevir/pibrentasvir treatment in liver or kidney transplant patients with hepatitis C virus infection. Hepatology. 2018;68(4):1298–307.
52. WHO Global report on access to hepatitis C treatment – focus on overcoming barriers. Disponible en: http://www.who.int/hepatitis/publications/hep-c-access-report/en/
53. Blach S, et al. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2(3):161–76. https://doi.org/10.1016/S2468-1253(16)30181-9. [PMID: 28404132; Epub 2016 Dec 16]
54. Saab S, Ghobrial RM, Ibrahim AB, Kunder G, Durazo F, Han S, et al. Hepatitis C positive grafts may be used in orthotopic liver transplantation: a matched analysis. Am J Transplant 2003; 3:1167–1172. [PMID: 12919097].
55. Northup PG, Argo CK, Nguyen DT, McBride MA, Kumer SC, Schmitt TM, et al. Liver allografts from hepatitis C positive donors can offer good outcomes in hepatitis C positive recipients: a US National Transplant Registry analysis. Transpl Int. 2010;23:1038–44. https://doi.org/10.1111/j.1432-2277.2010.01092.x. [PMID: 20444239]
56. Montenovo MI, Dick AA, Hansen RN. Donor hepatitis C sero-status does not impact survival in liver transplantation. Ann Transplant. 2015;22:44–50. https://doi.org/10.12659/AOT.892530. [PMID: 25608491]
57. Ballarin R, Cucchetti A, Spaggiari M, Montalti R, Di Benedetto F, Nadalin S, et al. Longterm follow-up and outcome of liver transplantation from antihepatitis C virus-positive donors: a European multicentric case-control study. Transplantation. 2011;91:1265–72. https://doi.org/10.1097/TP.0b013e318219eb8f. [PMID: 21478815]
58. Colombo M, Aghemo A, Liu H, Zhang J, Dvory-Sobol H, Hyland R, Yun C, Massetto B, Brainard DM, McHutchison JG, Bourlière M, Peck-Radosavljevic M, Manns M, Pol S. Treatment with ledipasvir-sofosbuvir for 12 or 24 weeks in kidney transplant recipients with chronic hepatitis C virus genotype 1 or 4 infection: a randomized trial. Ann Intern Med. 2017;166(2):109–17. https://doi.org/10.7326/M16-1205. [PMID: 27842383; Epub 2016 Nov 15]
59. Saxena V, Khungar V, Verna EC, Levitsky J, Brown RS Jr, Hassan MA, Sulkowski MS, O'Leary JG, Koraishy F, Galati JS, Kuo AA, Vainorius M, Akushevich L, Nelson DR, Fried MW, Terrault N, Reddy KR. Safety and efficacy of current direct-acting antiviral regimens in kidney and liver transplant recipients with hepatitis C: results from the HCV-TARGET study. Hepatology. 2017;66(4):1090–101. https://doi.org/10.1002/hep.29258. [PMID: 28504842; Epub 2017 Sep 4]
60. Fernández I, Muñoz-Gómez R, Pascasio JM, Baliellas C, Polanco N, Esforzado N, Arias A, Prieto M, Castells L, Cuervas-Mons V, Hernández O, Crespo J, Calleja JL, Forns X, Londoño

MC. Efficacy and tolerability of interferon-free antiviral therapy in kidney transplant recipients with chronic hepatitis C. J Hepatol. 2017;66(4):718–23. https://doi.org/10.1016/j.jhep.2016.12.020. [PMID: 28039098; Epub 2016 Dec 28]
61. Chute DF, Chung RT, Sise ME. Direct-acting antiviral therapy for hepatitis C virus infection in the kidney transplant recipient. Kidney Int. 2018;93:560–7. https://doi.org/10.1016/j.kint.2017.10.024. [PMID: 29325996; Epub 2018 Jan 9]
62. Casanova T, Roca J, Niubó J. Successul treatment of hepatitis C virus infection combining daclatasvir and simeprevir in a heart transplant recipient with decompensated cirhosis. J Heart Lung Transplant. 2016;35:949–51. https://doi.org/10.1016/j.healun.2016.05.005. [PMID: 27311374; Epub 2016 May 17]
63. Trakroo S, Qureshi K. Successful treatment of chronic hepatitis C infection with directacting antivirals in a heart transplant recipient: a case report. Transplant Proc. 2015;47:2295–7. https://doi.org/10.1016/j.transproceed.2015.06.003. [PMID: 26361703]
64. Liu C-H, Chen Y-S, Wang S-S, Liu C-J, Su T-H, Yang H-C. Sofosbuvir-based interferon-free direct acting antiviral regimens for heart transplant recipients with chronic hepatitis C virus infection. Clin Infect Dis. 2018;66:289–92. https://doi.org/10.1093/cid/cix787. [PMID: 29020359]
65. Vitrone M, Andini R, Mattucci I, Maiello C, Atripaldi L, Durante-Mangoni E, et al. Direct antiviral treatment of chronic hepatitis C in heart transplant recipients. Transpl Infect Dis. 2018;20:e12813. https://doi.org/10.1111/tid.12813. [PMID: 29139181; Epub 2017 Dec 21]
66. Doucette K, Sumner S, Weinkauf J. Treatment of hepatitis C in a lung transplant recipient with sofosbuvir and daclatasvir. J Heart Lung Transplant. 2016;35:840–1. https://doi.org/10.1016/j.healun.2016.01.1219. [PMID: 26944671; Epub 2016 Feb 8]
67. D'Ambrosio R, Aghemo A, Rossetti V, Carrinola R. Sofosbuvir-based regimens for the treatment of hepatitis C virus in patients who underwent lung transplant: case series and review of the literature. Liver Int. 2016;36:1585–9. https://doi.org/10.1111/liv.13203. [PMID: 27429162; Epub 2016 Aug 21]
68. Spanish hepatology and liver transplant societies (ONT · AEEH · GESITRA · SEC · SEN · SEPAR · SET SETH) consensus statements for the evaluation of donors with positive serology for hepatitis C virus infection. Available in http://www.ont.es/infesp/Paginas/DocumentosdeConsenso.aspx
69. Halleck F, Budde K, Duerr M, Staeck O, Hofmann J, Eisenbergr U, et al. Transplanting VHC-infected kidneys into uninfected recipients. N Engl J Med. 2017;377:1103–4. https://doi.org/10.1056/NEJMc1709315. [PMID: 28902583]
70. Schlendorf KH, Zalawadiya S, Shah AS, Wigger M, Chung CY, Smith S, et al. Early outcomes using hepatitis C-positive donors for cardiac transplantation in the era of effective direct-acting anti-viral therapies. J Heart Lung Transplant. 2018;37:763–9. https://doi.org/10.1016/j.healun.2018.01.1293. [PMID: 29530322; Epub 2018 Jan 31]
71. Goldberg DS, Abt PL, Blumberg EA, et al. Trial of transplantation of HCV-infected kidneys into uninfected recipients. N Engl J Med. 2017;376(24):2394–5.
72. Reese PP, Abt PL, Blumberg EA, et al. Twelve-month outcomes after transplant of hepatitis C-infected kidneys into uninfected recipients: a single-group trial. Ann Intern Med. 2018;169(5):273–81.
73. Durand CM, Bowring MG, Brown DM, et al. Direct-acting antiviral prophylaxis in kidney transplantation from hepatitis C virus-infected donors to noninfected recipients: an open-label nonrandomized trial. Ann Intern Med. 2018;168(8):533–40. https://doi.org/10.1002/hep.31011.
74. Gupta G, Yakubu I, Bhati CS, et al. Ultra-short duration direct acting antiviral prophylaxis to prevent virus transmission from hepatitis C viremic donors to hepatitis C negative kidney transplant recipients. Am J Transplant. 2020;20(3):739–751. https://doi.org/10.1111/ajt.15664. [PMID: 31652392; Epub 2019 Nov 15].
75. Schlendorf KH, Zalawadiya S, Shah AS, et al. Expanding heart transplant in the era of direct-acting antiviral therapy for hepatitis C. JAMA Cardiol. 2020;5(2):167–174. https://doi.org/10.1001/jamacardio.2019.4748. [PMID: 31851352; PMCID: PMC6990812].

West Nile Virus and Transplantation

40

Marilyn E. Levi and Marian G. Michaels

Contents

Epidemiology 971
Disease Manifestation 974
Diagnostic Considerations and Laboratory Testing for WNV 975
Geographic Regions at Higher Risk for WNV 975
Laboratory Testing 976
Diagnostic Imaging 977
Management of West Nile Virus Infection 977
Prevention 978
Blood Donor Screening in the United States 979
Organ Donor Screening 979
Environmental Prevention of West Nile Virus Infection 981
Key Points 981
Conclusion 982
References 982

Epidemiology

West Nile virus (WNV) takes its name from the first identified case of a woman with fever in the West Nile district of Uganda in 1937 [1]. The virus is a single-stranded RNA virus in the *Flaviviridae* family and is related to other flaviviruses including St. Louis encephalitis virus, dengue, yellow fever, Japanese encephalitis virus, and hepatitis C (Table 1). It is an avian zoonosis that is maintained in an enzootic cycle

M. E. Levi
Health Systems Bureau, Division of Transplantation, Human Resources Services Administration, Rockville, MD, USA

M. G. Michaels (✉)
Pediatric Infectious Diseases, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA
e-mail: michmg@upmc.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_39

Table 1 List of flaviviruses

West Nile virus
St. Louis encephalitis virus
Japanese encephalitis virus
Powassan virus
Zika virus
Dengue virus
Chikungunya virus
Jamestown Canyon virus
Murray Valley virus
La Crosse encephalitis
Eastern equine encephalitis
Yellow fever virus
Tick-borne encephalitis
Hepatitis C

between mosquito vectors, predominantly of the *Culex* species, and over 330 species of birds. The birds serve as amplifying hosts and may remain viremic for up to 100 days, providing ample time for other mosquitoes to become infected during blood meals [2]. In the United States, WNV peaks during the midsummer and fall (Fig. 1) when mosquitoes are most infectious for humans. Following mosquito bites, humans and horses generate only lower levels of WNV viremia [3] and are considered dead-end hosts.

Since 1937, epidemics of WNV infection developed intermittently in the Mediterranean basin [4], but the Western Hemisphere was generally free until 1999, when 59 individuals presented with WNV encephalitis and/or meningitis in Queens, New York [5]. Over the ensuing years, WNV quickly spread westward with an intermittent predominance of infection based on the migratory pattern of the infected birds and predominantly *Culex* spp. mosquitoes. Between 1999 and 2017, there were 48,183 confirmed cases of WNV disease reported to the Centers for Disease Control and Prevention (CDC), 22,999 cases of which were neuroinvasive [6]. West Nile virus has become endemic and the leading cause of arboviral disease in the continental United States [7].

Concern for WNV in transplant recipients came shortly after its spread to the United States with donor transmission via blood or organs in 2002 when WNV was recognized in four recipients who received organs from a woman who had sustained a fatal motor vehicle accident [8, 9]. Three of the four recipients developed encephalitis, while the fourth had a fever with mild cognitive impairment; one of the patients died. The donor was subsequently documented to be infected by WNV with positive reverse transcriptase polymerase chain reaction (RT-PCR) and positive culture from blood obtained on the day of organ procurement. The donor had a negative RT-PCR and WNV IgM results on samples obtained 1 and 2 days prior to procurement [9]. The infection was eventually confirmed to have originated from

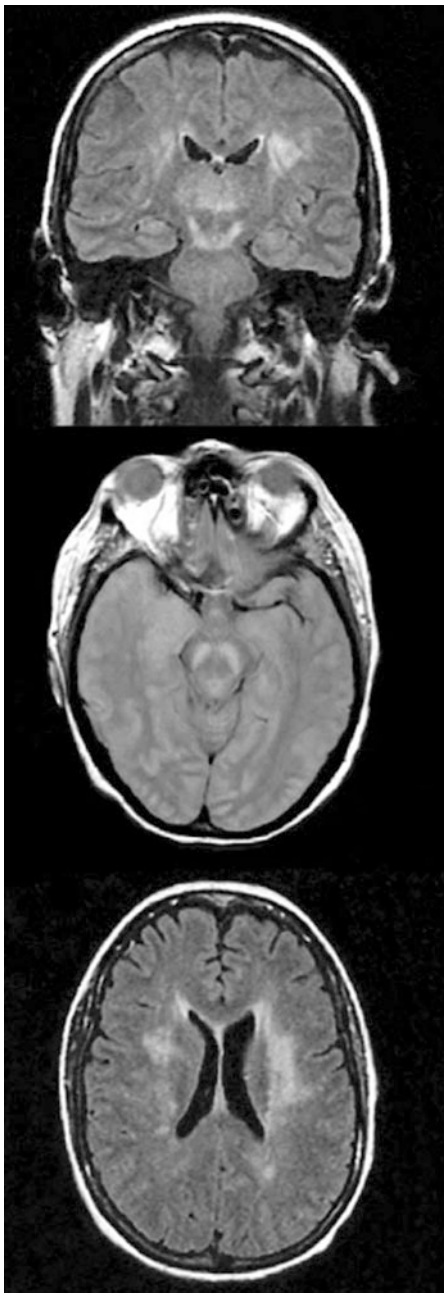


Fig. 1 Coronal fluid-attenuated inversion recovery (FLAIR) image shows an area of abnormally increased signal in the thalami, substantia nigras (extending superiorly toward the subthalamic nuclei), and white matter. (Figure reproduced with permission from Ref. [34])

1 of 53 units of blood products given to the donor prior to procurement [8, 9]. The Food and Drug Administration provided guidance for donor blood screening starting in 2002, and screening of all blood donors for WNV RNA was implemented by the summer of 2003 [10]. Mosquito-borne community-acquired WNV in organ transplant recipients was also recognized early in the spread to the Western Hemisphere [11, 27].

West Nile virus is now considered endemic within the Western Hemisphere.

Disease Manifestation

In immunocompetent individuals, WNV infection is asymptomatic in 80% of individuals with an incubation period typically of 2–14 days. Only 20–25% develop symptoms predominantly manifesting as an acute febrile illness (WNV fever) often accompanied by nonspecific symptoms of headache, myalgia, arthralgia, vomiting diarrhea, or rash, with <1% developing West Nile neuroinvasive disease (WNND) [6, 7, 12–16]. Increasing age was found to be a particular risk for the development of WNND [12–14]. West Nile neurologic features can be manifold presenting with encephalitis, meningitis, acute flaccid paralysis, Parkinsonian-like cogwheel rigidity, or a combination of these symptoms [15, 16]. Involvement of the brain stem, basal ganglia, thalamus, or anterior horn cells of the spinal cord can account for variable clinical presentations. In comparison to healthy individuals, immunocompromised patients may have a more prolonged incubation period with symptoms developing between 4 and 20 days after donor-derived infection [9, 17–25]. In addition, transplant recipients appear to have an increased incidence of WNND occurring in up to 40% with mosquito-acquired infection and over 50% with donor-derived infections [9, 17–27]. However, these data must be taken with consideration that mild or asymptomatic infections may not undergo diagnostic evaluation so that neuroinvasive and severe disease may be overestimated.

The cerebrospinal fluid findings are similar to other viral meningoencephalitides with mild leukocytosis (usually <500 cells/mm^3), normal glucose, and elevated protein [12, 15, 26–28]. Similar findings were noted in transplant recipients [9, 11, 17–25]. MRI of the brain and spine may identify anterior horn cell signal changes and enhancement of the ventral nerve root (as shown in Figs. 1 and 2) [27].

Prognosis and recovery in transplant patients with neuroinvasive disease varies but has a high rate of sustained morbidity and mortality [17]. In 23 reported donor-derived infections after organ transplantation, 13 (56%) had encephalitis, 3 died, and at the time of reporting, 5 were noted to still be in a coma or requiring rehabilitation [17–25]. In a more recent series of 11 kidney transplant patients, 7 had altered mental status, while 4 had fever and headache; 9 had a full recovery, but 2 had ongoing neurologic deficits [29].

Recipients of bone marrow transplantation are less frequently reported to have WNV [27, 30–36]. In a review of ten cases, only one was proven to be donor-derived despite negative routine testing 3 weeks prior to donation [36]. In this review, one pediatric

Fig. 2 MRI of a patient with WNV encephalitis, acute flaccid paralysis of the upper extremities, and respiratory failure. Increased T2 signal involving the anterior cervical spinal cord. (Courtesy of Dr. Lyle Peterson, Centers for Diseases Control, Fort Collins, Colorado)

recipient developed fatal encephalitis and repeat testing of the donor documented seroconversion. In three cases, the acquisition was felt most likely to be environmental from mosquitoes, and in six it was not known if it was due to blood transfusions or natural acquisition [30–35]. In total, seven of the ten died with WNV encephalitis; two of the surviving recipients were over 9 months after transplant and on minimal to no immunosuppression which may have accounted for their better outcomes [34].

Diagnostic Considerations and Laboratory Testing for WNV

Arboviral infections particularly WNV should be considered when transplant recipients present with fever, headache, and viral-like symptoms especially with altered mental status, flaccid paralysis, Parkinson-like cogwheel rigidity, or other neurologic symptoms during mosquito season, especially in regions of reported cases in humans, birds, or other animals.

Geographic Regions at Higher Risk for WNV

Of great importance is for clinicians to also consider the epidemiology of the donor particularly if the symptoms are within a month after transplantation. Consideration

of possible donor associated infection is important not only for the individual patient but also to rapidly inform other recipients of organs from the same donor so that evaluation and management can be optimized. The CDC maintains up-to-date tracking of WNV activity by state both in humans and in nonhumans including veterinary cases and infections of mosquitoes, birds, or sentinel animals [6].

Laboratory Testing

Testing of recipients can be multipronged as one method may not always be accurate particularly in immunocompromised hosts. Interpretation of WNV laboratory testing is dependent on the stage of the patient's illness. Upon human infection, a period of asymptomatic West Nile viremia is detectable by nucleic acid amplification testing (NAT) usually starting 1–2 days after a bite from an infected mosquito, lasting for 5–7 days in immunocompetent individuals with negative testing generally by the time symptoms develop [37]. Serum WNV IgM seropositivity develops 3–5 days after viremia develops and generally 2 days within the onset of symptoms, so it is commonly considered consistent with acute infection lasting on average 156 days [37]. However, IgM persistence for at least 3 years has been reported in healthy adults [38].

In cases of WNV neuroinvasive disease, IgM may be detected in the CSF before serum and is pathognomonic for central nervous system disease, as the antibody does not penetrate the blood-brain barrier [36]. Positive nucleic acid testing of CSF is also pathognomonic for WNND but is less sensitive. If the clinical presentation is concerning for WNV with an initial negative serum or CSF IgM assay, repeat testing may be performed after 1 week. Serum WNV IgG becomes detectable generally several days after IgM and may be persistent for years. Cross-reactivity with other arboviruses, particularly St. Louis encephalitis [39] may occur, and laboratory confirmation with plaque reduction neutralization test (PRNT) may be obtained through local and state health department laboratories and the CDC Division of Vector-Borne Diseases. Commercial assays for WNV-specific antibodies are available but may vary in sensitivity and specificity. Table 1 lists all flaviviruses given the need to consider serologic cross-reactivity with WNV.

In transplant recipients, antibody response to WNV may be delayed or absent, and viremia may persist particularly in the presence of severe immunosuppression or use of B-cell-depleting agents such as rituximab [35, 40–42]. Both serum and CSF WNV NAT, IgM, and IgG should be obtained when WNND is suspected in a transplant recipient. Delay in serum WNV IgM seropositivity has been reported in several transplant recipients maintained on tacrolimus with initial negative serology upon neurologic presentation. Seroconversion was noted with repeat testing [18, 43]. Accordingly, if initial testing is negative for WNV and diagnostic workup does not identify other sources to account for the patient's symptomatology, repeat testing for WNV by serology and NAT after 4–7 days are warranted. This was highlighted in a case report of severe West Nile virus infection in a kidney transplant recipient who presented with neurologic involvement, thrombotic microangiopathy,

pancreatitis, and irreversible loss of kidney function, where the standard US Centers for Disease Control and Prevention diagnostic serology criteria did not initially identify WNV. Due to persistent symptoms, a subsequent plaque reduction neutralization test confirmed the diagnosis months later, with additional diagnostic and therapeutic procedures performed that contributed to morbidity and graft loss [44]. More recently, newer methodologies such as metagenomic deep sequencing of nucleic acids have been noted in a case report to identify WNV encephalitis in a 14-year-old renal transplant recipient whose CSF and serum antibody and NAT tests were initially negative and may become a technologic advance to consider in cases that are diagnostic dilemmas [43]. However, in this case report, subsequent testing documented the development of a positive antibody; for now, issues including significant cost, limited availability, and potential contaminating microbes may be problematic with this technique.

Diagnostic Imaging

Imaging studies including MRI images with T2-weighted imaging should be considered for all transplant patients with neurologic symptoms. However, here too significant variation in findings may occur, and brain MRI scans may be normal in the presence of severe WNV encephalitis particularly early in the course [45]. Examples of MRI imaging are shown in Figs. 1 and 2. While positive findings are suggestive of neuroinvasive viral diseases such as enhancement of the thalamus, basal ganglia, and anterior horn cells of the spinal cord, they are not diagnostic for WNV over other etiologies.

Management of West Nile Virus Infection

There are no WNV-specific antiviral therapies or adjunctive therapies that have been shown to be definitively effective for the treatment of WNV. Accordingly, supportive care and minimizing immunosuppression as tolerated are the mainstay of management. However, in a series of 11 kidney transplant patient with WNV infection and who all had immunosuppression reduced, 3 had acute rejection within the following year, and the entire cohort had a mean drop in estimated glomerular filtration rate of 8.4 ± 13 mL/min/1.73 m^2, suggesting a need for careful immunosuppression management after clearance of WNV [29]. Hospital protocols used in many institutions for the prevention of secondary bacterial infections in patients with compromised respiratory function should be implemented such as elevating the head and suctioning. Long-term care in nursing facilities may be required for further rehabilitative care as well and should be planned early in the course as an expectation for infected patients and their families.

Several agents have been used with variable results in case reports and case series. While ribavirin had in vitro activity, a retrospective review of a large outbreak in Israel noted an increased mortality risk for those treated with ribavirin [46]. Likewise,

while interferon alpha-2b had some anecdotal success, a clinical trial has not as yet reported definitive clinical benefit [17, 47] and carries a concern for potentially triggering organ rejection after SOT. Intravenous immunoglobulin products have been most widely used in an attempt to treat WNV disease particularly in immunocompromised patients with both standard preparations of intravenous immunoglobulin (IVIG) and high titer IVIG against WNV (Omr-IgG-am™, OMRIX Biopharmaceuticals) that have been anecdotally reported to be of benefit [18, 21–25, 31–36, 48, 49]. A phase I/II randomized, double-blind, placebo-controlled study failed to show a benefit of a single dose (500 mg/kg) of high-titer anti-WNV IVIG (Omr-IgG-am™) compared to placebo (standard WNV-negative IVIG or normal saline) in a study conducted by the NIAID Collaborative Antiviral Study Group in 55 adults with confirmed WNV neuroinvasive disease (personal communication Richard Whitley [50]). However, the majority of the patients in the study had been symptomatic for several days before receiving the investigational therapy which may have impacted efficacy.

Predictors of death or persistent neurologic deficits included age over 65 years and presenting with coma [50]. High-titer WNV IgG (OMR-IgGam) is no longer available in the United States; over time polyclonal immune globulin intravenous (IVIG) from plasma-derived immunoglobulin in the United States has been shown to have measurable WNV-neutralizing antibody although its efficacy is not proven [51, 52]. Multiple case reports in the literature have described the use of US polyclonal IVIG for patients with varying results in neurologic sequelae and survival. Dosing regimens are not standardized, but if there is efficacy, it is suggested that early treatment may improve the outcome and decrease mortality. Further studies are needed to assess this as well as to determine dosage and timing.

A WNV recombinant humanized monoclonal antibody, MGAWN1 (Macrogenics Inc., Rockville, MD) was also evaluated in a clinical trial from 2009 to 2011 but terminated due to inability to adequately enroll subjects [53]. Deaths occurred in 2 of 6 patients receiving study drug compared to 1 death in 7 placebo recipients. In addition, a phase 1 safety and pharmacokinetic study of MGAWN1 in 40 healthy adults (30 received study-drug) noted adverse reactions in 6 (20%) subjects ranging from neutropenia, leukopenia, diarrhea, chest discomfort, dizziness, and somnolence; 1 subject was diagnosed with schizophrenia [53]. This agent is no longer available.

Finally, corticosteroids have been used in some case reports with varying results [54, 55] regarding neurologic improvement; there is one report of topical corticosteroids for the treatment of ocular signs of WNV in four patients also with varying results [56].

Prevention

Preventive strategies for WNV in transplant patients can take two forms, screening of blood products and donors and environmental protection from mosquitoes for living donors, candidates, and recipients.

Blood Donor Screening in the United States

Since the middle of 2003, blood banks in the United States and Canada have been screening all blood donations for WNV using NAT platforms, and since 2005 the FDA has two approved NAT assays that can be used to combine samples from 6 to 16 individuals (mini-pool, MP-NAT) or to test individual donor samples (ID-NAT) for WNV RNA [20, 57, 58]. Due to the great variability of WNV circulation in a specific geographic locality, blood banks in the United States either test all specimens by ID-NAT or use an algorithm to use MP-NAT testing until a reactive result is found indicating that WNV may be circulating in that local region. Once a reactive mini-pool is found, each blood specimen is individually tested, and the blood bank screening is converted to ID-NAT until no further positive results are found [58, 59]. After a period of time where ID-NAT testing remains negative, screening of blood reverts back to MP-NAT. A positive NAT may represent either active infection or a false positive which is less likely during WNV season (June–December in North America). A positive WNV NAT during low-incidence seasons or the absence of human infections is more likely to represent a false-positive result. Unlike deceased organ donors, blood donors can be contacted, counseled, and told to defer donating products for 120 days [58]. Nucleic acid testing of blood products has worked well to preserve the blood bank safety in the United States as viremia corresponds to the majority of the time when blood products are transmissible. Data suggest that once IgM antibody is detected, blood donors no longer transmit WNV [37].

Organ Donor Screening

Unlike blood donation, similar data are not available with respect to WNV transmissibility from tissue or organs, and it may be transmissible after an antibody has developed, even when WNV RNA is no longer detectable. Organ Procurement Transplantation Network (OPTN) policies differ between deceased and live donation and require that individual transplant centers have written plans to screen for specific pathogens based on variable geographic risks including WNV and trypanosomiasis [14, 15]. Laboratory screening should be based on geography and seasonal risk of mosquito bites, although the potential limitations of a family's knowledge of a donor's travel and exposure histories need to also be considered.

Limitations of serologic and NAT screening are highlighted by reports of transmission of WNV in at least two deceased organ donors who were WNV antibody positive but NAT negative [18, 23, 24]. The timing of screening for live donors should be close to the time of transplant. WNV was transmitted from a human stem cell donor who initially tested negative 3 weeks prior to donation but was found to be antibody positive on repeat testing when the pediatric recipient developed the disease [21]. These cases emphasize the importance of considering geographic risks, timing of testing, as well as education for prevention around the time of donation, as highlighted later in this chapter.

Animal models of WNV have demonstrated persistence of the infectious virus in tissues particularly of the kidney for significant periods after clearance of plasma viremia, suggesting that tissue transmissibility in the absence of viremia may be possible [60, 61]. Of concern, a small study found persistent WNV viruria in 5 of 25 (20%) of patients up to 6 years after severe WNV encephalitis [62], although two subsequent larger studies failed to confirm these observations. Gibney et al. found no viruria in 40 patients 6 years after acute WNV infection, and Baty and colleagues observed very low-level viruria in only 1 of 63 patients (1.6%) <5 months after acute WNV infection [63, 64]. Further studies by Murray and colleagues found WNV by electron microscopy in urine sediment in 12 individuals up to 9 years after their initial infection [65]. The infectivity of organs from WNV RNA-positive donors who are also positive for IgM as well as those who are negative for WNV RNA but have either persistent WNV IgM or viruria remains uncertain.

In 2011 an American Society of Transplantation Consensus Conference on donor-derived WNV was held, co-sponsored by the Canadian Society of Transplantation and The Transplantation Society, and results were presented at ATC 2011 (personal communication Marian Michaels). Conclusions and recommendations included:

- WNV appears to carry a risk of more severe disease in transplant recipients compared to healthy hosts.
- There was no evidence for one organ recipient type being at higher risk than others.
- Universal testing was NOT recommended due to the concern for organ wastage due to false-positive results during seasons when WNV is not circulating in a particular geographic area. Rather it was recommended that organ procurement agencies develop communication with their local blood banks so that they can be made aware of circulating WNV in the blood donor population; at that time, consideration for testing organ donors could be put into place.
- The consensus was to not use organs from donors testing positive with the caveat that use could be considered when there was an increased chance of the result being a false positive or a potential recipient had an emergent life-threatening condition without other life-sustaining therapeutic options so that they, their family, and healthcare team might consider the risk: benefit ratio to be in favor of using the organ.

In the United States, individual screening of living organ donors for West Nile virus is performed locally when positive surveillance testing or infection of vectors (mosquitoes, birds) or "innocent bystanders" such as humans is reported. Screening deceased donors when WNV is circulating is also generally followed. In addition, deceased donors with history of unexplained fever, altered mental status, or other symptoms of unknown etiology during WNV season should be evaluated before organ acceptance.

In the case of live donation, WNV NAT screening should be performed within 7–14 days of donation [66]. If positive, confirmatory testing by repeat NAT or serology

may be performed, and if testing remains positive, the donor is considered to be infected with WNV. If repeat NAT or serologies are negative, the donor may have cleared the viremia or there may be a delay in the development of WNV antibodies. Similar to the FDA recommendations for a 120-day deferral period for blood donors, the Organ Procurement and Transplantation Network (OPTN) recommends deferral of donor candidates for 120 days followed by repeat NAT screening prior to organ donation [66, 67]. Serologic testing is not recommended, since WNV IgG is likely to be associated with past infection and WNV IgM can last for variable periods of time. The OPTN recommends that "local expertise should be used in making decisions regarding timing of organ donation and additional testing on initial NAT reactive donors" [67]. If a recipient or donor tests positive for WNV or is suspected to have WNV after transplantation, OPTN safety portal and the OPO should be notified immediately so that all recipients of organs from that donor can be evaluated and managed expeditiously [68, 69].

Environmental Prevention of West Nile Virus Infection

Since transmission of WNV is primarily through mosquito bites, avoidance is key to prevention. Knowledge of the epidemiology and reports of WNV in the locale are helpful to counsel potential live donors, transplant candidates, and recipients during mosquito season in order to prevent infection.

Specific effective insect repellents have been listed by the CDC [6]:

- Picaridin (known as KBR 3023 and icaridin outside the United States).
- N,N-Diethyl-meta-toluamide (DEET).
- Ethyl butylacetylaminopropionate (IR3535).
- Oil of lemon eucalyptus (OLE).
- Para-menthane-diol (PMD).
- 2-Undecanone.

Behavioral and lifestyle adjustments are helpful to prevent bites, with avoidance of outside activities from dusk to dawn when *Culex* spp. mosquito activity is increased. In addition, standing pools of water should be minimized or avoided to decrease mosquito breeding grounds.

Key Points

- Great variability exists (both year-to-year and geographically) in WNV activity.
- Immunocompromised populations may have a higher risk for neuroinvasive disease than immunocompetent populations:
  - The full spectrum of WNV epidemiology in transplant recipients is still emerging information.
- Transplant recipients can acquire West Nile virus at transplantation from donors or transfusions.

- Transplant recipients can also acquire WNV from infected mosquitoes.
- West Nile virus should be considered in transplant patients with fever and neurologic symptoms early after transplant or during mosquito season in their geographic area.
- Treatment of WNV disease at this time is supportive:
  - Decreasing immunosuppression, when possible, is advised; transplant teams may wish to increase immunosuppression again once the infection is cleared so as to avoid rejection.
  - Trials evaluating specific treatment strategies have failed to document a successful intervention to date.
  - Intravenous immunoglobulin may be of benefit early in the disease, but dosing and timing require further study.
- Transplant candidates, recipients, and potential living donors should be counseled on the means for mosquito avoidance.

Conclusion

While rare, WNV can cause severe and fatal disease after transplantation. Accordingly, candidates and recipients of transplantation should be educated about preventive strategies. In addition, screening should be considered for donors during periods of WNV circulation. Evaluation and differential diagnosis of transplant patients with neurological symptoms with fever should include WNV. If infection is found early after transplantation, consideration should be given for a potential donor-derived infection and reporting should be made to the OPTN safety portal to allow for timely evaluation of other recipients from the same donor. The mainstay of treatment is supportive care with some evidence of response to intravenous immunoglobulin.

Disclaimer Dr. Levi's contribution to this chapter represents her own opinions and work and does not represent the policies or opinions of the United States government.

References

1. Smithburn KC, Hughes TP, Burke AW, Paul JH, et al. A neurotropic virus isolated from the blood of the native of Uganda. Am J Trop Med Hyg. 1940;20:471–92.
2. Komar NLS, Hinten S, Nemeth N, Edwards E, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis. 2003;9:311–22.
3. van der Meulen KM, Pensaert MB, Nauwynck HJ. West Nile in the vertebrate world. Arch Virol. 2005;150:637–57.
4. Murgue B, Murri S, Triki H, Deubel V, et al. West Nile in the Mediterranean basin: 1950–2000. Ann N Y Acad Sci. 2001;951:117–26.
5. Nash D, Mostashari F, Fine A, Miller J, et al. The outbreak of West Nile virus in the New York City area in 1999. N Engl J Med. 2001;344(24):1807–14.
6. https://www.cdc.gov/westnile/. Accessed 11 June 2020.

7. Curren EJ, Lehman J, Kolsin J, Walker WL, Martin SW, Staples JE, Hills SL, Gould CV, Rabe IB, Fishcer M, Lindsey NP. West Nile virus and other nationally notifiable arboviral diseases – United States, 2017. MMWR Morb Mortal Wkly Rep. 2018;67:1137–42.
8. Centers for Disease Control and Prevention (CDC). Update: investigations of West Nile virus infections in recipients of organ transplantation and blood transfusion. MMWR Morb Mortal Wkly Rep. 2002;51:833–6.
9. Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003;348:2196–203.
10. Stramer SL, Fang Chyang T, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med. 2005;353:451–9.
11. Kumar D, Ramesh Prasad GV, Zaltzman J, Levy GA, Humar A. Community-acquired West Nile virus infection in solid-organ transplant recipients. Transplantation. 2004;77:399–402.
12. Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, et al. Neurologic manifestations and outcome of West Nile virus infection. JAMA. 2003;290:511–5.
13. Emig M, Apple DJ. Severe West Nile virus disease in healthy adults. Clin Infect Dis. 2004;38:289–92.
14. Lindsey NP, Staples JE, Lehman JA, Fischer M. Surveillance for human West Nile virus disease – United States, 1999–2008. MMWR Surveill Summ. 2010;59(2):1–17.
15. Tyler KL, Pape J, Goody RJ, et al. CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis. Neurology. 2006;66:361–5.
16. Davis LE, DeBiasi R, Goade DE, Haaland KY, Harrington JA, Harnar JB, Pergam SA, King MK, DeMasters BK, Tyler KL. West Nile virus and neuroinvasive disease. Ann Neurol. 2006;60:286–300.
17. Anesi JA, Silveira FP. Arenaviruses and West Nile virus in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13576.
18. Centers for Disease Control and Prevention. West Nile virus infections in organ transplant recipients – New York and Pennsylvania, August–September, 2005. MMWR Morb Mortal Wkly Rep. 2005;54:1021–3.
19. Centers for Disease Control and Prevention. West Nile virus infections in organ transplantation and blood transfusion – Louisiana, 2008. MMWR Morb Mortal Wkly Rep. 2009;58:1263–7.
20. Nett RJ, Kuehnert MJ, Ison MG, Orlowski JP, Fischer M, Staples JE. Current practices and evaluation of screening solid organ donors for West Nile virus. Transpl Infect Dis. 2012;14:268–77.
21. Rhee C, Eaton EF, Concepcion W. West Nile virus encephalitis acquired via liver transplantation and clinical response to intravenous immunoglobulin: case report and review of the literature. Transpl Infect Dis. 2011;13:312–7.
22. Morelli MC, Sambri V, Grazi GL, et al. Absence of neuro invasive disease in the liver transplant recipient who acquired West Nile virus (WNV) infection from the organ donor and new received WNV antibodies prophylactically. Clin Infect Dis. 2010;51(4):e34–7.
23. Costa AN, Capobianchi MR, Ippolito G, Palu G, Barzon L, Piccolo G, Andreetta B, Filippetti M, Fehily D, Lombardini L, Grossi P. West Nile virus: the Italian national transplant network reaction to and alert in the North Eastern region, Italy 2011. Euro Surveill. 2011;16(41):19991.
24. Inojosa WO, Scotton PG, Fuser R, et al. West Nile virus transmission through organ transplantation in North–Eastern Italy: a case report and implications for pre-procurement screening. Infection. 2012;40:557–62.
25. Winston DJ, Vikram HR, Rabe IB, Dhillon G, et al. Donor-derived West Nile virus infection in solid organ transplant recipients: report of four additional cases and review of clinical, diagnostic, and therapeutic features. Transplantation. 2014;97(9):881–9.
26. Kumar D, Debrot MA, Wong SJ, et al. A seroprevalence study of West Nile virus infection in solid organ transplant recipients. Am J Transplant. 2004;4(11):1883–8.
27. Kleinschmidt-DeMasters BK, Marder B, Levi ME, Laird SP, et al. Naturally acquired West Nile virus encephalomyelitis in transplant recipients: clinical, laboratory, diagnostic, and neuropathological features. Arch Neurol. 2004;61(8):1210–20.

28. Crichlow R, Bailey J, Gardner C. Cerebrospinal fluid neutrophilic pleocytosis in hospitalized West Nile virus patients. J Am Board Fam Pract. 2004;17:470–2.
29. Aziz F, Saddler C, Jorgenson M, Smith J, Mandelbrot D. Epidemiology, management, and graft outcomes after West Nile virus encephalitis in kidney transplant recipients. Transpl Infect Dis. 2020;22:e13317.
30. Hiatt B, DesJardin L, Carter T, Gingrich R, Thompson C, de Magalhaes-Silverman M. A fatal cases of West Nile virus infection the bone marrow transplant recipient. Clin Infect Dis. 2003;37:e129–31.
31. Hong DS, Jacobson KL, Raad II, et al. West Nile encephalitis in 2 hematopoietic stem cell transplant recipients: case series and literature review. Clin Infect Dis. 2003;37:1044–7.
32. Martin SE, Grubbs S, Della Valla J, et al. Fatal West Nile virus encephalitis following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. 2004;34:1007–8.
33. Reddy P, Davenport R, Ratanatharathorn V, et al. West Nile virus encephalitis causing fatal CNS toxicity after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2004;33:109–12.
34. Robertson KB, Barron MA, Nieto Y. West Nile virus infection and bone marrow transplant patients. Bone Marrow Transplant. 2004;34:823–4.
35. Brenner W, Storch G, Buller R, Vij R, Devine S, DiPersio J. West Nile virus encephalopathy in an allogeneic stem cell transplant recipient: use of quantitative PCR for diagnosis and assessment of viral clearance. Bone Marrow Transplant. 2005;36:369–70.
36. Kitagawa MG, Ettinger N, Breen D, Erklauer J, Chang E, Herce H, King K, Naik S. Transmission of West Nile virus through a hematopoietic stem cell transplant. J Pediatr Infect Dis. 2018;7:e52–4.
37. Busch MP, Kleinman SH, Toblet LH, et al. Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis. 2008;198(7):984–93.
38. Papa A, Anastasiadou A, Delianidou M. West Nile virus IgM and IgG antibodies three years post-infection. Hippokratia. 2015;19:34–6.
39. Vanichanan J, Salazar L, Wootton SH, et al. Use of testing for West Nile virus and other arboviruses. Emerg Infect Dis. 2016;22(9):1587–93.
40. Levi ME, Quan D, Ho JT, et al. Impact of rituximab-associated B-cell defects on West Nile virus meningoencephalitis in solid organ transplant recipients. Clin Transpl. 2010;24(2):223–8.
41. Koepsell SA, Freifeld AG, Sambol AR, et al. Seronegative naturally acquired West Nile virus encephalitis in a renal and pancreas transplant recipient. Transpl Infect Dis. 2010;12:459–64.
42. Penn RG, Guarner J, Sejvar JJ, et al. Persistent neuroinvasive West Nile virus infection in an immunocompromised patient. Clin Infect Dis. 2006;42:680–3.
43. Massa J, Manthena P. Delayed seropositivity for West Nile virus in post-transplant patients: a case series. Neurology. 2016;86(16 Suppl):337.
44. Zanoni F, Alfieri C, Moroni G, Passerini P, Regalia A, Meneghini M, Messa P. Delayed diagnosis of West Nile virus infection in a kidney transplant patient due to inaccuracies in commonly available diagnostic tests. Exp Clin Transplant. 2020;18(3):385–9.
45. Wilson MR, Zimmermann LL, Crawford ED, et al. Acute West Nile virus meningoencephalitis diagnosed via metagenomic deep sequencing of cerebrospinal fluid in a renal transplant patient. Am J Transplant. 2017;17:803–8.
46. Petropoulou KA, Gordon SM, Prayson RA, Ruggierri PM. West Nile virus infection: MR imaging findings in the nervous system. Am J Neuroradiol. 2005;26(8):1986–95.
47. Chowers MY, Lang R, Nassar F, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. Emerg Infect Dis. 2001;7:675–8.
48. Kalil AC, Devetten MP, Singh S, Lesiak B, Poage DP, Bargenquast K, Fayad P, Freifeld AG. Use of interferon-alpha in patients with West Nile encephalitis: report of 2 cases. Clin Infect Dis. 2005;40:764–6.
49. Haley M, Retter AS, Fowler D, Gea-Banacloche J, O'Grady NP. The role for intravenous immunoglobulin in the treatment of West Nile virus encephalitis. Clin Infect Dis. 2003;37:e88–90.
50. Shimoni Z, Niven MJ, Pitlick S, Bulvik S. Treatment of West Nile virus encephalitis with intravenous immunoglobulin. Emerg Infect Dis. 2001;7:759.

51. Hart J Jr, Tailman G, Kraut MA, et al. West Nile neural invasive disease: neurological manifestations and prospective longitudinal outcomes. BMC Infect Dis. 2014;14:248–58.
52. Planitzer CB, Modrof J, Kreil TR. West Nile virus neutralization by US plasma-derived immunoglobulin products. J Infect Dis. 2007;196:435–40.
53. Planitzer CB, Modrof J, Yu MY, et al. West Nile virus infection in plasma of blood and plasma donors, United States. Emerg Infect Dis. 2009;15:1668–70.
54. Beigel JH, Nordstrom JL, Pillemer SR, et al. Safety and pharmacokinetics of single intravenous dose of MGAWN1, a novel monoclonal antibody to West Nile virus. Antimicrob Agents Chemother. 2010;54:2431–6.
55. Alker A. West Nile virus-associated acute flaccid paralysis. BMJ Case Rep. 2015;2015: bcr2014206480.
56. Pyrgos V, Younus F. High-dose steroids in the management of acute flaccid paralysis due to West Nile virus infection. Scand J Infect Dis. 2004;36:509–12.
57. Bakri SJ, Kaiser PK. Ocular manifestations of West Nile virus. Curr Opin Ophthalmol. 2004;15:537–40.
58. Tilley PAG, Fox JD, Jayaraman GC, Preiksaitis JK. Nucleic acid testing for West Nile virus RNA in plasma enhances rapid diagnosis of acute infection in symptomatic patients. J Infect Dis. 2006;193:1361–4.
59. Food and Drug Administration Guidance for Industry. Use of nucleic acid test to reduce the risk of transmission of West Nile virus from donors of whole blood and blood components intended for transfusion. 2019. https://www.fda.gov/media/124300/download. Accessed 15 June 2019.
60. Custer B, et al. Triggers for switching from minipool testing by nucleic acid technology to individual-donation nucleic acid testing for West Nile virus: analysis of 2003 data to inform 2004 decision making. Transfusion. 2004;44:1547–54.
61. Pogodina VV, Frolova MP, Malenko GV, et al. Study on West Nile virus persistence in monkeys. Arch Virol. 1983;75:71–86.
62. Saxena V, Xie G, Li B, et al. A hamster-derived West Nile virus isolate induces persistent renal infection in mice. PLoS Negl Trop Dis. 2013;7:e2275. https://doi.org/10.1371/journal.pntd.0002275.
63. Murray K, Walker C, Herrington E, et al. Persistent infection with West Nile virus years after initial infection. J Infect Dis. 2010;201:2–4.
64. Gibney KA, Lanciotti RS, Sejvar JJ, et al. West Nile virus RNA not detected in urine of 40 people tested 6 years after acute West Nile virus disease. J Infect Dis. 2011;203:344–7.
65. Baty SA, Gibney KB, Staples JE, et al. Evaluation of West Nile virus (WNV) RNA in newborn of patients within 5 months of WNV infection. J Infect Dis. 2012;205:1476–7.
66. Murray KO, Kolodziej S, Ronca SE, et al. Visualization of West Nile virus in your urine sediment using electron microscopy and immunogold up to nine years post infection. Am J Trop Med Hyg. 2017;97:1913–9.
67. Levi ME, Kumar D, Green M, et al. Considerations for screening live kidney donors for endemic infections: a viewpoint on the UNOS policy. Am J Transplant. 2014;14:1003–11.
68. https://optn.transplant.hrsa.gov/resources/guidance/identifying-risk-factors-for-west-nile-virus-wnv-during-evaluation-of-potential-living-donors/
69. OPTN Policy 15. Identification of transmissible diseases. 2019. https://optn.transplant.hrsa.gov/governance/policies/. Accessed 23 June 2019.

Emerging Tropical Viral Infections: Dengue, Chikungunya, and Zika

41

Alexandre Machado de Sant' Anna Carvalho and
Clarisse Martins Machado

Contents

Introduction 988
Disease Epidemiology 989
Dengue 989
Chikungunya 990
Zika 991
Clinical Impact on Transplantation 992
Dengue 992
Chikungunya 994
Zika 995
Diagnosis of DENV, CHIKV, and ZIKV 995
Dengue 995
Chikungunya 996
Zika 996
Management of DENV, CHIKV, and ZIKV Infections in Transplant Recipients 997
Dengue 997
Chikungunya 998
Zika 998
Preventive Measures 999
Vector Control 999
Vaccines 999
Blood Supply Safety 1001

A. M. d. S. A. Carvalho
Department of Internal Medicine, Saint Joseph Mercy Ann Arbor, Ann Arbor, MI, USA
e-mail: alexandre.carvalho@stjoeshealth.org

C. M. Machado (✉)
Virology Laboratory (LIM 52 - HCFMUSP), Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil

HSCT Program – Amaral Carvalho Foundation, Jahu, Brazil

Israel Institute of Education and Research, Hospital Albert Einstein, São Paulo, Brazil
e-mail: clarimm@usp.br

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_40

Screening Recommendations and Donor Deferral 1001
Conclusions 1002
Key Points 1003
References 1003

Introduction

Currently, more than 500 arboviruses (arthropod-borne viruses) have been identified, of which nearly 150 are associated with human infection [1]. Five arboviruses (West Nile, yellow fever, dengue, chikungunya, and Zika) have recently re-emerged in both hemispheres, and outbreaks have been reported mostly in tropical and subtropical countries (Fig. 1).

Diseases caused by arboviruses bring particular challenges to the transplant community whenever donors or immunocompromised recipients travel or live in endemic regions. Although dengue is the most common mosquito-borne disease in tropical and subtropical regions, chikungunya and Zika have been circulating in the Americas for several years now, bringing renewed attention to how these infections behave in immunocompromised hosts.

These three arboviruses have demonstrated the potential for blood and graft transmission, extended viremia, and complicated engraftment, which has affected donor selection or deferrals, among other impacts. In the acute phase, the diseases have a similar clinical presentation and, if contracted in the perioperative window, may be confused with adverse transplant reactions, hindering timely diagnosis and management and occasionally resulting in severe or fatal outcomes. Thus, diseases caused by arboviruses may represent a threat to immunocompromised hosts living in or traveling to endemic regions.

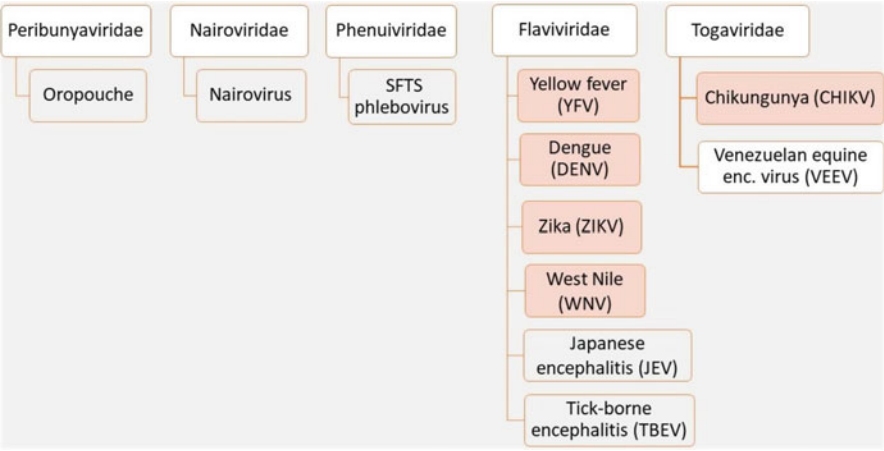


Fig. 1 Arbovirus families (re-emergent arboviruses in color)

Disease Epidemiology

Dengue

Dengue virus (DENV) is a small single-stranded RNA virus belonging to the genus *Flavivirus* and family *Flaviviridae* and transmitted by mosquitoes of the genus *Aedes (Aedes aegypti* and *Aedes albopictus)* [2]. Dengue occurs both as an endemic and epidemic disease. A recent study estimates that 390 million dengue infections worldwide occur every year, of which 96 million manifest clinically, with variable severity [3]. An estimated 3.9 billion people in 128 countries are at risk of infection with DENV [4].

Dengue virus comprises four main serotypes, DENV-1, DENV-2, DENV-3, and DENV-4. Infection by one serotype provides lifelong immunity against that serotype, but only partial protection against subsequent infections by other serovars. In immunocompetent hosts, the risk of severe disease is greater in secondary infection, due to the mechanism known as antibody-dependent enhancement (ADE). A robust immunologic response seems to be a prerequisite for the development of severe dengue, when elevated circulating levels of IL-8, IL-10, TGF-β, and interferon-γ may be detected [5].

Besides mosquito bite, other routes of dengue transmission may occur and have recently been reviewed [6]. Nonvector transmission of dengue can occur through blood transfusion, solid organ transplant (SOT) and hematopoietic stem cell transplantation (HSCT), perinatal transmission, breast milk, percutaneous (needlestick or laboratory accident), and mucocutaneous (blood splash) [6].

In the last decade, an increasing number of dengue infections have been described in transplant recipients due to the expansion of DENV [7–12]. In endemic areas, this number may be much higher, since most cases are mild and present as a flu-like illness, with some manifestations resembling posttransplant syndromes.

In general, dengue infections in transplant recipients are acquired by vector transmission during travel to endemic areas, or in recipients living in such areas [9, 10, 12]. However, other sources of infection are relevant in the transplant setting, such as the graft itself and blood transfusions. Most transfusion-transmitted (TT) dengue cases are asymptomatic, and viremic donors have been detected during outbreaks [13–15]. Although rare, an increasing number of allograft transmission cases have been reported in transplant recipients.

In HSCT, two cases of dengue transmitted by the graft have been well documented [16, 17]. The first case occurred during the 1994–1995 dengue epidemic in Puerto Rico. The donor developed fever 2 days after marrow harvesting, and DENV-4 serotype was confirmed in donor samples [16]. The recipient died of severe dengue 11 days after HSCT. DENV-4 was detected in blood, ascitic fluid, and tissue samples. The other case of graft transmission occurred in a HSCT recipient whose unrelated donor had returned from Sri Lanka 3 days before donation [17]. In SOT recipients, graft transmission has also been reported [18–23] and has been recently reviewed [21–23]. In one case report, the evidence of graft transmission is

weak, as both donor and recipient were from an endemic area and the living donor had acquired dengue 6 months before donation. Such long persistence has not been demonstrated in the healthy population infected with DENV. Since the genetic identity between donor and recipient viruses was not demonstrated, vector transmission is more likely to have occurred in this setting [18].

In cases of graft transmission, dengue symptoms usually start in the first 10 days after transplantation. A living donor who developed symptoms 2 days after donation transmitted dengue to a liver transplant recipient in Delhi [20]. Mathew and colleagues also reported on a liver transplant recipient who acquired DENV through the organ allograft. Using phylogenetic analysis, they were able to rule out other infections and match the molecular signature to the donor, who presented with fever, thrombocytopenia, and abdominal pain on postoperative day (POD) 6 and had positive RT-PCR for DENV-1. On POD 9, the recipient developed abdominal pain, thrombocytopenia, and encephalopathy that quickly progressed to renal dysfunction and graft failure, leading to death despite aggressive treatment [23].

Chikungunya

Chikungunya virus (CHIKV) belongs to the family *Togaviridae* genus *Alphavirus* and is transmitted by *Aedes* mosquitoes *(Aedes albopictus* and *A. aegypti).* Until now, four lineages distinguished by genotypes have been identified [24].

CHIKV was first isolated in the 1952 outbreak in a southern province of Tanzania [25, 26]. The re-emergence of CHIKV in the twenty-first century involved the dispersal of two endemic genotypes, the African genotype and the Asian genotype. CHIKV quickly spread to the islands along the coast of Africa in the Indian Ocean and caused a massive outbreak on Réunion Island in 2004, affecting approximately 34% of the population [27]. In 2013, cases of CHIKV infection were reported in St. Martin's Island, later spreading throughout the Caribbean, Central America, South America, and North America [28, 29]. In 2006, several cases of CHIKV infection were reported in Europe [30]. In 2017, up to 1,010,724 cases were reported in the region of the Americas, mainly in Mexico, the USA, and Brazil [31]. In 2018, there were 26,840 cases of probable CHIKV infection reported in Brazil, and by March 2019, more than 15,352 cases of probable CHIKV infections have already been registered [32].

Regarding other modes of CHIKV transmission, few articles discuss transmission by blood transfusion or organ transplantation. The maximum viremic period of CHIKV infection is 2 days before and 17 days after symptom onset. The mean and maximal risks of CHIKV viremic blood donation were estimated to be 38–52 per 100,000 donations (0.04–0.06%), respectively [33]. In the 2005 outbreak in La Réunion, CHIKV was detected in 4 of 12 healthy donors of corneal grafts, demonstrating the potential risk of transmission during tissue transplantation [34].

Zika

Zika virus (ZIKV) is a *Flavivirus* in the family *Flaviviridae* and was first identified in 1947 in a sentinel rhesus monkey in the Zika Forest of Uganda [35]. Two species are considered the main competent vectors, *Aedes aegypti* and, less commonly, *A. albopictus*. Before 2007, only sporadic human disease cases were reported from countries in Africa and Southeast Asia [36]. In 2007, an outbreak of ZIKV occurred in the island of Yap, Micronesia, with approximately 5000 cases reported [37]. In 2013, an outbreak began in French Polynesia, and the virus subsequently spread to other Pacific Islands [35]. After reaching Brazil in May 2015, ZIKV was subsequently detected through much of the Americas. Microcephaly, a devastating effect of ZIKV infection during pregnancy, was first observed (and later confirmed) during the ZIKV epidemic in Brazil [38]. From January 2015 through March 2017, a total of 754,460 autochthonous cases of ZIKV disease were reported to the Pan American Health Organization (PAHO) from countries and territories in the Americas, 27% of which were laboratory confirmed. So far, a total of 84 countries or territories globally have reported autochthonous mosquito-borne ZIKV transmission. In the Americas, the only countries that have not reported cases of vector-transmitted ZIKV are Bermuda, Canada, Uruguay, and continental Chile [36].

Alternative ways of ZIKV transmission include sexual, perinatal, and via blood products [39]. A relevant concern related to ZIKV transmission is the capacity of viral persistence in fluids and organs. ZIKV RNA can persist in semen up to 370 days [40], although infectious ZIKV can be detected during a much shorter period, around 40 days [41]. Although never definitively demonstrated, the risk for ZIKV transmission through organ or tissue transplantation is plausible. Chen et al. have demonstrated that ZIKV infects and persists in the human renal proximal tubular epithelium suggesting that these cells are a potential reservoir of ZIKV in the human urinary system, explaining the prolonged persistency of ZIKV in patient urine and highlighting the possibility of organ transmission through kidney transplantation [42].

So far, the majority of ZIKV cases in transplant patients were acquired by mosquito bite. During the ZIKV epidemic in Brazil, some cases were reported in SOT and HSCT recipients. Nogueira and colleagues described four cases in SOT recipients (two for the liver, two for the kidney) who developed symptomatic ZIKV infection diagnosed by RT-PCR [43]. In a nearby region, a heart transplant recipient with fever, malaise, and seizures was diagnosed with ZIKV meningoencephalitis [44]. A prospective study conducted in 2016 in Brazil described the clinical findings and outcomes in four cases of ZIKV in HSCT recipients and hematologic malignancy patients [45]. Transfusion transmission is another possibility. Musso et al. reported a 2.8% incidence of blood donors positive for ZIKV by PCR at the time of donation during the French Polynesian ZIKV outbreak [46]. Similarly, 0.5–1% of donors were found to be ZIKV RNA positive in Puerto Rico and Brazil, respectively, during the recent ZIKV epidemic [47, 48]. The first case of

Zika in a liver transplant recipient was transmitted by the platelet component transfused during surgery. The blood donor was asymptomatic at the time of donation [49]. Two other cases of ZIKV transmission by platelet transfusion have also been documented [50].

Clinical Impact on Transplantation

Dengue

Dengue has a wide clinical spectrum varying from asymptomatic to severe clinical manifestations. Seroprevalence studies suggest that the number of asymptomatic carriers is three times that of dengue fever cases. Since 2016, symptomatic dengue is grouped into three categories: dengue without warning signs (DNWS), dengue with warning signs (DWWS), and severe dengue (SD), as shown in Fig. 2. Severe disease and lethal outcome may occur even in the absence of warning signs [51].

After a 3- to 15-day incubation period, the disease presents abruptly as a flu-like illness in three phases – febrile, critical, and recovery. The three phases are not clearly distinguishable in transplanted patients, in whom symptom duration is longer, thrombocytopenia occurs in more than 80% of cases, and incubation period is shorter in case of graft transmission [22]. Atypical presentations of dengue in SOT and HSCT recipients include acute colitis and encephalitis [52, 53].

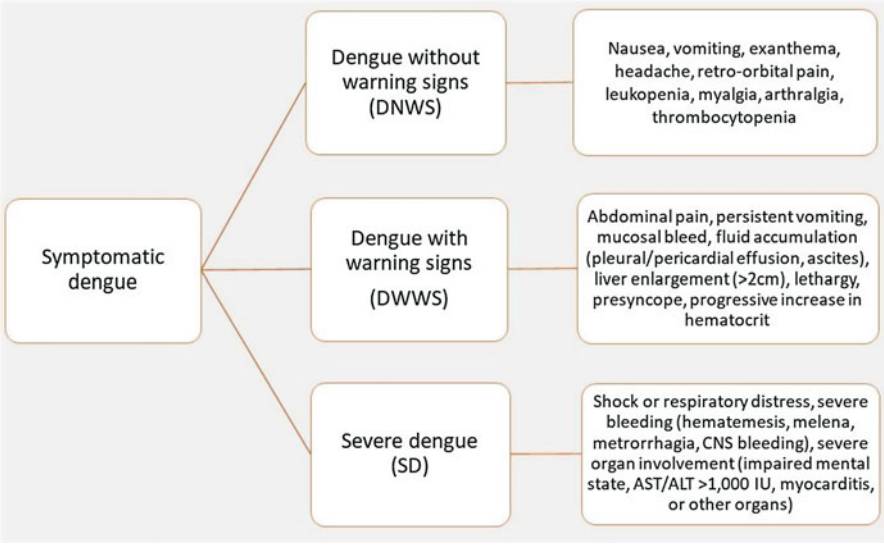


Fig. 2 Clinical classification of dengue

A recent prospective study in HSCT recipients conducted during dengue season in Brazil showed the main clinical findings to be fever (100%), thrombocytopenia (80%), myalgia (80%), and rash (60%), with or without hemorrhagic episodes, similar to immunocompetent individuals. Severe dengue occurred in one patient (20%). An important finding of this study was the observation of prolonged viremia (more than 15 days) in all patients who had blood samples collected serially [12]. In the immunocompetent population, dengue viremia persisted from 4 to 7 days (median 5 days) in 69% of the patients and was uncommon after the fifth day (12.5%) [54, 55].

Among SOT recipients, the largest case series was published in 2013 and reported the clinical findings of 102 DENV cases after renal transplantation [7]. Forty-four patients (43%) had primary dengue infection, and 58 (56.8%) had secondary dengue infection. Most patients presented with fever (80%), which was less frequent in patients receiving high-dose steroids. DWWS or DNWS occurred in 88%, and SD occurred in 11.7%, with graft dysfunction in 66.7% of these. Thrombocytopenia was seen in 95% of cases, with a mean duration of 11 ± 9 days. Interestingly, patients on a cyclosporine (CSA) containing regimen had less severe disease [7]. Some authors have shown that CSA can be a potential drug for the treatment of flavivirus infections [56].

In recent literature reviews, the incidence of SD in transplant vs immunocompetent patients was reported as 16% vs. 3.7% [8], with dengue mortality rates varied from 0.026% in DNWS up to 5% in SD in immunocompetent patients [57], while studies in transplant populations show mortality rates ranging from 0% to 38% [58]. There are some reports of complicated dengue in kidney transplantation presenting early in the postoperative period with or without fever, abdominal pain, and third-spacing, with imaging demonstrating bleeding around the graft requiring blood products and sometimes surgical evacuation to achieve hemostasis. Poor outcomes such as graft failure, protracted hospitalization, and two deaths were reported [59, 60]. Furthermore, an excellent article by Pinsai and colleagues summarizing and comparing recent dengue case series in kidney recipients shows data suggesting that mycophenolate mofetil (MMF) may increase disease severity [58]. Another article by Subbiah and colleagues also reported the need to de-escalate from MMF in 60% of patients due to refractory cytopenias, with the medication resumed later [61]. To clarify these discrepancies, a meta-analysis or prospective trials comparing these different scenarios could have significant impact on future recommendations, especially in hyperendemic countries.

In this uncertain scenario, practitioners should be aware of the increasingly recognized association between hemophagocytic lymphohistiocytosis (HLH) and DENV [58, 62, 63]. HLH is a sporadic or familial disease characterized by loss of immune homeostasis and a dysregulated immune response manifesting as a cytokine storm to exogenous triggers in a genetically predisposed host. It presents with fever, hepatosplenomegaly, rash, neurologic symptoms, cytopenia in two or three lines, and elevated ferritin that quickly progresses to multi-organ system failure and death. Chung and colleagues summarized recent DENV-associated HLH with outcomes and important clinical pearls [64]. In many ways, HLH behaves similarly to severe DENV making it hard to diagnose if a high index of suspicion is not maintained. In

endemic countries, an intervening DENV infection in pediatric HSCT recipients transplanted due to familial forms of HLH may be misdiagnosed as disease relapse or graft-versus-host disease (GVHD) [65].

Chikungunya

After a mosquito bite, CHIKV reaches the bloodstream and spreads through the liver, muscles, joints, spleen, lymph nodes, and brain. Unlike ZIKV and DENV, CHIKV is symptomatic in more than 95% of cases [26]. In immunocompetent patients, the incubation period varies between 2 and 10 days [30]. After this, an abrupt onset of fever is observed, followed in a few days by symmetric polyarthralgia in 90% of patients, with maculopapular rash in about 50%. Myalgia, malaise, nausea, and headache are also typical, and the acute illness lasts 1–2 weeks [66]. Persistent or recurring arthralgia is characteristic, with 20–43% developing incapacitating chronic polyarthralgia that persists for months or years after the acute phase [67, 68]. Chronic CHIKV arthritis often causes severe pain and disability, and pathogenesis is not well understood. It is hypothesized that continued low-level viral replication in the joints, viral RNA persistence in the synovium, and induction of autoimmunity are the possible causes [69]. There are also reports of neurologic, cardiac, renal, and hepatic manifestations with a fatality ratio of 17% in cases of encephalitis [70, 71].

The intensity of joint symptoms and overall course in transplant recipients may not be as severe as is seen in the immunocompetent population. In general, the disease has been reported as mild with favorable outcomes [72–74]. In general, most of the patients presented with arthralgia, 85% had fever, and 46% developed chronic arthritis by 3 months of follow-up, but no graft dysfunction or deaths were reported [72–74]. Rosso and colleagues recently reported similar findings in Colombia during the 2015 outbreak. Ten SOT recipients that acquired CHIKV were followed for 3 months. Diagnosis was confirmed through RT-PCR and graft transmission ruled out by testing donor samples. Initial clinical manifestations were arthralgia in 100%, fever in 90%, headache in 40%, and myalgia. Interestingly, there were no reports of graft dysfunction, severe complications, or death, and only 30% had mild arthralgia lasting >5 days. All patients achieved full recovery in 3 months without protracted joint pain [75].

Another recent publication from Brazil retrospectively describes 32 cases of CHIKV in kidney transplant recipients. All presented with arthralgia, 47% with overt joint inflammation, and 44% evolving to chronic arthralgia. During the acute phase, seven patients (21.9%) showed acute kidney injury (AKI), but kidney function tended to recover, with transitory graft dysfunction resolving after 3 months. Previous corticosteroid use was not associated with AKI or chronic arthralgia [76]. Similarly, CHIKV infections in HSCT recipients have been reported without increased morbidity [45]. The use of immunosuppressive drugs may play a role in the paucity of symptoms.

Zika

It is estimated that around 80% of ZIKV infections are asymptomatic. Immunocompetent hosts infected with ZIKV by mosquito bite may present with mild fever, maculopapular rash, nonpurulent conjunctivitis, headache, retro-orbital pain, arthralgia, myalgia, and, less commonly, vomiting and edema [35]. In pregnant women, ZIKV infection has been associated with severe congenital complications. Microcephaly is the most devastating feature of congenital infection caused by ZIKV. Other neurological disorders include Guillain–Barre syndrome (GBS), acute myelitis and meningoencephalitis, isolated single or multiple cranial nerve palsies, and stroke [35, 77].

There are few interesting reports of ZIKV in transplant recipients from Brazil [43–45]. In the case series reported by Nogueira and colleagues, four SOT recipients (two for the kidney, two for the liver) infected with ZIKV had symptoms such as fever, headache, cough, myalgia, diarrhea, and vomiting, and three developed concomitant severe bacterial sepsis, with complications such as biliary abscess, graft dysfunction, and one hepatic artery thrombosis requiring re-transplantation. All survived despite these critical events [43], and it is unclear which manifestations can be attributed to ZIKV or solely to bacterial sepsis. In 2017, a fatal case of ZIKV meningoencephalitis was reported in a heart transplant recipient; however, the cause of death was determined to be arrhythmia due to acute cardiac allograft rejection in the setting of abrupt interruption of immunosuppressive drugs [44]. Also in Brazil, Machado and colleagues described a total of four cases of ZIKV in malignant hematology and HSCT recipients, with prolonged RNA detection and delayed engraftment (HSCT) being observed with no further complications [45]. As the symptoms of ZIKV, CHIKV, and DENV are similar, these infections should be considered in the differential of neutropenic fever or rash in posttransplant settings, alongside GVHD and graft rejection, in patients living in endemic areas.

Diagnosis of DENV, CHIKV, and ZIKV

Dengue

Fever and rash are good markers of arbovirus infection, especially during mosquito season, and should prompt DENV, ZIKV, and CHIKV investigation [45]. Thrombocytopenia is a hallmark of dengue and helps to differentiate it from other arboviruses [12, 45]. After disease onset, DENV can be detected in serum, plasma, circulating blood cells, and other tissues for 4–5 days. Currently, nucleic acid tests (NAT) and NS1 antigen detection are recommended to diagnose dengue in the acute phase since results are available within a few hours. Due to the occurrence of prolonged RNAemia (over 15 days), frequently observed in transplant patients, NAT can be used to diagnose dengue even after the first week of illness in this population [12, 58].

After the acute phase of infection, serology used to be the method of choice for diagnosis. Previous studies showed that specific IgM antibodies are detectable in 50% of patients by days 3–5 after illness onset, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about 2 weeks after the onset of symptoms and then decline to undetectable levels over 2–3 months. IgG is detectable at low titers at the end of the first weeks, increasing slowly thereafter with serum IgG still detectable after several months, probably persisting for life [2]. In recent years, with the spread of ZIKV and CHIKV in dengue regions, cross-reactivity in serological tests has unfortunately been observed, with some Zika patients testing false-positive for dengue [78, 79]. As a result, both serological diagnosis and seroprevalence estimates are compromised.

Chikungunya

The diagnosis of CHIKV infection requires laboratory corroboration. Lymphopenia is a key finding, though increased AST/ALT, thrombocytopenia, and hypocalcemia are frequently seen [24]. The laboratory diagnosis is based on NAT in blood samples, viral culture, or detection of IgM and IgG antibodies through serological tests. Viral isolation from blood cells is the gold standard, but technical complexity and delay in results hinder its use in clinical practice [30]. IgM is useful in patients presenting after the acute phase, being detectable between 2 and 12 days and persisting for several weeks [66]. Antigenic cross-reactivity between CHIKV and other alphaviruses has been reported [80]. ELISA assays using recombinant envelope protein-2 antigen for diagnosis of CHIKV without cross-reactivity with other alphaviruses were reported by Fumagalli and colleagues. CHIKV detection parameters for IgG were 89.7% sensitivity and 100% specificity while for IgM were 92.5% sensitivity and 79.0% specificity, showing similar results to immunochromatography assays (LumiQuick, USA) for CHIKV IgM and IgG [81].

In the first week of symptoms, NAT is the method of choice. High viral load ($>1 \times 10^6$ copies/mL) in quantitative RT-PCR was observed in 80% of the patients in the series reported by Rosso and colleagues. However, this was not associated with worse outcomes, which are more likely to occur in immunocompetent patients [75]. In transplant recipients, CHIKV viremia seems to be shorter than in DENV infection [45].

Zika

In symptomatic patients, laboratory confirmation of ZIKV infection can be made through NAT and serology, but older generation immunological assays are known to exhibit cross-reactivity with other *Flaviviridae*, particularly DENV [82]. However, a new Zika NS1 blockade-of-binding ELISA was found to be 91.8% sensitive and 88.9% specific in discriminating ZIKV from DENV and can be used >10 days after

disease onset or presumed exposure, which is an advantage [83]. The definitive diagnosis is established by detection of viral nucleic acid in urine or serum, with the former being more reliable since most patients exhibit transient viremia making serum diagnosis elusive. Recent studies have shown that viral shedding in urine lasts 8–15 days in 41.4% of patients and 16–30 days in an additional 11.2% of patients [84]. Thus, detection by RT-PCR in the urine allows for a broader detection window and, per a recent study, performs better when compared to serum [85].

A method using Trioplex RT-PCR was found to detect and discriminate between ZIKV, CHIKV, and DENV, which is a great addition to the diagnostic armamentarium in tropical countries where these arboviruses commonly co-circulate. No cross-reaction or PCR inhibition was observed in the clinical samples tested [86].

In conclusion, transplant recipients with suspected arbovirus infection living in or returning from endemic areas should be tested by NAT during the acute phase of the disease. Recent developments in simultaneous multiplex RT-PCR technology have made fast, specific, and definitive diagnosis possible in geographic regions where the three arboviruses share the same biting arthropods and seasonality, with high specificity and sensitivity [86]. Serological diagnosis has been hindered by the occurrence of cross-reactivity among *Flavivirus.* The commercially available ELISAs to detect dengue IgG and/or IgM have shown low specificity when used for patients infected by ZIKV [79].

Management of DENV, CHIKV, and ZIKV Infections in Transplant Recipients

Dengue

Overall assessment includes history and physical exam, laboratory tests, and evaluation of disease severity [10]. Investigation of previous DENV infection is mandatory as seropositive patients have a greater risk of developing SD if infected with a different serotype. Based on the findings, clinicians are able to determine the phase of the disease, the presence of warning signs, and the patient's need for hospital admission (see Fig. 2) [2].

There is no specific antiviral drug to treat dengue, and generous fluid replacement is the mainstay of therapy. Freeman and colleagues summarize new developments in DENV-targeted therapy [87], including a combination of sunitinib (VEGF receptor tyrosine kinase inhibitor) and TNF-alpha inhibitors to halt the ADE phenomenon and prevent progression to SD [88]. VIS513, a pan-serotype monoclonal antibody targeting domain III of dengue E protein, has been shown to reduce viral load in the acute phase of dengue [89]. Some authors have shown that CSA can be a potential drug for the treatment of flavivirus infections [56]. On the other hand, balapiravir, celgosivir, and chloroquine have proved ineffective in trials [90–92]. Anecdotally, there are reports of successful use of IVIG to reverse capillary leak in a renal

transplant patient [61], though a randomized controlled trial in immunocompetent patients demonstrated that IVIG does not significantly change outcomes but also causes no additional harm [93]. Prospective studies to evaluate the role of IVIG in dengue are warranted in the transplant setting. Currently, there is not enough evidence to recommend decreasing or switching immunosuppression regimens in infected transplanted patients. De-escalating immunosuppressants may trigger graft rejection [7]. As mentioned previously, some authors have transiently held MMF in case of severe cytopenias, and patients on a cyclosporine (CSA) containing regimen appeared to have less severe disease [7, 58]. More studies are needed to clarify the best immunosuppressive strategy in this scenario.

Chikungunya

Treatment of acute CHIKV is mainly supportive and symptomatic. The co-administration of nonsteroidal anti-inflammatory drugs (NSAIDs) with low-dose systemic steroids has been recommended to reduce pain and improve quality of life in treating acute CHIKV with arthralgia. Aspirin should be avoided [94]. Antiviral agents and monoclonal antibodies are in initial stages of testing. Niclosamide, nitazoxanide, and suramin have shown anti-CHIKV activity [95, 96]. A combination of ribavirin and doxycycline has shown antiviral activity in vitro and in an animal model [97]. Giancotti and colleagues identified about 69 small-molecule compounds that were derived using a benzylidene-acrylohydrazide antiviral scaffold with potential direct anti-CHIKV activity in Vero cell lines [98]. A recent review describes the antigenic sites and mechanisms of action of 18 different monoclonal antibodies (mAbs) against CHIKV [99]. Other excellent reviews of potential therapeutic compounds have been recently published [100, 101]. Although promising, these compounds need to be tested in clinical trials.

Zika

So far, there is no specific FDA-licensed therapy for ZIKV infection. Treatment for uncomplicated disease includes rest, hydration, and symptomatic management for fever and arthritis. NSAIDs and aspirin should be avoided until DENV has been ruled out. Freeman and colleagues point recent advances in research toward direct antiviral therapy including specific DENV anti-E dimer epitope antibodies that cross neutralizes ZIKV in rhesus monkeys [87, 102]. Chloroquine administered to ZIKV-infected pregnant mouse models reduced vertical transmission and viral load in fetal brain. There are ongoing studies using small molecules, but there are also ethical barriers to the inclusion of pregnant women in clinical trials without extra safety precautions [103]. Another study showed in vitro therapeutic effects of atovaquone against ZIKV, particularly the Brazilian and Ugandan strains, likely via depletion of nucleotides blocking pyrimidine biosynthesis [104]. The drug is considered FDA category C in pregnancy, which makes it attractive for such vulnerable populations.

Preventive Measures

Transplant donors or recipients living in or traveling to endemic regions should receive information about arbovirus transmission and *Aedes* habits to avoid exposure. Insect bites should be minimized by application of mosquito repellent (N, N-diethyl-m-toluamide DEET, picaridin or IR3535) and protective clothing [2].

Vector Control

The backbone of dengue, zika, and chikungunya prevention is based on vector control and community programs to keep the environment free of potential breeding sources (flower vases, uncovered water storage barrels, discarded tires, etc.). The vector is well adapted to urban and domestic environments, and *Aedes* distribution maps have been shown to help predict the local, regional, and international spread of arboviruses. Local outbreaks of these arboviruses have typically followed within 5–15 years of infestation by *Aedes aegypti* and *Aedes albopictus,* emphasizing the importance of vector density control [105].

Fascinating interdisciplinary work is being done in mathematical forecasting models using climate parameters to predict the next *Aedes aegypti* mosquito season and its associated risk of arbovirus epidemics. Huber and colleagues examined the influences of seasonal temperature mean, variation, and temperature at the start of outbreaks in disease dynamics. The model projects that intermediate temperatures at about 24–25 °C produced the largest epidemics, while warmer temperatures at the start of epidemics promote more rapid outbreaks due to fast burnout of the susceptible population [106].

Another interesting line of research attempts to make mosquitos resistant to arboviruses. One study identified an insect symbiont, *Wolbachia* wStri, that was shown to inhibit by >99.9% of the replication of ZIKV, DENV, and CHIKV in *Aedes albopictus* cells. RNA transfection and metabolic labeling studies demonstrated that the replication defect is at the level of RNA translation [107]. Moretti and colleagues and Xue and colleagues are also working in *Wolbachia*-induced sterility and mathematical modeling of such strategies, respectively. This interdisciplinary work is essential in a future where climate volatility and change will expand the mosquito geographical reach and its associated diseases [108, 109].

Vaccines

The first licensed Sanofi Pasteur CYD-TDV vaccine (Dengvaxia) is a tetravalent, recombinant, live-attenuated vaccine with a yellow fever 17D vaccine virus backbone, administered in a three-dose schedule at 6-month intervals. The vaccine was initially licensed for individuals aged 9–45 years living in areas with dengue seroprevalence >70%. Pooled results from two parallel phase-3 trials showed a clear benefit in DENV-seropositive individuals, with efficacy of 81.9% [110].

However, there was less protection observed for infections caused by DENV-2 and a significant excess of dengue hospitalization in the vaccinated group aged 2–5 years (RR = 7.45; 95% CI 1.15–313.80) [110, 111]. This was also the age group with the lowest pre-vaccination seroprevalence (51%), strongly suggesting that the ADE phenomena are not merely a theoretical concern, but a real issue with live-attenuated dengue vaccines. Safety data of 4-year follow-up from the abovementioned clinical trials showed a relative risk of 1.327 (95% CI 0.56–3.48) for patients seronegative at baseline to have virologically confirmed dengue hospitalizations, irrespective of age [112].

In September 2018, the WHO published a position paper reviewing safety data for Dengvaxia. Due to the excess risk in seronegative subjects, individual pre-vaccination screening is recommended with only seropositive individuals advised to receive CYD-TDV. Alternatively, vaccination without individual screening could be considered in areas with recent documentation of seroprevalence rates of at least >80% by age 9 years. The position paper did not recommend use in immunocompromised patients due to paucity of data and suggested research priorities including highly discriminative rapid diagnostic tests (RDTs) to evaluate serostatus prior to vaccination at the point of care and the development of safe, effective, and affordable DENV vaccines that could be administered irrespective of serostatus [113].

In that regard, there is emerging research on next-generation vaccines that offer the promise of no increased ADE risk. Two candidates, DSV4 and T-mVLP, do not utilize pre-membrane (prM) and envelope domain (ED)-II epitopes, which have been shown to produce serotype cross-reactive immune responses, focusing instead on ED-III which is also serotype-specific and has been shown in mouse AG129 models to produce neutralizing antibodies and not predispose to ADE. These candidate vaccines make use of virus-like particles (VLP) delivery platforms and can be mass-produced using the yeast *Pichia pastoris.* Both candidates require clinical trials to assess their performance and safety profiles before expanding their use to the broader population and specifically to transplant recipients [114].

Vaccines against Zika and chikungunya are in fast-track development and show promise in recent studies. Prow and colleagues report on a single-vector construct SCV vaccine that encodes the structural polyprotein cassettes of both ZIKV and CHIKV. Vaccination in wild-type (WT) and interferon alpha/beta receptor (IFNAR) −/− mice induces neutralizing antibodies to both viruses that halt viremia in CHIKV and ZIKV infected mice, as well as preventing arthritis from CHIKV and placental/fetal infection in female and male testes infection from ZIKV in IFNAR −/− mice [115]. Another group reported an additional new vaccine candidate for both CHIKV and ZIKV. It is a single dose, bivalent recombinant vaccine, based on a chimeric vesicular stomatitis virus (VSV) that expresses the CHIKV envelope polyprotein E3-E2-6K-E1 in place of the VSV glycoprotein and the membrane envelope glycoproteins of ZIKV. This vaccine induced neutralizing antibodies to both CHIKV and ZIKV in WT and A129 mice [116]. No large-scale commercial vaccine is currently available, but its development appears promising. When these vaccines do become available, safety and efficacy studies in immunocompromised animal models followed by clinical trials will be necessary before recommending their use in

transplant recipients. One recently published double-blind, randomized, placebo-controlled phase-2 trial demonstrated immunogenicity, safety, and tolerability for a CHIKV-specific vaccine enrolling healthy volunteers in Austria and Germany [117].

Blood Supply Safety

There are also ongoing efforts to increase blood supply safety and prevent TT of arboviruses. Amotosalen and ultraviolet A light (UVA) have been demonstrated to inactivate ZIKV in platelet and plasma components [118]. TT-ZIKV through platelets has been reported in the past [49, 50]. Another study demonstrated the efficacy of amustaline and glutathione (S-303/GSH) for CHIKV pathogen inactivation in red blood cell concentrates [119].

Screening Recommendations and Donor Deferral

There are several international guidelines on tissue and blood donor screening [120–122]. Most of them focus on epidemiological risk factors and the risk-benefit ratio of each particular case and note the impact that unduly restrictive policies might have in the organ supply especially in tropical and resource-deprived countries. Two recent articles discuss the similar course of ZIKV in immunosuppressed as compared to immunocompetent patients as important factors for decision-makers to consider [123, 124].

Since August 2016 the FDA has a universal ZIKV NAT screening policy for the blood supply in place, initially as NAT for every individual donation that was later revised in July 2018 toward a mini-pool NAT strategy with a trigger for individual screening during local autochthonous transmission or travel-related risk [47, 125]. However, a recently published Monte Carlo simulation questions this strategy by projecting ZIKV transfusion infections and cost-effectiveness per quality-adjusted life-years (QALY) gained, showing screening was cost-effective only in the high mosquito season in Puerto Rico, and no evaluated screening policy was cost-effective in the 50 states of the US mainland. Even without screening, the simulation showed a very low risk of ZIKV complications (one GBS case every 16 years in Puerto Rico and every 84 years in US mainland). The analysis does not account for more subjective aspects of blood safety policies, like public trust in the blood supply [126].

Some guidelines suggest avoiding traveling to endemic areas 30 days prior to donation and avoiding sexual intercourse for 6 months with a male traveler returning from a region with an active outbreak of ZIKV [66].

Although viral RNA can be detected in semen more than 350 days after initial symptoms, a recent study showed that infectious ZIKV is detected up to 38 days after symptoms onset [41]. Some authors suggest ruling out acute infection in donors traveling or living in regions with active outbreaks, with organs still utilized and follow-up monitoring of recipients after transplantation [124]. Donor screening for

Action		DENV	CHIKV	ZIKV
Blood screening		No	No	According to country recommendation
Donor screening	Endemic regions	Yes, in symptomatic donors	Yes, in symptomatic donors and corneal donors (during outbreaks)	Yes, in symptomatic donors
	Non-endemic regions	No. Check recent travel and consider screen specially if symptoms suggestive of arbovirus in the last 30 days.		
Donor deferral		Discard organs from donors with documented DENV within 30 days.	Discard organs from donors with documented CHIKV within 30 days. Defer corneal donors during local CHIKV outbreaks	Defer donors with documented ZIKV for a period longer than 1 month
Diagnosis during acute phase		NAT, NS1	NAT	NAT

Fig. 3 Screening and deferral recommendations

arboviruses is not routinely recommended. However, in areas of arbovirus endemicity, screening for DENV, ZIKV, and CHIKV should be considered, particularly in symptomatic donors [127]. If screening is not feasible, donors from endemic regions should be deferred if they have a recent history of febrile illness suggestive of arbovirus in the last 30 days [121, 122]. Due to the high frequency of silent eye infection, some authors recommend screening of corneal donors for CHIKV during outbreaks [34].

The FDA has recommended a 4-week deferral for donor candidates with a recent travel history arriving from ZIKV endemic countries [125]. However, a risk analysis performed in Canada using the Monte Carlo simulation showed that a 21-day travel deferral would provide an extremely safe margin [128]. Current screening and deferral recommendations are depicted in Fig. 3.

Conclusions

Clinical manifestations of DENV, CHIKV, and ZIKV are similar in transplant recipients. Therefore, in case of suspected arbovirus infection, transplant recipients living in or returning from endemic areas should be tested by NAT during the acute phase of the disease. Recent developments in simultaneous multiplex RT-PCR technology have made fast, specific, and definitive diagnosis possible in geographic regions where the three arboviruses share the same biting arthropods and seasonality, with high specificity and sensitivity.

Serological diagnosis is currently a challenge, as recent data showed cross-reactivity of dengue tests in patients with proven ZIKV. So far, NAT is preferred in

blood bank and pretransplant donor selection, as well as in symptomatic or suspect cases after transplantation.

Among the three discussed arbovirus infections, DENV showed greater morbidity with a protracted course, prolonged viremia, and more associated complications. No specific FDA-approved treatment is available, and current vaccines can elicit ADE in previously exposed individuals, while safe vaccine alternatives are still in development. Management of arboviruses is mainly supportive, with attention to warning signs of decompensation. Reduction of immunosuppression is not recommended as it may trigger rejection or GVHD.

Key Points

- Clinical manifestations of DENV, CHIKV, and ZIKV are similar in transplant recipients, and laboratory corroboration is necessary.
- NAT is preferred in the acute phase of the disease. DENV and ZIKV serology may cross-react.
- DENV has a more aggressive course than ZIKV and CHIKV with more complications and prolonged viremia. Management of DENV is mainly supportive, with attention to warning signs of decompensation.
- Wise management of immunosuppression is paramount. Avoid abrupt reduction.
- No specific treatment is currently available. DENV vaccine is not recommended in transplant recipients. CHIKV and ZIKV vaccines are in development.
- Screening and deferral recommendations are still controversial and vary among endemic and non-endemic regions.

References

1. Higuera A, Ramírez JD. Molecular epidemiology of dengue, yellow fever, Zika and chikungunya arboviruses: an update. Acta Trop. 2019;190:99–111.
2. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control: new edition. Geneva: WHO; 2009.
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
4. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.
5. Pandey N, Jain A, Garg RK, Kumar R, Agrawal OP, Lakshmana Rao PV. Serum levels of IL-8, IFNγ, IL-10, and TGF β and their gene expression levels in severe and non-severe cases of dengue virus infection. Arch Virol. 2015;160(6):1463–75.
6. Chen LH, Wilson ME. Update on non-vector transmission of dengue: relevant studies with Zika and other flaviviruses. Trop Dis Travel Med Vaccines. 2016;2(1):15.
7. Nasim A, Anis S, Baqi S, Akhtar SF, Baig-Ansari N. Clinical presentation and outcome of dengue viral infection in live-related renal transplant recipients in Karachi, Pakistan. Transpl Infect Dis. 2013;15(5):516–25.

8. Weerakkody R, Palangasinghe D, Dalpatadu KP, Rankothkumbura J, Cassim MR, Karunanayake P. Dengue fever in a liver-transplanted patient: a case report. J Med Case Rep. 2014;8(1):378. Available from: http://www.jmedicalcasereports.com/content/8/1/378
9. Machado CM. Transplant infections [internet]. In: Ljungman P, Snydman D, Boeckh M, editors. 4th ed. Cham: Springer International Publishing; 2016. p. 129–50. Available from: https://doi.org/10.1007/978-3-319-28797-3
10. Weerakkody RM, Patrick JA, Sheriff MHR. Dengue fever in renal transplant patients: a systematic review of literature. BMC Nephrol. 2017;18(1):15.
11. Fernandes PFCBC, Siqueira RA, Girão ES, Siqueira RA, Mota MU, Marques LCBF, et al. Dengue in renal transplant recipients: clinical course and impact on renal function. World J Transplant. 2017;7(1):57.
12. de Souza Pereira BB, Darrigo Junior LG, de Mello Costa TC, Felix AC, Simoes BP, Stracieri AB, et al. Prolonged viremia in dengue virus infection in hematopoietic stem cell transplant recipients and patients with hematological malignancies. Transpl Infect Dis. 2017;19(4): e12721.
13. Busch MP, Linnen JM, Vinelli E, Sabino EC, Tobler LH, Hyland C, et al. Dengue viremia in blood donors from Honduras, Brazil, and Australia. Transfusion. 2008;48(7):1355–62.
14. Dias LL, Amarilla AA, Poloni TR, Covas DT, Aquino VH, Figueiredo LTM. Detection of dengue virus in sera of Brazilian blood donors. Transfusion. 2012;52(8):1667–71.
15. Levi JE, Nishiya A, Félix AC, Salles NA, Sampaio LR, Hangai F, et al. Real-time symptomatic case of transfusion-transmitted dengue. Transfusion. 2015;55(5):961–4.
16. Rigau-Pérez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994–1995. Am J Trop Med Hyg. 2001;64(1–2):67–74.
17. Punzel M, Korukluoglu G, Caglayik DY, Menemenlioglu D, Bozdag SC, Tekgündüz E, et al. Dengue virus transmission by blood stem cell donor after travel to Sri Lanka. Emerg Infect Dis. 2014;20(8):1366–9.
18. Tan FL-SS, Loh DLSK, Prabhakaran K, Tambyah PA, Yap H-K. Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant. 2005;20(2):447–8.
19. Saigal S, Choudhary NS, Saraf N, Kataria S, Mohanka R, Soin A. Transmission of dengue virus from a donor to a recipient after living donor liver transplantation. Liver Transpl. 2013;19:1413–4.
20. Gupta RK, Gupta G, Chorasiya VK, Bag P, Shandil R, Bhatia V, et al. Dengue virus transmission from living donor to recipient in liver transplantation: a case report. J Clin Exp Hepatol. 2016;6(1):59–61.
21. Rosso F, Pineda JC, Sanz AM, Cedano JA, Caicedo LA. Transmission of dengue virus from deceased donors to solid organ transplant recipients: case report and literature review. Braz J Infect Dis. 2018;22(1):63–9.
22. Cedano JA, Mora BL, Parra-Lara LG, Manzano-Nuñez R, Rosso F. A scoping review of transmission of dengue virus from donors to recipients after solid organ transplantation. Trans R Soc Trop Med Hyg. 2019;74:625–34.
23. Shaji Mathew J, Menon VP, Menon VP, Mallick S, Sivasankara Pillai Thankamony Amma B, Balakrishnan D, et al. Dengue virus transmission from live donor liver graft. Am J Transplant. 2019;19(6):1838–46.
24. Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. N Engl J Med. 2015;372(13):1231–9.
25. Robison MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–53. I. Clinical features. Trans R Soc Trop Med Hyg. 1955;49(1):28–32.
26. Morens DM, Fauci AS. Chikungunya at the door – déjà vu all over again? N Engl J Med. 2014;371(10):885–7.
27. Borgherini G, Poubeau P, Staikowsky F, Lory M, Moullec NL, Becquart JP, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin Infect Dis. 2007;44(11):1401–7.
28. Nhan T-X, Claverie A, Roche C, Teissier A, Colleuil M, Baudet J-M, et al. Chikungunya virus imported into French Polynesia, 2014. Emerg Infect Dis. 2014;20(10):1773–4.

29. Rodrigues Faria N, Lourenço J, Marques de Cerqueira E, Maia de Lima M, Pybus O, Carlos Junior Alcantara L. Epidemiology of chikungunya virus in Bahia, Brazil, 2014–2015. PLoS Curr. 2016;8:2014–5.
30. da Cunha RV, Trinta KS. Chikungunya virus: clinical aspects and treatment – a review. Mem Inst Oswaldo Cruz. 2017;112(8):523–31.
31. Pan American Health Organization; World Health Organization. Number of reported cases of chikungunya fever in the Americas, vol. 2017. Washington, DC: Pan American Health Organization; 2016.
32. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Monitoramento dos casos de arboviroses urbanas transmitidas pelo Aedes (dengue, chikungunya e Zika) até a Semana Epidemiológica 12 de 2019 [internet], vol 50. 2019. Available from: http://portalarquivos2.saude.gov.br/images/pdf/2019/abril/30/2019-013-Monitoramento-dos-casos-de-arboviroses-urbanas-transmitidas-pelo-Aedes-publicacao.pdf
33. Appassakij H, Promwong C, Rujirojindakul P, Wutthanarungsan R, Silpapojakul K. The risk of blood transfusion-associated chikungunya fever during the 2009 epidemic in Songkhla Province, Thailand. Transfusion. 2014;54(8):1945–52.
34. Couderc T, Gangneux N, Chretien F, Caro V, Le Luong T, Ducloux B, et al. Chikungunya virus infection of corneal grafts. J Infect Dis. 2012;206(6):851–9.
35. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. N Engl J Med. 2016;374(16):1552–63.
36. Hills SL, Fischer M, Petersen LR. Epidemiology of Zika virus infection. J Infect Dis. 2017;216(Suppl 10):S868–74. Table 2.
37. Duffy MR, Chen T-H, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536–43.
38. Kleber de Oliveira W, Cortez-Escalante J, WTGH DO, GMI d C, CMP H, Coelho GE, et al. Increase in reported prevalence of microcephaly in infants born to women living in areas with confirmed Zika virus transmission during the first trimester of pregnancy – Brazil, 2015. MMWR Morb Mortal Wkly Rep. 2016;65(9):242–7.
39. Lowe R, Barcellos C, Brasil P, Cruz OG, Honório NA, Kuper H, et al. The Zika virus epidemic in Brazil: from discovery to future implications. Int J Environ Res Public Health. 2018;15(1):pii: E96.
40. Barzon L, Percivalle E, Pacenti M, Rovida F, Zavattoni M, Del Bravo P, et al. Virus and antibody dynamics in travelers with acute Zika virus infection. Clin Infect Dis. 2018;66(8):1173–1180. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29300893
41. Medina FA, Torres G, Acevedo J, Fonseca S, Casiano L, De León-Rodríguez CM, et al. Duration of the presence of infectious Zika virus in semen and serum. J Infect Dis. 2019;219(1):31–40.
42. Chen J, Yang YF, Chen J, Zhou X, Dong Z, Chen T, et al. Zika virus infects renal proximal tubular epithelial cells with prolonged persistency and cytopathic effects. Emerg Microbes Infect. 2017;6(8):e77.
43. Nogueira ML, Estofolete CF, ACB T, do Vale EPB M, de Cássia R, da Silva RF, et al. Zika virus infection and solid organ transplantation: a new challenge. Am J Transplant. 2016;17(3):791–5. https://doi.org/10.1111/ajt.14047.
44. Schwartzmann PV, Ramalho LNZ, Neder L, Vilar FC, Ayub-Ferreira SM, Romeiro MF, et al. Zika virus meningoencephalitis in an immunocompromised patient. Mayo Clin Proc. 2017;92(3):460–6.
45. Machado CM, Pereira BB, Felix AC, Oliveira MC, Darrigo LG, de Souza MP, et al. Zika and chikungunya virus infections in hematopoietic stem cell transplant recipients and oncohematological patients. Blood Adv. 2017;1(10):624–7.
46. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill. 2014;19(14):pii: 20761.
47. Kuehnert MJ, Basavaraju SV, Moseley RR, Pate LL, Galel SA, Williamson PC, et al. Screening of blood donations for Zika virus infection – Puerto Rico, April 3–June 11, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(24):627–8.

48. Slavov SN, Hespanhol MR, Rodrigues ES, Levi JE, Ubiali EMA, Covas DT, et al. Zika virus RNA detection in asymptomatic blood donors during an outbreak in the northeast region of São Paulo State, Brazil, 2016. Transfusion. 2017;57(12):2897–901.
49. Barjas-Castro ML, Angerami RN, Cunha MS, Suzuki A, Nogueira JS, Rocco IM, et al. Probable transfusion–transmitted Zika virus in Brazil. Transfusion [Internet]. 2016, 56(7):1684-1688. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27329551
50. Motta IJF, Spencer BR, Cordeiro da Silva SG, Arruda MB, Dobbin JA, Gonzaga YBM, et al. Evidence for transmission of Zika virus by platelet transfusion. N Engl J Med. 2016;375(11):1101–3.
51. Alfaro A, Guzman MG, Martinez E, Pizarro D, Pleites E, Gutierrez FH, et al. Dengue: guidelines for patient care in the region of the Americas [internet]. 2nd ed. Washington, DC: Pan American Health Organization (PAHO), World Health Organization (WHO); 2016. 136 p. Available from: http://iris.paho.org/xmlui/handle/123456789/31207
52. Park SB, Ryu SY, Jin KB, Hwang EA, Han SY, Kim HT, et al. Acute colitis associated with dengue fever in a renal transplant recipient. Transplant Proc. 2008;40(7):2431–2.
53. Barroso KSN, Kaufman J, Brunetta DM, de Carvalho Araújo FM, Barroso-Duarte F. Dengue encephalitis in allogenic hematopoietic stem cell transplantation recipient. Bone Marrow Transplant. 2017;52(10):1455–6.
54. Petersen LR, Tomashek KM, Biggerstaff BJ. Estimated prevalence of dengue viremia in Puerto Rican blood donations, 1995 through 2010. Transfusion. 2012;52(8):1647–51.
55. Guilarde AO, Turchi MD, Siqueira JB, Feres VCR, Rocha B, Levi JE, et al. Dengue and dengue hemorrhagic fever among adults: clinical outcomes related to viremia, serotypes, and antibody response. J Infect Dis. 2008;197(6):817–24.
56. Qing M, Yang F, Zhang B, Zou G, Robida JM, Yuan Z, et al. Cyclosporine inhibits flavivirus replication through blocking the interaction between host cyclophilins and viral NS5 protein. Antimicrob Agents Chemother. 2009;53(8):3226–35.
57. Cox J, Mota J, Sukupolvi-Petty S, Diamond MS, Rico-Hesse R. Mosquito bite delivery of dengue virus enhances immunogenicity and pathogenesis in humanized mice. J Virol. 2012;86(14):7637–49.
58. Pinsai S, Kiertiburanakul S, Watcharananan SP, Kantachuvessiri S, Boongird S, Bruminhent J. Epidemiology and outcomes of dengue in kidney transplant recipients: a 20-year retrospective analysis and comparative literature review. Clin Transpl. 2019;33(1):e13458.
59. Costa SD, Bezerra da Silva G, Jacinto CN, Mesquita Martiniano LV, Amaral YS, Villar Nogueira Paes FJ, et al. Dengue fever among renal transplant recipients: a series of 10 cases in a tropical country. Am J Trop Med Hyg. 2015;93(2):394–6.
60. Maia SHF, Brasil IRC, Esmeraldo RDM, Da Ponte CN, Costa RCS, Lira RA, et al. Severe dengue in the early postoperative period after kidney transplantation: two case reports from Hospital Geral de Fortaleza. Rev Soc Bras Med Trop. 2015;48(6):783–5.
61. Subbiah A, Bagchi S, Bhowmik D, Mahajan S, Yadav RK, Chhabra Y, et al. Dengue fever in renal allograft recipients: clinical course and outcome. Transpl Infect Dis. 2018;20(3):e12875.
62. Tan LH, Lum LCS, Omar SFS, Kan FK. Hemophagocytosis in dengue: comprehensive report of six cases. J Clin Virol. 2012;55(1):79–82.
63. Ray U, Dutta S, Mondal S, Bandyopadhyay S. Severe dengue due to secondary hemophagocytic lymphohistiocytosis: a case study. IDCases. 2017;8:50–3.
64. Chung SM, Song JY, Kim W, Choi MJ, Jeon JH, Kang S, et al. Dengue-associated hemophagocyticlymphohistiocytosis in an adult. Medicine. 2017;96(8):e6159.
65. Messina C, Zecca M, Fagioli F, Rovelli A, Giardino S, Merli P, et al. Outcomes of children with hemophagocytic lymphohistiocytosis given allogeneic hematopoietic stem cell transplantation in Italy. Biol Blood Marrow Transplant. 2018;24(6):1223–31.
66. Morris MI, Grossi P, Nogueira ML, Azevedo LS. Arboviruses recommendations for solid-organ transplant recipients and donors. Transplantation. 2018;102(2S Suppl 2):S42–51.
67. Paixão ES, Rodrigues LC, Costa M d CN, Itaparica M, Barreto F, Gérardin P, et al. Chikungunya chronic disease: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg. 2018;112(7):301–16.

68. McCarthy MK, Morrison TE. Chronic chikungunya virus musculoskeletal disease: what are the underlying mechanisms? Future Microbiol. 2016;11(3):331–4.
69. Amaral J, Taylor P, Teixeira M, Morrison T, Schoen R. The clinical features, pathogenesis and methotrexate therapy of chronic chikungunya arthritis. Viruses. 2019;11(3):289.
70. Renault P, Solet J-L, Sissoko D, Balleydier E, Larrieu S, Filleul L, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005–2006. Am J Trop Med Hyg. 2007;77(4):727–31.
71. Gérardin P, Couderc T, Bintner M, Tournebize P, Lémant J, Boisson V, et al. Chikungunya virus-associated encephalitis: a cohort study on La Réunion Island, 2005–2009. Neurology. 2016;86(1):94–102.
72. Dalla Gasperina D, Balsamo ML, Garavaglia SD, Rovida F, Baldanti F, Grossi PA. Chikungunya infection in a human immunodeficiency virus-infected kidney transplant recipient returning to Italy from the Dominican Republic. Transpl Infect Dis. 2015;9:876–9.
73. Pierrotti LC, Lopes MIBF, Nascimento APD, Caiaffa-Filho H, Lemos FBC, Reusing JO, et al. Chikungunya in kidney transplant recipients: a series of cases. Int J Infect Dis. 2017;64:96–9.
74. Girão ES, Rodrigues Dos Santos BG, do Amaral ES, Costa PEG, Pereira KB, de Araujo Filho AH, et al. Chikungunya infection in solid organ transplant recipients. Transplant Proc. 2017;49(9):2076–81.
75. Rosso F, Rodríguez S, Cedano JA, Mora BL, Moncada PA, Velez JD. Chikungunya in solid organ transplant recipients, a case series and literature review. Transpl Infect Dis. 2018;20(6):e12978.
76. Tavares BM, Fernandes PF, Oliveira CMC, Silva SL, Mota MU, Andrade TH, et al. Chikungunya on kidney transplant recipients: is it the same? Transplantation. 2019;103(2):441–5.
77. Lannuzel A, Fergé J-L, Lobjois Q, Signate A, Rozé B, Tressières B, et al. Long-term outcome in neuroZika: when biological diagnosis matters. Neurology. 2019;92(21):e2406–e2420.
78. Keasey SL, Pugh CL, Jensen SMR, Smith JL, Hontz RD, Durbin AP, et al. Antibody responses to Zika virus infections in environments of flavivirus endemicity. Clin Vaccine Immunol. 2017;24(4):1–16.
79. Felix AC, Souza NCS, Figueiredo WM, Costa AA, Inenami M, da Silva RMG, et al. Cross reactivity of commercial anti-dengue immunoassays in patients with acute Zika virus infection. J Med Virol. 2017;89(8):1477–9.
80. Mascarenhas M, Garasia S, Berthiaume P, Corrin T, Greig J, Ng V, et al. A scoping review of published literature on chikungunya virus. PLoS One. 2018;13:e0207554.
81. Fumagalli MJ, De Souza WM, Espósito DLA, Silva A, Romeiro MF, Martinez EZ, et al. Enzyme-linked immunosorbent assay using recombinant envelope protein 2 antigen for diagnosis of chikungunya virus. Virol J. 2018;15(1):1–7.
82. Landry ML, George KS. Laboratory diagnosis of Zika virus infection. Arch Pathol Lab Med. 2017;141(1):60–7.
83. Balmaseda A, Stettler K, Medialdea-Carrera R, Collado D, Jin X, Zambrana JV, et al. Antibody-based assay discriminates Zika virus infection from other flaviviruses. Proc Natl Acad Sci U S A. 2017;114(31):8384–9.
84. Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, et al. Persistence of Zika virus in body fluids – final report. N Engl J Med. 2018;379(13):1234–43.
85. del Pilar Martinez Viedma M, Puri V, Oldfield LM, Shabman RS, Tan GS, Pickett BE. Optimization of qRT-PCR assay for Zika virus detection in human serum and urine. Virus Res. 2019;263:173–8.
86. Santiago GA, Vázquez J, Courtney S, Matías KY, Andersen LE, Colón C, et al. Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses. Nat Commun. 2018;9(1):1391.
87. Freeman MC, Coyne CB, Green M, Williams JV, Silva LA. Emerging arboviruses and implications for pediatric transplantation: a review. Pediatr Transplant. 2019;23(1):1–12.
88. Branche E, Tang WW, Viramontes KM, Young MP, Sheets N, Joo Y, et al. Synergism between the tyrosine kinase inhibitor sunitinib and anti-TNF antibody protects against lethal dengue infection. Antivir Res. 2018;158:1–7.

89. Budigi Y, Ong EZ, Robinson LN, Ong LC, Rowley KJ, Winnett A, et al. Neutralization of antibody-enhanced dengue infection by VIS513, a pan serotype reactive monoclonal antibody targeting domain III of the dengue E protein. PLoS Negl Trop Dis. 2018;12(2):1–20.
90. ricou V, Minh NN, Van TP, Lee SJ, Farrar J, Wills B, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLoS Negl Trop Dis. 2010;4(8): e785.
91. Nguyen NM, Tran CNB, Phung LK, Duong KTH, Huynh HLA, Farrar J, et al. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. J Infect Dis. 2013;207(9):1442–50.
92. Low JG, Sung C, Wijaya L, Wei Y, Rathore APS, Watanabe S, et al. Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. Lancet Infect Dis. 2014;14(8):706–15.
93. Inoue S, Natividad FF, Alonzo MTG, Saito M, Honda S, Kumaori A, et al. Lack of efficacy of high-dose intravenous immunoglobulin treatment of severe thrombocytopenia in patients with secondary dengue virus infection. Am J Trop Med Hyg. 2018;77(6):1135–8.
94. Silva JVJ, Ludwig-Begall LF, Oliveira-Filho EF, Oliveira RAS, Durães-Carvalho R, Lopes TRR, et al. A scoping review of chikungunya virus infection: epidemiology, clinical characteristics, viral co-circulation complications, and control. Acta Trop. 2018;188:213–24.
95. Ho YJ, Wang YM, Lu JW, Wu TY, Lin LI, Kuo SC, et al. Suramin inhibits chikungunya virus entry and transmission. PLoS One. 2015;10(7):1–18.
96. Wang Y-M, Lu J-W, Lin C-C, Chin Y-F, Wu T-Y, Lin L-I, et al. Antiviral activities of niclosamide and nitazoxanide against chikungunya virus entry and transmission. Antivir Res. 2016;135:81–90.
97. Rothan HA, Bahrani H, Mohamed Z, Teoh TC, Shankar EM, Rahman NA, et al. A combination of doxycycline and ribavirin alleviated chikungunya infection. PLoS One. 2015;10(5):1–15.
98. Giancotti G, Cancellieri M, Balboni A, Giustiniano M, Novellino E, Delang L, et al. Rational modifications on a benzylidene–acrylohydrazide antiviral scaffold, synthesis and evaluation of bioactivity against chikungunya virus. Eur J Med Chem. 2018;149:56–68.
99. Jin J, Simmons G. Antiviral functions of monoclonal antibodies against chikungunya virus. Viruses. 2019;11(4):305.
100. Abdelnabi R, Neyts J, Delang L. Chikungunya virus infections: time to act, time to treat. Curr Opin Virol. 2017;24:25–30.
101. Powers AM. Vaccine and therapeutic options to control chikungunya virus. Clin Microbiol Rev. 2018;31(1):1–29.
102. Abbink P, Larocca RA, Dejnirattisai W, Peterson R, Nkolola JP, Borducchi EN, et al. Therapeutic and protective efficacy of a dengue antibody against Zika infection in rhesus monkeys. Nat Med. 2018;24(6):721–3.
103. Shiryaev SA, Mesci P, Pinto A, Fernandes I, Sheets N, Shresta S, et al. Repurposing of the antimalaria drug chloroquine for Zika virus treatment and prophylaxis. Sci Rep. 2017;7(1):1–9.
104. Cifuentes Kottkamp A, De Jesus E, Grande R, Brown JA, Jacobs AR, Lim JK, et al. Atovaquone inhibits arbovirus replication through the depletion of intracellular nucleotides. J Virol. 2019;93(11):e00389–19.
105. Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nat Microbiol. 2019;4(5):854–63.
106. Huber JH, Childs ML, Caldwell JM, Mordecai EA. Seasonal temperature variation influences climate suitability for dengue, chikungunya, and Zika transmission. PLoS Negl Trop Dis. 2018;12(5):1–20.
107. Schultz MJ, Tan AL, Gray CN, Isern S, Michael SF, Frydman HM, et al. Wolbachia wStri blocks Zika virus growth at two independent stages of viral replication. MBio. 2018;9(3):1–15.
108. Moretti R, Yen P, Houé V, Lampazzi E, Desiderio A, Failloux A, et al. Combining Wolbachia-induced sterility and virus protection to fight *Aedes albopictus*-borne viruses. PLoS Negl Trop Dis. 2018;12(7):e0006626.

109. Xue L, Fang X, Hyman JM. Comparing the effectiveness of different strains of Wolbachia for controlling chikungunya, dengue fever, and Zika. PLoS Negl Trop Dis. 2018;12(7):1–24.
110. Wichmann O, Vannice K, Asturias EJ, de Albuquerque Luna EJ, Longini I, Lopez AL, et al. Live-attenuated tetravalent dengue vaccines: the needs and challenges of post-licensure evaluation of vaccine safety and effectiveness. Vaccine. 2017;35(42):5535–42.
111. Swaminathan S, Khanna N. Dengue vaccine development: global and Indian scenarios. Int J Infect Dis. 2019;84S:S80–6.
112. Arredondo-García JL, Hadinegoro SR, Reynales H, Chua MN, Rivera Medina DM, Chotpitayasunondh T, et al. Four-year safety follow-up of the tetravalent dengue vaccine efficacy randomized controlled trials in Asia and Latin America. Clin Microbiol Infect. 2018;24(7):755–63.
113. WHO. Background paper on dengue vaccines – Strategic Advisory Group of Experts on immunization meeting [internet]. Geneva: WHO SAGE; 2018. p. 165–226. Available from: http://www.who.int/immunization/sage/meetings/2018/april
114. Shukla R, Ramasamy V, Rajpoot RK, Arora U, Poddar A, Ahuja R, et al. Next generation designer virus-like particle vaccines for dengue. Expert Rev Vaccines. 2019;18(2):105–17.
115. Prow NA, Liu L, Nakayama E, Cooper TH, Yan K, Eldi P, et al. A vaccinia-based single vector construct multi-pathogen vaccine protects against both Zika and chikungunya viruses. Nat Commun. 2018;9(1):1230.
116. Chattopadhyay A, Aguilar PV, Bopp NE, Yarovinsky TO, Weaver SC, Rose JK. A recombinant virus vaccine that protects against both chikungunya and Zika virus infections. Vaccine. 2018;36(27):3894–900.
117. Reisinger EC, Tschismarov R, Beubler E, Wiedermann U, Firbas C, Loebermann M, et al. Immunogenicity, safety, and tolerability of the measles-vectored chikungunya virus vaccine MV-CHIK: a double-blind, randomised, placebo-controlled and active-controlled phase 2 trial. Lancet. 2019;392(10165):2718–27.
118. Santa Maria F, Laughhunn A, Lanteri MC, Aubry M, Musso D, Stassinopoulos A. Inactivation of Zika virus in platelet components using amotosalen and ultraviolet A illumination. Transfusion. 2017;57(8):2016–25.
119. Aubry M, Laughhunn A, Santa Maria F, Lanteri MC, Stassinopoulos A, Musso D. Amustaline (S-303) treatment inactivates high levels of chikungunya virus in red-blood-cell components. Vox Sang. 2018;113(3):232–41.
120. Silveira FP, Campos SV. The Zika epidemics and transplantation. J Heart Lung Transplant. 2016;35(5):560–3.
121. Darrigo LG, de Sant'Anna Carvalho AM, Machado CM. Chikungunya, dengue, and Zika in immunocompromised hosts. Curr Infect Dis Rep. 2018;20(4):5.
122. Clemente WT, Pierrotti LC, Abdala E, Morris MI, Azevedo LS, López-Vélez R, et al. Recommendations for management of endemic diseases and travel medicine in solid-organ transplant recipients and donors: Latin America. Transplantation. 2018;102(2):193–208.
123. Aguilar C, Husain S, Lortholary O. Recent advances in understanding and managing infectious diseases in solid organ transplant recipients. F1000Res. 2018;7:661.
124. Grossi PA. Donor-derived infections, lessons learnt from the past, and what is the future going to bring us. Curr Opin Organ Transplant. 2018;23(4):417–22.
125. Food and Drug Administration – FDA. Revised recommendations for reducing the risk of Zika virus transmission by blood and blood components; guidance for industry [internet]. 2018. Available from: https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guid
126. Russell WA, Stramer SL, Busch MP, Custer B. Screening the blood supply for Zika virus in the 50 U.S. states and Puerto Rico: a cost-effectiveness analysis. Ann Intern Med. 2019, 170(3):164–174. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30615781
127. Nam H, Nilles KM, Levitsky J, Ison MG. Donor-derived viral infections in liver transplantation. Transplantation. 2018;102(11):1824–1836. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29979345
128. Katz LM, Rossmann SN. Zika and the blood supply: a work in progress. Arch Pathol Lab Med. 2017;141(1):85–92.

Yellow Fever: Prevention in Transplant Candidates and Emerging Treatment Data for Liver Transplant

42

Alice Tung Wan Song and Luiz Augusto Carneiro D'Albuquerque

Contents

Introduction 1011
Epidemiology 1011
Prevention in Transplant Candidates 1016
Hematological Malignancies and HSCT Candidates 1017
Kidney Transplant Candidates 1018
Liver Transplant Candidates 1018
Liver Transplantation as an Emerging Treatment Option 1019
Conclusions 1021
Key Points 1021
References 1021

Introduction

Epidemiology

Yellow fever (YF) is caused by a single-strand RNA virus of the genus *Flavivirus* and is transmitted to humans by blood-feeding mosquitoes mainly belonging to the genera *Haemagogus* and *Sabethes* (in the Americas) and the genera *Aedes* (in Africa and the Americas), respectively [1]. YF is often called the "original" hemorrhagic fever. The sylvatic cycle consists of transmission through the bite of

A. T. W. Song (✉) · L. A. Carneiro D'Albuquerque
Disciplina de Transplante de Figado e Orgaos do Aparelho Digestivo, Departamento de Gastroenterologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Liver Surgery and Transplantation Laboratory LIM37, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
e-mail: alicetwsong@gmail.com; alice.song@hc.fm.usp.br; profluizcarneiro@gmail.com

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_41

a mosquito that has previously fed on a viremic primate, and urban YF occurs through interhuman transmission. In the Americas, YF is transmitted by *Haemagogus* mosquitoes in the jungle environment (sylvatic cycle) and *Aedes aegypti* in urban settings (urban cycle), whereas in Africa, transmission to humans occurs via *Aedes* spp.

There is an estimated occurrence of 200,000 cases of YF cases worldwide annually with 30,000 deaths [2], with 90% of cases occurring in Africa. Thirty-four countries in Africa and 13 countries in Central and South America have regions that are endemic for YF. In South America, the most affected countries are Peru, Bolivia, Brazil, and Colombia.

There are three conditions that must be present in order to result in a YF epidemic: the introduction of the virus in a susceptible population, the presence of competent mosquito vectors, and inadequate prevention strategies (vector control and immunization of human populations) [3].

In Africa, the epidemiology is commonly mixed due to a high density of vector mosquito populations in close proximity to large unvaccinated populations, thus provoking far greater epidemics than in South America [1, 4]. A large urban outbreak occurred in 2015 in Angola and subsequently spread to the Democratic Republic of Congo, with 961 confirmed cases and 137 deaths [5]. In Asia, the only reported cases were of 11 Chinese workers who had been infected in Angola and were therefore imported cases [6]. The potential international spread to countries with susceptible populations and presence of mosquito vectors is of great concern. It has been recently reported that the YF virus has also been detected in *Aedes albopictus* [7].

In South America, the possible explanations for the recent resurgence in YF include low vaccine coverage, migration of susceptible individuals to forested endemic regions, and increasing urbanization [4]. In Brazil, after the major epidemic of urban yellow fever in the late 1930s, cases were limited to endemic areas such as the Amazonian states, with a few outbreaks in the southeast (Minas Gerais and São Paulo in 2002 and 2008, respectively) and the south (Paraná and Rio Grande do Sul in 2008) [8]. Since 2014, there have been epizootics in monkeys, and between December 2016 and May 2018, there have been 2050 confirmed YF cases with 681 deaths (case fatality rate of 33.2%) [9]. It was demonstrated through phylogenetic and phylodynamic tools that the outbreak originated in Northeastern Brazil, moving southward to areas of unvaccinated populations, at a rapid rate of 4.25 km per day [10].

During the Angola and the Democratic Republic of Congo outbreak, the novel strategy EYE (Eliminating Yellow fever Epidemics) began, as an initiative of the World Health Organization, and aims to eliminate yellow fever by 2026.

Clinical Manifestations

The incubation period following mosquito bite ranges from 3 to 9 days. The clinical presentation of YF ranges from a subclinical infection to severe systemic disease. The majority of patients are asymptomatic.

In symptomatic patients, there are typically three phases of disease. The majority of patients present only the first phase, in which viremia is present. This phase may be subclinical or characterized by fever, malaise, generalized myalgia, nausea, vomiting, irritability, insomnia, and prostration [4]. Laboratory abnormalities include thrombocytopenia, leukopenia, and elevated serum transaminase levels. The second phase is not always present and is characterized by up to 48 h of remission of symptoms. The third phase occurs in approximately 15% of cases – using a Bayesian model, one study estimated that for every severe YF case identified, there may be up to 70 infections that are either asymptomatic or oligosymptomatic [11]. The severe cases are characterized by nausea, vomiting, mild jaundice, and coagulopathy, with involvement of various organs and systems, notably severe liver damage with encephalopathy and/or seizures, cardiovascular dysfunction with bradycardia or arrhythmias, circulatory shock, and varying degrees of acute kidney injury [4, 12]. Hemorrhagic manifestations include epistaxis, mucosal bleeding, hematemesis, and catheter-site bleeding. In convalescent individuals, the elevation of bilirubin is delayed and is seen approximately 2–3 weeks after onset of symptoms.

Case fatality rates in severe forms range from 20 to 60%. The few studies on prognostic factors demonstrated that serum aminotransferase and bilirubin levels were independently associated with higher mortality [13]. Also renal failure, notably blood urea nitrogen levels above 100 mg/mL, was associated with a higher risk of death [13]. Overall, case fatality rates are lower in Africa (approximately 20%) compared to South America (30–60%), suggesting that there may be genetic factors that determine prognosis [1].

Diagnosis

YF diagnosis may be confirmed by virologic or serologic tests [14], as clinical symptoms generally overlap with a wide range of diseases, including dengue, leptospirosis, viral hepatitis, and malaria [15].

Virologic tests include detection of viral RNA in serum samples via molecular methods such as conventional or real-time reverse transcription polymerase chain reaction (RT-PCR), with confirmation of YF infection given by a positive result [14], viral isolation (rarely used because of its complexity), and immunohistochemistry in tissues, commonly performed in necropsy samples, although molecular diagnostics can also be performed in tissue samples. In the past, it was believed that YF viremia only lasted for the first 5 days of the illness [16], but more recent studies using molecular tools have shown that the virus can still be detected in blood up to 20 days after symptom onset in more severe cases [17–19]. It has also been demonstrated that viral RNA was still present in urine and semen samples up to 21 days after symptom onset [20].

Serologic tests may be performed by ELISA or plaque reduction neutralization test (PRNT) [21]. Both techniques may present cross-reactivity with other flavivirus diseases, such as dengue and Zika, especially in areas of high co-circulation with these viruses. Prior immunity to dengue virus is the most frequent confounder [22]. Moreover, serology is unable to differentiate between vaccine-induced

immunity and post-disease immunity. There are no validated ELISA commercial kits for detection of YF IgM. The confirmation requires seroconversion in samples of an interval of at least 1 week or a fourfold increase in IgG testing in acute and convalescent samples [21, 22].

In fatal cases, diagnosis can be confirmed by molecular diagnostics of tissue or immunohistochemistry [14]. YF virus has been isolated from the liver, spleen, kidneys, lungs, heart, and brain of symptomatic patients [23, 24].

Prevention

Vector control and immunization are the mainstay of prevention strategies for YF control. As has previously been seen with dengue, chikungunya, and Zika, *Aedes aegypti*-mediated epidemics can spread rapidly in unvaccinated populations. A recent study estimated that between 393.7 million and 472.9 million people still require YF vaccination in areas at risk of disease transmission in order to achieve the 80% coverage threshold recommended by the World Health Organization (WHO), representing between only 43% and 52% of the population in these risk zones [25].

The Asibi strain of YF virus was successfully attenuated, resulting in the first vaccine developed in the 1930s [26]. There were two vaccines – the 17D vaccine and the French neurotropic vaccine. Production of the latter was interrupted in 1982 due to high rates of adverse neurological events. Nowadays, there are the 17DD (manufactured in Brazil) and the 17D-204 strain vaccine (YF-VAX, manufactured by Sanofi Pasteur, Swiftwater, Pennsylvania), with similar immune responses [27]. In clinical trials, 80–100% of vaccinated individuals develop protective immunity within 10 days [28] and 99% within 30 days [29].

YF vaccine is recommended for persons aged $\geq$9 months who are traveling to or living in areas at risk for YF transmission [26]. In April 2013, the WHO Strategic Advisory Group of Experts on Immunization concluded that a single primary dose of YF vaccine is sufficient to confer sustained immunity and lifelong protection, with no need for a booster dose every 10 years, as previously recommended [30]. In 2015, a committee of experts issued recommendations for certain populations who would benefit from YF vaccine booster doses [31]: women who were pregnant at receipt of first dose; HSCT recipients when sufficiently immunocompetent to be revaccinated; people infected with human immunodeficiency virus; travelers in high-risk settings, including those planning on spending a prolonged period in highly endemic areas; and laboratory workers of wild-type YF virus laboratories.

After YF vaccine administration, recipients usually develop low-level viremia within 3–7 days, which persists for 1–3 days, followed by the development of YF virus IgM antibodies [32]. Although the viremia is low, it is high enough to be transmitted through blood products [33], as reported in 2010, when five patients received six blood products from individuals who had been vaccinated for YF 4 days prior to donation. One patient died without further information regarding viremia or serologic testing, and of the 4 other blood product recipients, all were asymptomatic with no laboratory abnormalities compatible with YF infection. Three of these

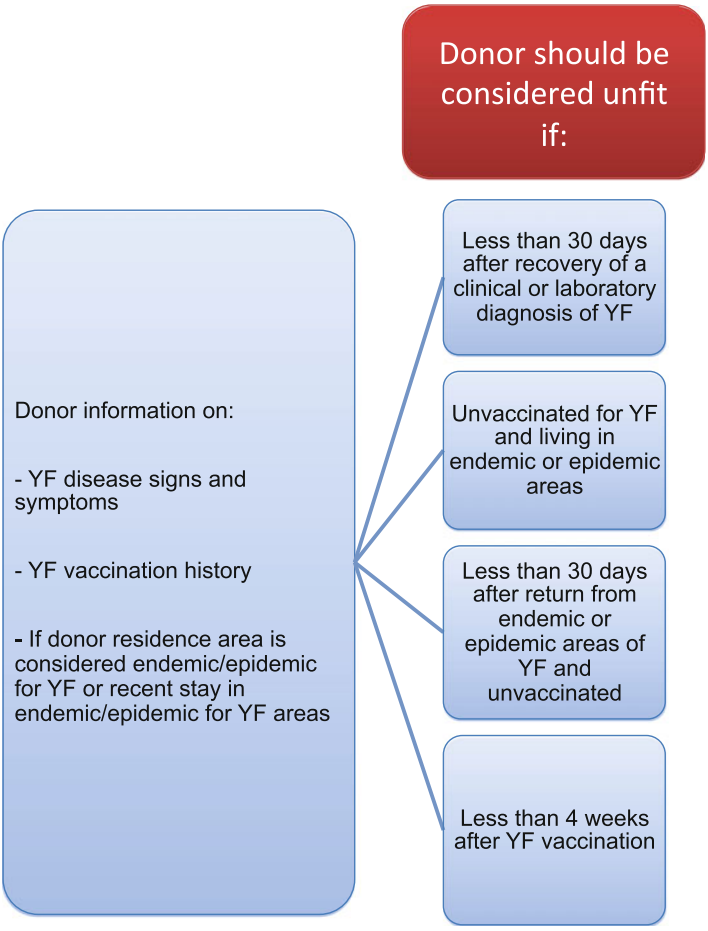


Fig. 1 Recommendations for the acceptance of solid organ donors with previous infection, living or with a recent stay in endemic or epidemic areas, or a recent history of YF vaccination. (Adapted from Brazilian Ministry of Health [35])

patients presented positive IgM and PRNT titers approximately 30 days after transfusion. The American Red Cross recommends deferral from blood donation for 2 weeks after receipt of YF vaccine [34].

In 2017, the Brazilian Ministry of Health issued recommendations for the acceptance of tissue and SOT donors with past infection or a recent history of YF vaccination (Fig. 1) [35].

Adverse events due to YF vaccination are usually mild: fever, myalgia, prostration, malaise, and headache may occur for 5–10 days after vaccination, in 10–30% of vaccine recipients [29]. Serious adverse events include vaccine-associated neurological disease, viscerotropic disease, and egg allergy-associated hypersensitivity reactions such as anaphylaxis [36].

The rate of adverse reactions following YF vaccine depends on the population studied. Data on US and European travelers report a rate of viscerotropic disease ranging from 0.25 to 0.4 per 100,000 doses, while in Peru, the rate was 7.9 per 100,000 doses [37]. The rate is also higher in elderly persons (over 60 years), reaching 2.3–12.6 per 100,000 doses [38, 39]. In the same elderly population, nonserious adverse events were considerably higher in first-dose vaccines (17.5%) compared to those receiving booster doses (9.5%) in a Brazilian study of over 700 vaccinated travelers [40]. In Brazil, a study based on the passive National Surveillance System following immunizations estimated overall rates of serious YF vaccine-related adverse events as hypersensitivity, 0.9 per 100,000 doses; anaphylactic shock, 0.023 per 100,000 doses; neurotropic disease, 0.084 per 100,000 doses; and viscerotropic disease, 0.019 per 100,000 doses [41].

Due to the increased risk of developing adverse events, current contraindications for YF vaccination as issued by the WHO are [42] children below 9 months, pregnant or breastfeeding women, people with severe hypersensitivity to egg antigens, severe immunodeficiency, and age over 60 years.

Recently vaccinated individuals (less than 30 days) that present with YF infection should undergo molecular testing in order to differentiate infection by wild-type YF virus or vaccine strain [14].

Prevention in Transplant Candidates

Clinical manifestations in transplant recipients have been previously described in other arboviruses. In renal transplant recipients, dengue manifestations appear to be similar to the general population, with some degree of graft dysfunction [43], with similar findings with Chikungunya infection [44]. Dengue virus shedding has been demonstrated to be more prolonged in immunocompromised persons (up to 80 days) compared to immunocompetent patients (up to 7 days) [45]. Literature is scarce regarding YF clinical manifestations in SOT patients, with only one fatal case in a kidney recipient [46] (Fig. 2).

In real life, the compliance with vaccine administration in transplant candidates may be suboptimal, as observed in a single-center study where patients were poorly informed by transplant physicians or general practitioners regarding pretransplantation vaccination [47]. Another single-center study involving 105 kidney transplant candidates showed YF vaccine coverage of only 3.8% [48]. However, efforts should be focused on this strategy especially where live vaccines are concerned, as they are contraindicated after transplantation. A French recommendation endorses the YF vaccine prior to transplantation whenever immunologic conditions allow. If the transplant recipient is traveling, then a contraindication medical certificate may be issued, though this increases the risk of wild-type disease if the individual enters a YF risk zone [49].

Most studies on live vaccine posttransplant are limited to observational and case series or reports. One of them, including 19 SOT recipients vaccinated for YF [50], showed no serious adverse events. A meta-analysis of live attenuated

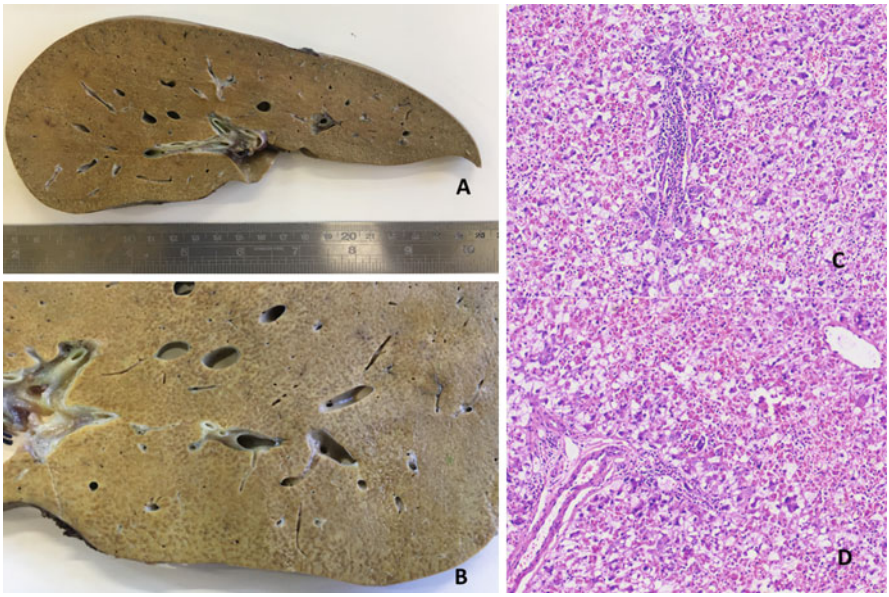


Fig. 2 (**a** and **b**) Explant of a liver transplant recipient with the severe form of yellow fever showing the liver with a yellowish to tan, homogeneous, and soft cut surface. (**c** and **d**) Characteristic midzonal necrosis with numerous Councilman-Rocha Lima bodies, sparing hepatocytes in periportal and perivenular zones

vaccination in immunocompromised individuals observed 1 fatal case of vaccine-related YF disease in a patient with rheumatoid arthritis and systemic erythematous lupus receiving treatment with methotrexate and corticosteroids, while 20 SOT recipients did not report serious adverse events or infection related to vaccination [51]. Nonetheless, the numbers are small, and therefore recommendations cannot be made for the administration of live vaccines in this population, thus limiting use of these vaccines to the pretransplant period.

The persistence of YF antibodies in SOT recipients has been demonstrated in those vaccinated prior to transplantation. Of 53 individuals (29 kidney recipients and 18 liver recipients), all but one had protective titers of antibodies after a median period of 3 years (0.8–21), and of 46 patients with a known estimated date of vaccination, the antibodies were still detectable after a median time of 13 years [2–32] after YF vaccination [52].

In the following sections, the literature on YF vaccination in hematologic malignancies and HSCT, kidney, and liver transplant recipients will be reviewed. Heart and lung recipients will not be discussed due to scarcity of data.

Hematological Malignancies and HSCT Candidates

Data on YF vaccination in this population is scarce, as the vaccine is contraindicated in this population. YF vaccination is possible after a HSCT and a 2-year period

without graft-versus-host disease or immunosuppressive treatment, with a case series of 21 recipients immunized with no adverse events at a median of 39 months after transplantation and a median of 33 months after withdrawal of immunosuppression [53]. Among those individuals vaccinated prior to transplantation, one third had persistent protective immunity after the procedure [53].

One single case report presents the case of a patient with acute myeloid leukemia who started chemotherapy 7 days after receiving a first dose of YF vaccine, resulting in prolonged viremia (15 days) with no other complications, while surprisingly maintaining antibody titers for more than 30 months after transplantation [54].

Kidney Transplant Candidates

In renal failure, an impairment of cell-mediated immunity is observed [55]. There is a reduced absolute number of peripheral blood T lymphocytes as a result of depressed activation and proliferation of T lymphocytes [56]. Regarding humoral response, chronic renal failure individuals are known to show poor protective efficacy from vaccines [57]. Early studies indicate defective macrophage function as a result of a uremic environment and impaired generation of activated antigen-specific T helper cells without adequate B cell antibody synthesis as a result of diminished interleukin 1 and 2 production [58].

Despite these findings in early literature, there have been safety studies on live, attenuated varicella-zoster vaccine in end-stage renal disease recipients that demonstrated that the vaccine was safe and antibody responses were protective [59]. In a larger safety study of the herpes zoster vaccine, 300 individuals, including 61 with chronic renal disease, had similar rates of adverse events compared to immunocompetent patients [60].

Literature is scarce concerning YF vaccine efficacy in hemodialysis patients. One study analyzed the YF vaccine status of 130 hemodialysis patients, and unvaccinated individuals were referred for immunization. Of the 86 referred patients, 45 were actually vaccinated, and there were no serious adverse events attributable to the YF vaccine, including anaphylaxis and neurologic or viscerotropic disease [61].

In a French study on YF vaccination in 84 elderly individuals after a median of 14 years after vaccine, there was one case of renal insufficiency among the studied population, with persistence of neutralizing antibodies [62].

Liver Transplant Candidates

In view of the morbidity of infection in cirrhotic patients, prevention through immunization is crucial in the management of this population, particularly those who are candidates for liver transplantation, as posttransplantation vaccine response is less efficient [63]. The literature mostly addresses influenza, hepatitis A and B, and pneumococcal vaccines in cirrhotic patients [64].

In inactivated influenza vaccine studies, although the percentage of cirrhotic patients who develop seroprotection is lower overall than healthy controls, the indication still stands, due to the high morbidity and mortality in this population [63]. Specific humoral immunity in cirrhotic patients against influenza was demonstrated to be comparable to age-matched controls, while cell-mediated response was only worse in Child C cirrhotic patients [65].

There are some studies on other live vaccines, such as the herpes zoster vaccine. One cohort study demonstrated clinical efficacy in patients with chronic conditions, including liver diseases [66].

There are no studies in the current literature concerning YF vaccine and cirrhotic patients. However, in light of the recent epidemic, the Brazilian Ministry of Health issued YF vaccine recommendations for liver transplant candidates [35]: indicated for all candidates below 60 years of age; if over 60 years, the patient is evaluated for comorbidities and requires authorization from the attending physician and consultation with the transplantation team, with a recommendation of transplantation deferral for 4 weeks after vaccination.

Liver Transplantation as an Emerging Treatment Option

Some drugs have been evaluated for YF treatment, such as interferon-alfa and antivirals such as high-dose ribavirin in experimental models though, to date, none has proven effective, and management of severe YF consists of supportive treatment [67–69]. YF virus has recently been demonstrated to be susceptible in vitro and in vivo to the antiviral sofosbuvir [70, 71], and randomized clinical trials are currently ongoing in Brazil.

It was believed that the clinical outcome was correlated with the severity of liver damage, leading to coagulopathy-related disorders and consequently multiorgan failure [1]. Until 2018, epidemics of YF had occurred in areas lacking high-complexity medical care facilities. In the epidemic that struck the southeast of Brazil from December 2017 to April 2018, cases occurred in close proximity to the city of São Paulo, and patients were therefore referred to quaternary hospitals. In this context, the first liver transplantation in a case of severe YF was performed in December 2017 in Brazil, with a successful outcome [72], and subsequently another six cases were performed at the same center, in the context of high mortality rate among severe cases of YF. Of seven transplanted patients, three survived, and four died at a median of 5.5 days after transplant [4–16]. One of the surviving patients progressed to severe pancreatitis and required several debridements (surgical and interventional radiology-guided) with secondary bacterial infections. The other two liver recipients presented with neurologic impairment after transplant, mainly apraxia, with gradual improvement. The outcome of the liver graft was unremarkable in all three cases, and none underwent liver biopsies after transplantation. The four patients who did not survive had signs of YF reinfection

of the liver graft, and two patients had severe necrotizing pancreatitis at necropsy. A key feature that could define prognosis is acute pancreatitis, which has not previously been described in YF. Management of these cases includes abdominal imaging, and the presence of signs of necrotizing pancreatitis should constitute a contraindication for liver transplantation.

Owing to the limited experience, precise recommendations cannot be made. However, in an analysis of other cases of the same epidemic in which patients receiving vasopressors and/or mechanical ventilation did not survive except for the three cases in which liver transplantation was performed, it is safe to say that the procedure was life-saving for these patients.

Liver transplantation in YF has also been performed in other transplant centers in São Paulo, Minas Gerais, and Rio de Janeiro, adding an additional 16 liver transplantations in Brazil. Overall, of 23 transplanted patients in Brazil, six (26%) survived. As a consequence of the high lethality in severe cases, many underwent the procedure in extremely severe clinical conditions and succumbed shortly after transplantation.

As a consequence of travelers returning from endemic or epidemic regions in Brazil or Africa, liver transplantation for severe YF has also been successfully performed in Argentina (G. A. Soteras, personal communication) and Holland (S. Darwish Murad, personal communication). In France, it has also been performed, but the patient succumbed due to posttransplantation necrotizing pancreatitis (O. Soubrane, personal communication).

The precise indications for liver transplantation in the context of YF are unknown, as the classical indications of Clichy [73] and King's College [74] criteria were not defined for systemic diseases like YF. The clinical picture of liver failure in YF patients is distinct from that of fulminant hepatitis due to other classical causes such as hepatotropic viruses or drug hepatoxicity, because YF is a systemic disease. Other successful cases of liver transplantation due to acute liver failure related to infectious systemic diseases have been performed, such as in herpes simplex virus (HSV) [75], Epstein-Barr virus (EBV) [76], parvovirus B19 [77], and dengue [78]. Similarly, there is the risk of a posttransplant viral recurrence following immunosuppression, and in the context of HSV and EBV, acyclovir may be administered as an attempt to prevent recurrence. However, in YF, there are to date no antivirals proven to be efficacious.

More recently, in the epidemic that began in January 2019 in Brazil, data from the State of São Paulo showed that there were fewer cases, with 65 autochthonous confirmed cases [79] as opposed to 504 cases in 2018, and a different management strategy was undertaken in our center. Plasma exchange was performed in patients considered at high risk of death, yielding good results (unpublished data), based initially on previous experiences of plasma exchange in acute liver failure [80]. Current management therefore includes plasma exchange with replacement with fresh frozen plasma, and liver transplantation may be indicated in severe YF cases where there is suspicion of baseline chronic hepatopathy. Supportive management also includes anticonvulsive prophylactic drugs and antimicrobial prophylaxis, as for acute liver failure [81].

Conclusions

Adequate vaccine coverage should be the focus of YF control in countries in which there are epidemics or endemic regions for YF. Because YF vaccine is composed of live, attenuated virus, it is contraindicated in SOT and HSCT recipients, and vaccine should be preferably administered prior to transplantation. Despite the scarcity of data, SOT candidates living in or traveling to endemic or epidemic areas should be vaccinated, and transplantation should preferably be deferred for a period of 4 weeks after vaccination.

Management of severe YF is based on emerging data on liver transplantation and plasma exchange and should include a multidisciplinary approach to this serious disease with high rates of lethality.

Key Points

- The resurgence of yellow fever epidemics continues to occur in non-endemic areas of Africa and Latin America.
- Although the majority of cases are oligosymptomatic, case fatality rates in severe forms may reach up to 60%.
- The diagnosis can be made by virological or serological tests, as clinical manifestations may overlap with a wide range of diseases.
- Vector control and immunization are the mainstay of prevention strategies for the control of yellow fever, and adverse event rates vary according to existing literature.
- As it is a live attenuated virus vaccine, contraindications for the yellow fever vaccine includes immunocompromised populations, thus limiting the use of these vaccines to the pretransplant period, with albeit scarce data in transplant candidates as well.
- Liver transplantation has been recently performed as a form of treatment with some successful cases, although precise indications and contraindications are difficult to establish. Plasma exchange, along with supportive intensive care management, has also been successfully performed in severe cases.

References

1. Monath TP, Vasconcelos PFC. Yellow fever. J Clin Virol. 2015;64:160–73.
2. PAHO. Yellow fever. March 2015. Available at https://www.paho.org/hq/index.php?option=com_content&view=article&id=9476:yellow-fever&Itemid=40721&lang=en. Accessed 27 Mar 2019.
3. Chippaux J-P, Chippaux A. Yellow fever in Africa and the Americas: a historical and epidemiological perspective. J Venom Anim Toxins Trop Dis [Internet]. 2018 Dec [cited 2019 Mar 27];24(1). Available from: https://jvat.biomedcentral.com/articles/10.1186/s40409-018-0162-y
4. Barnett ED. Yellow fever: epidemiology and prevention. Clin Infect Dis. 2007;44(6):850–6.

5. World Health Organization. Yellow fever situation report. 7 October 2016 [Internet]. 2016. http://www.who.int/emergencies/yellow-fever/situation-reports/7-october-2016/en/.
6. Song R, Guan S, Lee SS, Chen Z, Chen C, Han L, et al. Late or lack of vaccination linked to importation of yellow fever from Angola to China. Emerg Infect Dis. 2018;24(7):1383.
7. Couto-Lima D, Madec Y, Bersot MI, Campos SS, de Motta MA, dos Santos FB, et al. Potential risk of re-emergence of urban transmission of yellow fever virus in Brazil facilitated by competent Aedes populations. Sci Rep [Internet]. 2017 Dec [cited 2019 Mar 27];7(1). Available from: http://www.nature.com/articles/s41598-017-05186-3
8. Yellow fever in Africa and the Americas. 2016. Releve Epidemiol Hebd. 2017;92(32):442–52. Available at https://apps.who.int/iris/bitstream/handle/10665/258707/WER9232.pdf;jsessionid=9F91371F998C52A77710D06B8A46FF42?sequence=1
9. Ministry of Health. Monitoramento da Situação Epidemiológica da Febre Amarela no Brasil. 2018. Available at http://portalarquivos2.saude.gov.br/images/pdf/2018/maio/18/Informe-FA-26.pdf. Accessed 30 May 2018.
10. Faria NR, Kraemer MUG, Hill SC, Goes de Jesus J, Aguiar RS, Iani FCM, et al. Genomic and epidemiological monitoring of yellow fever virus transmission potential. Science. 2018;361(6405):894–9.
11. Johansson MA, Vasconcelos PFC, Staples JE. The whole iceberg: estimating the incidence of yellow fever virus infection from the number of severe cases. Trans R Soc Trop Med Hyg. 2014;108(8):482–7.
12. Litvoc MN, Novaes CTG, Lopes MIBF, Litvoc MN, Novaes CTG, Lopes MIBF. Yellow fever. Rev Assoc Méd Bras. 2018;64(2):106–13.
13. Tuboi SH, Costa ZGA, da Costa Vasconcelos PF, Hatch D. Clinical and epidemiological characteristics of yellow fever in Brazil: analysis of reported cases 1998–2002. Trans R Soc Trop Med Hyg. 2007;101(2):169–75.
14. PAHO. Laboratory diagnosis of yellow fever virus infection. Pan American Health Organization. September 2018. https://www.paho.org/hq/index.php?option=com_docman&view=download&category_slug=guidelines-5053&alias=46877-laboratory-diagnosis-of-yellow-fever-virus-infection&Itemid=270&lang=pt. Accessed 26 Mar 2019.
15. Domingo C, Patel P, Yillah J, Weidmann M, Mendez JA, Nakoune ER, et al. Advanced yellow fever virus genome detection in point-of-care facilities and reference laboratories. J Clin Microbiol. 2012;50(12):4054–60.
16. Shope RE, Woodall JP, Rosa AT. The epidemiology of diseases in groups C and Guama (Bunyaviridae). In: Monath TP, editor. Arboviruses: epidemiology and ecology, vol. III. Boca Raton: CRC Press; 1985. p. 139–231.
17. Nassar E da S, Chamelet ELB, Coimbra TLM, de Souza LTM, Suzuki A, Ferreira IB, et al. Jungle yellow fever: clinical and laboratorial studies emphasizing viremia on a human case. Rev Inst Med Trop São Paulo. 1995;37(4):337–41.
18. Centers for Disease Control and Prevention (CDC). Fatal yellow fever in a traveler returning from Amazonas, Brazil, 2002. MMWR Morb Mortal Wkly Rep. 2002;51(15):324–5.
19. Reusken CBEM, Knoester M, GeurtsvanKessel C, Koopmans M, Knapen DG, Bierman WFW, et al. Urine as sample type for molecular diagnosis of natural yellow fever virus infections. J Clin Microbiol. 2017;55(11):3294–6.
20. Barbosa CM, Di Paola N, Cunha MP, Rodrigues-Jesus MJ, Araujo DB, Silveira VB, et al. Yellow fever virus DNA in urine and semen of convalescent patient, Brazil. Emerg Infect Dis. 2018;24(1):176–8.
21. Centers for Disease Control and Prevention (CDC). Yellow fever: clinical & laboratory evaluation. 21 August 2015. Available online at: https://www.cdc.gov/yellowfever/healthcareproviders/healthcareproviders-clinlabeval.html. Accessed 25 Mar 2019.
22. Domingo C, Charrel RN, Schmidt-Chanasit J, Zeller H, Reusken C. Yellow fever in the diagnostics laboratory. Emerg Microbes Infect. 2018;7(1):1–15.

23. de Filippis AMB, Nogueira RMR, Schatzmayr HG, Tavares DS, Jabor AV, Diniz SCM, et al. Outbreak of jaundice and hemorrhagic fever in the Southeast of Brazil in 2001: detection and molecular characterization of yellow fever virus. J Med Virol. 2002;68(4):620–7.
24. de Filippis AMB, Nogueira RMR, Jabor AV, Schatzmayr HG, Oliveira JC, Dinis SCM, et al. Isolation and characterization of wild type yellow fever virus in cases temporally associated with 17DD vaccination during an outbreak of yellow fever in Brazil. Vaccine. 2004;22(9–10):1073–8.
25. Shearer FM, Moyes CL, Pigott DM, Brady OJ, Marinho F, Deshpande A, et al. Global yellow fever vaccination coverage from 1970 to 2016: an adjusted retrospective analysis. Lancet Infect Dis. 2017;17(11):1209–17.
26. Staples JE, Gershman M, Fischer M, Centers for Disease Control and Prevention (CDC). Yellow fever vaccine: recommendations of the Advisory Committee On Immunization Practices (ACIP). MMWR Recomm Rep Morb Mortal Wkly Rep Recomm Rep. 2010;59 (RR-7):1–27.
27. Monath TP, Nichols R, Archambault WT, Moore L, Marchesani R, Tian J, et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase III multicenter, double-blind clinical trial. Am J Trop Med Hyg. 2002;66 (5):533–41.
28. Monath TP, McCarthy K, Bedford P, Johnson CT, Nichols R, Yoksan S, et al. Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections. Vaccine. 2002;20(7–8):1004–18.
29. Monath T, Cetron MS, Teuwen DE. Yellow fever vaccine. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 959–1055.
30. Vaccines and vaccination against yellow fever. WHO position paper – June 2013. Releve Epidemiol Hebd. 2013;88(27):269–83.
31. Staples JE, Bocchini JA, Rubin L, Fischer M, Centers for Disease Control and Prevention (CDC). Yellow fever vaccine booster doses: recommendations of the advisory committee on immunization practices, 2015. MMWR Morb Mortal Wkly Rep. 2015;64(23):647–50.
32. Reinhardt B, Jaspert R, Niedrig M, Kostner C, L'age-Stehr J. Development of viremia and humoral and cellular parameters of immune activation after vaccination with yellow fever virus strain 17D: a model of human flavivirus infection. J Med Virol. 1998;56(2):159–67.
33. Centers for Disease Control and Prevention (CDC). Transfusion-related transmission of yellow fever vaccine virus – California, 2009. MMWR Morb Mortal Wkly Rep. 2010;59(2):34–7.
34. American Red Cross. Donating blood: eligibility requirements. Available at http://www.redcrossblood.org/donating-blood/eligibility-requirements/eligibility-criteria-alphabetical-listing. Accessed 27 May 2019.
35. Ministry of Health, Brazil. Nota Tecnica Conjunta ANVISA/SAS/MS – Critérios tecnicos para a triagem clinica de potenciais doadores de orgaos e tecidos para o virus da febre amarela. 2017. Available online at: http://portal.anvisa.gov.br/documents/4048533/4920270/Nota+Técnica+Conjunta+n°+01+de+2017.pdf/a0195af8-d595-4307-b3fe-7a965e712a77. Accessed 10 Apr 2019.
36. Porudominsky R, Gotuzzo EH. Yellow fever vaccine and risk of developing serious adverse events: a systematic review. Rev Panam Salud Pública [Internet]. 2018 [cited 2019 Mar 27];42. Available from: http://iris.paho.org/xmlui/handle/123456789/49083
37. Whittembury A, Ramirez G, Hernández H, Ropero AM, Waterman S, Ticona M, et al. Viscerotropic disease following yellow fever vaccination in Peru. Vaccine. 2009;27(43):5974–81.
38. Khromava AY, Eidex RB, Weld LH, Kohl KS, Bradshaw RD, Chen RT, et al. Yellow fever vaccine: an updated assessment of advanced age as a risk factor for serious adverse events. Vaccine. 2005;23(25):3256–63.
39. Lindsey NP, Rabe IB, Miller ER, Fischer M, Staples JE. Adverse event reports following yellow fever vaccination, 2007–13. J Travel Med. 2016;23(5):taw045.

40. Miyaji KT, Luiz AM, Lara AN, do Socorro Souza Chaves T, Piorelli Rde O, Lopes MH, et al. Active assessment of adverse events following yellow fever vaccination of persons aged 60 years and more. Hum Vaccin Immunother. 2013;9(2):277–82.
41. de Menezes Martins R, Maia M de L de S, dos Santos EM, de Cruz RLS, dos Santos PRG, Carvalho SMD, et al. Yellow fever vaccine post-marketing surveillance in Brazil. Procedia Vaccinol. 2010;2(2):178–83.
42. World Health Organization. Updates on yellow fever vaccination recommendations for international travelers related to the current situation in Brazil. 16 January 2018. Available at https://www.who.int/ith/updates/20180116/en/. Accessed 10 Apr 2019.
43. Weerakkody RM, Patrick JA, Sheriff MHR. Dengue fever in renal transplant patients: a systematic review of literature. BMC Nephrol. 2017;18(1):15.
44. Tavares BM, Fernandes PF, Oliveira CMC, Silva SL, Mota MU, Andrade TH, et al. Chikungunya on kidney transplant recipients: is it the same? Transplantation. 2019;103(2):441–5.
45. de Souza Pereira BB, Darrigo Junior LG, de Mello Costa TC, Felix AC, Simoes BP, Stracieri AB, et al. Prolonged viremia in dengue virus infection in hematopoietic stem cell transplant recipients and patients with hematological malignancies. Transpl Infect Dis. 2017;19(4):e12721.
46. Pierrotti LC, Duarte-Neto AN, Song ATW, Ventura CG, David-Neto E, Sergio de Azevedo L. Fatal Yellow Fever in a kidney transplant patient. Clin Infect Dis [Internet]. 2019 May 11 [cited 2019 May 26]; Available from: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciz389/5488071
47. Mazzola A, Minh MT, Jauréguiberry S, Bernard D, Lebray P, Chrétien Y, et al. Are cirrhotic patients awaiting liver transplantation protected against vaccine-preventable diseases? Clin Microbiol Infect. 2018;24(7):787–8.
48. Camargo LF, Lother AM, Mazzali M, Stucchi RSB. Immunization in end stage renal disease: the perception of waiting list patients. Transpl Infect Dis. 2018;20(3):e12831.
49. Wyplosz B, Van der Vliet D, Consigny PH, Calmus Y, Mamzer-Bruneel MF, Guillemain R, et al. Vaccinations du voyageur adulte transplanté d'organes (à l'exclusion des receveurs de cellules souches hématopoïétiques). Médecine Mal Infect. 2009;39(4):225–33.
50. Azevedo LS, Lasmar EP, Contieri FLC, Boin I, Percegona L, Saber LTS, et al. Yellow fever vaccination in organ transplanted patients: is it safe? A multicenter study. Transpl Infect Dis Off J Transplant Soc. 2012;14(3):237–41.
51. Croce E, Hatz C, Jonker EF, Visser LG, Jaeger VK, Bühler S. Safety of live vaccinations on immunosuppressive therapy in patients with immune-mediated inflammatory diseases, solid organ transplantation or after bone-marrow transplantation – a systematic review of randomized trials, observational studies and case reports. Vaccine. 2017;35(9):1216–26.
52. Wyplosz B, Burdet C, François H, Durrbach A, Duclos-Vallée JC, Mamzer-Bruneel M-F, et al. Persistence of yellow fever vaccine-induced antibodies after solid organ transplantation: yellow-fever antibodies in transplant recipients. Am J Transplant. 2013;13(9):2458–61.
53. Sicre de Fontbrune F, Arnaud C, Cheminant M, Boulay A, Konopacki J, Lapusan S, et al. Immunogenicity and safety of yellow fever vaccine in allogeneic hematopoietic stem cell transplant recipients after withdrawal of immunosuppressive therapy. J Infect Dis. 2018;217(3):494–7.
54. Avelino-Silva VI, Leal FE, Sabino EC, Nishiya AS, da Silva Freire M, Blumm F, et al. Yellow fever vaccine viremia following ablative BM suppression in AML. Bone Marrow Transplant. 2013;48(7):1008–9.
55. Raskova J, Ghobrial I, Shea SM, Eisinger RP, Raska K. Suppressor cells in end-stage renal disease. Functional assays and monoclonal antibody analysis. Am J Med. 1984;76(5):847–53.
56. Kurz P, Köhler H, Meuer S, Hütteroth T, Meyer zum Büschenfelde KH. Impaired cellular immune responses in chronic renal failure: evidence for a T cell defect. Kidney Int. 1986;29(6):1209–14.

57. Johnson DW, Fleming SJ. The use of vaccines in renal failure. Clin Pharmacokinet. 1992;22(6):434–46.
58. Dumann H, Meuer SC, Köhler H. Uremic serum inhibits monocyte-dependent, but not interleukin-2-dependent steps of T cell proliferation. Nephron. 1990;56(2):162–5.
59. Miller G, Schaefer H, Yoder S, Miller R, Winokur P, Kotloff K, et al. A randomized, placebo-controlled phase I trial of live, attenuated herpes zoster vaccine in subjects with end-stage renal disease immunized prior to renal transplantation. Transpl Infect Dis. 2018;20(3): e12874.
60. Ohfuji S, Ito K, Inoue M, Ishibashi M, Kumashiro H, Hirota Y, et al. Safety of live attenuated varicella-zoster vaccine in patients with underlying illnesses compared with healthy adults: a prospective cohort study. BMC Infect Dis [Internet]. 2019 Dec [cited 2019 Apr 8];19(1). Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-019-3719-7
61. Facincani T, Guimarães MNC, De Sousa dos Santos S. Yellow fever vaccination status and safety in hemodialysis patients. Int J Infect Dis. 2016;48:91–5.
62. Coulange Bodilis H, Benabdelmoumen G, Gergely A, Goujon C, Pelicot M, Poujol P, et al. Persistance à long terme des anticorps neutralisants de la fièvre jaune chez les personnes âgées de 60 ans et plus. Bull Soc Pathol Exot. 2011;104(4):260–5.
63. Leise MD, Talwalkar JA. Immunizations in chronic liver disease: what should be done and what is the evidence. Curr Gastroenterol Rep [Internet]. 2013 Jan [cited 2019 Apr 13];15(1). Available from: http://link.springer.com/10.1007/s11894-012-0300-6
64. Fagiuoli S, Ravasio R, Lucà MG, Baldan A, Pecere S, Vitale A, et al. Management of hepatitis C infection before and after liver transplantation. World J Gastroenterol: WJG. 2015;21(15):4447–56.
65. Cheong H-J, Song J-Y, Park J-W, Yeon J-E, Byun K-S, Lee C-H, et al. Humoral and cellular immune responses to influenza vaccine in patients with advanced cirrhosis. Vaccine. 2006;24(13):2417–22.
66. Tseng HF, Smith N, Harpaz R, Bialek SR, Sy LS, Jacobsen SJ. Herpes zoster vaccine in older adults and the risk of subsequent herpes zoster disease. JAMA. 2011;305(2):160–6.
67. Monath TP. Treatment of yellow fever. Antivir Res. 2008;78(1):116–24.
68. Julander JG, Ennis J, Turner J, Morrey JD. Treatment of yellow fever virus with an adenovirus-vectored interferon, DEF201, in a hamster model. Antimicrob Agents Chemother. 2011;55(5):2067–73.
69. Sbrana E, Xiao S-Y, Guzman H, Ye M, Travassos da Rosa APA, Tesh RB. Efficacy of post-exposure treatment of yellow fever with ribavirin in a hamster model of the disease. Am J Trop Med Hyg. 2004;71(3):306–12.
70. de Freitas CS, Higa LM, Sacramento CQ, Ferreira AC, Reis PA, Delvecchio R, et al. Yellow fever virus is susceptible to sofosbuvir both in vitro and in vivo. Scarpino SV, editor. PLoS Negl Trop Dis. 2019;13(1):e0007072.
71. Ferreira AC, Reis PA, de Freitas CS, Sacramento CQ, Villas Bôas Hoelz L, Bastos MM, et al. Beyond members of the *Flaviviridae* family, Sofosbuvir also inhibits chikungunya virus replication. Antimicrob Agents Chemother. 2018;63(2):e01389–18.
72. Song ATW, Abdala E, de Martino RB, Malbouisson LMS, Tanigawa RY, Andrade GM, et al. Liver transplantation for fulminant hepatitis attributed to yellow fever: hepatology. Hepatology. 2019;69(3):1349–52.
73. Bernuau J, Goudeau A, Poynard T, Dubois F, Lesage G, Yvonnet B, et al. Multivariate analysis of prognostic factors in fulminant hepatitis B. Hepatol Baltim Md. 1986;6(4):648–51.
74. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology. 1989;97(2):439–45.
75. Vincenzi R, Fonseca EA, Roda KMO, Porta G, Candido HL, Benavides MR, et al. Living donor liver transplantation for neonatal fulminant hepatitis due to herpes simplex virus infection. Pediatr Transplant. 2017;21(7):e13021.
76. Nakazawa A, Nakano N, Fukuda A, Sakamoto S, Imadome K-I, Kudo T, et al. Use of serial assessment of disease severity and liver biopsy for indication for liver transplantation in

pediatric Epstein-Barr virus-induced fulminant hepatic failure: LIVER TRANSPLANTATION FOR EBV-INDUCED FHF. Liver Transpl. 2015;21(3):362–8.
77. So K, Macquillan G, Garas G, Delriviere L, Mitchell A, Speers D, et al. Urgent liver transplantation for acute liver failure due to parvovirus B19 infection complicated by primary Epstein-Barr virus and cytomegalovirus infections and aplastic anaemia. Intern Med J. 2007;37(3):192–5.
78. Galante A, Adeyi O, Lau L, Humar A, Galvin Z, Selzner N, et al. Liver transplantation for acute liver failure due to dengue fever. Hepatology [Internet]. 2019 Aug 9 [cited 2019 Oct 13]; Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/hep.30803
79. Secretary of Health, State of São Paulo. Boletim Epidemiológico Febre Amarela, Centro de Vigilância Epidemiológica Prof. Alexandre Vranjac. 2019. Available at http://www.saude.sp.gov.br/resources/cve-centro-de-vigilancia-epidemiologica/areas-de-vigilancia/doencas-de-transmissao-por-vetores-e-zoonoses/doc/famarela/2019/fa19_boletim_epid_1204.pdf. Accessed 17 Apr 2019.
80. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. J Hepatol. 2016;64(1):69–78.
81. Ho Y-L, Joelsons D, Leite GFC, Malbouisson LMS, Song ATW, Perondi B, et al. Severe yellow fever in Brazil: clinical characteristics and management. J Travel Med [Internet]. 2019 Jun 11 [cited 2019 Oct 13];26(5). Available from: https://academic.oup.com/jtm/article/doi/10.1093/jtm/taz040/5509466

Viral Encephalitides Including Rabies and Lymphocytic Choriomeningitis Virus: Impact in Transplant Donors and Recipients

43

Aalok Khole and Nicole M. Theodoropoulos

Contents

Introduction 1027
Rabies Virus 1028
Signs and Symptoms 1029
Diagnosis 1030
Treatment 1031
Lymphocytic Choriomeningitis Virus (LCMV) 1031
Signs and Symptoms 1032
Diagnosis 1034
Treatment 1034
Emerging Viruses 1035
Prevention of Donor Association Transmission 1036
Prevention of Recipient-Acquired Infection 1038
Challenges and Next Steps 1038
Conclusions 1040
Key Points 1040
References 1041

Introduction

The rarely seen viral encephalitides, lymphocytic choriomeningitis virus (LCMV) and rabies virus encephalitis, are some of the most feared infections, especially in the immunosuppressed host. Luckily, although mortality is high from these viruses when seen after transplant, they remain a rare occurrence. There are no published reports of LCMV or rabies in hematopoietic stem cell recipients, not to say they are

A. Khole · N. M. Theodoropoulos (✉)
Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, USA
e-mail: Aalok.Khole@umassmemorial.org; Nicole.Theodoropoulos@umassmemorial.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_42

not microbiologically plausible, but this chapter will focus on solid organ transplant recipients.

Both LCMV and Rabies virus are zoonoses and therefore can be acquired through primary infection of the recipient via animal exposure. However, the published reports of these infections all focus on donor-derived transmissions [1].

Screening of organ donors and organ recipients for these infections is challenging and requires a high level of suspicion. The symptoms vary depending on the region of the brain affected and can present as global cerebral dysfunction or focal neurological deficits. Cardinal signs of encephalitis such as altered mentation carry a wide differential in transplant recipients, and clinical presentations may vary owing to the inoculum of infection and level of immunosuppression.

Since 2002, several clusters of infectious encephalitides have been reported in groups of transplant recipients. These donor-derived transmissions have been investigated in depth by the US Centers for Disease Control and Prevention (CDC) and other major healthcare bodies. Some of these emerging pathogens include rabies virus, LCMV, and Eastern equine encephalitis virus (EEEV), among several others. We will review these pathogens and their clinical impact on organ transplantation [2]. Of note, West Nile virus will be discussed in a separate chapter.

For a more detailed discussion on West Nile Virus in transplant patients, please refer to ► Chap. 40, "West Nile Virus and Transplantation."

Rabies Virus

Infection from rabies virus dates back to the twenty-third century BC and continues to be one of the most dreaded diseases [3]. The major way rabies is transmitted to humans is through animal saliva, specifically from bats or small mammals like raccoons, skunks, foxes, and dogs, but any mammal can become infected. Globally, 55,000 people die annually from rabies, mostly secondary to dog bites. The raccoon is known to be the most rabid animal in the United States, but disease in humans is mostly attributed to bat exposure [4]. The virus is neurotropic and can also be acquired through contact with infected nervous tissue. The virus typically causes disease 2–8 weeks after infection but has been reported to remain latent for many years [3], increasing the risk for asymptomatic infection that could be transmitted by transplantation.

The first case of transplant acquired rabies virus infection was in 1978 from a corneal transplant [5]. Infection transmitted from donor to recipients in solid organ transplantation (SOT) was first reported in 2004 [2]. Humans are usually dead-end hosts for the virus, but organ transplantation serves as a route for human-to-human transmission [6].

When the disease manifests in humans, it is virtually 100% fatal – in 2007, only five known cases existed that survived – four were immunized, while one survived apparently through natural immunity [7]. Developing countries serve as major reservoirs for the virus with dogs as the infected source in most nations [7]. Infection

of solid organ transplant recipients goes against previous dogma that retrograde axonal migration is the only mode of spread. It raises evidence for hematogenous spread – probably through infecting macrophages [8, 9].

Based upon our underlying knowledge of rabies in general, coupled with the information obtained from the cluster analyses of organ donor-derived rabies transmissions between 2003 and 2018, we shall review some of the important features of this disease.

Signs and Symptoms

Rabies can present in various forms – encephalitic (furious) form and paralytic (dumb) form. It also differs in presentation based upon the animal variant and mode of acquisition. The classic (encephalitic) form may include the cardinal signs of fluctuating consciousness, phobic spasms, and autonomic dysfunction, while the bat variant usually includes localized symptoms or neuropathic pain described as burning, itching, tingling, or numbness [3].

Donors often present with vague symptoms that overlap with several more common possible diagnoses. In the 1996 corneal donor, presenting symptoms included fever, abdominal pain, and diarrhea with no known exposure to a rabid animal [5]. In the 2003 rabies transmission, the donor presented with nausea, vomiting, and abdominal pain followed by intermittent episodes of confusion, agitation, and ballistic movements of the trunk. He also had a subarachnoid hemorrhage that was thought to be the cause of his symptoms [3]. The donor in the 2011 transmission case had nausea, vomiting, upper extremity paresthesias that progressed to seizures, worsening mental status, dysphagia, autonomic dysfunction, and respiratory failure, presumed at that time to have been secondary to ciguatera poisoning [4].

Transplant recipients have also presented with variable symptoms. The corneal recipients presented with confusion, agitation, photophobia, hydrophobia, hypersalivation, and finally cerebral encephalitis. Ocular symptoms were not seen in those cases, but prior reports have suggested some patients having redness and pain in the eyes for about 2 days prior to being diagnosed with rabies [5].

The lung transplant recipient in the 2003 cohort died of surgical complications. The other four recipients, including the one with vascular allograft transplant, presented back to the hospital around 27 days posttransplantation and died by 42 days with symptoms consisting of rapid neurological deterioration, agitation, restlessness, delirium, and hallucinations. Tremors and myoclonus were present in some of the patients. All cases were complicated by respiratory failure, increased secretions, cardiac rhythm disturbances, paralysis, coma, and absence of brain stem reflexes leading to death within 7–23 days after onset of neurological symptoms [3].

The incubation period was very prolonged, 17 months, in the 2011 kidney transplant recipient and, therefore, initially not expected to be donor-derived. This patient presented to the hospital with sciatica-like pain radiating down from his hip along with tenderness over his transplanted kidney. These symptoms then

progressed to bilateral lower extremity weakness with paresthesias, encephalopathy, excessive salivation, and hemodynamic instability, and the patient died within 22 days of hospitalization [4]. The effect of immunosuppression on rabies virus infection is not clearly understood [2]. The very long incubation period in the recipient in the 2011 case was possibly due to the donor being infected with a raccoon variant of rabies, as opposed to the much more common bat variant. Only one other case of raccoon rabies infection in a human is in the literature, and the incubation period of that infection was unknown [10].

Diagnosis

The differential diagnosis of symptoms in the case of rabies infection is very broad and includes CNS infection from herpes and varicella viruses, cryptococcal infection, noninfectious etiologies such as posterior reversible encephalopathy syndrome, and many others. CNS imaging with MRI and lumbar puncture with CSF analysis is necessary to investigate a diagnosis in patients presenting with neurologic symptoms.

In cases of rabies transmitted through corneal transplants, diagnosis was made by clinical signs and a brain biopsy. Donor corneal impressions can be helpful – since specificity is 100% but sensitivity is slated at 46% [5].

Apart from clinical signs and symptoms, the diagnosis of rabies may be aided by CSF analysis, serological markers, and the gold standard of brain biopsy histopathological analysis coupled with immunofluorescence (IF) testing.

Historically, rabies has been a postmortem diagnosis. Antibodies are slow to appear in serum and CSF. Corneal smears are not reliable, and MRI findings are not pathognomonic. Nucleic acid sequence-based amplification testing on saliva, urine, CSF, or hair follicles will be positive early in the course of infection; this testing should be coordinated with state department of public health laboratories in the United States. If initially negative, serial testing may be recommended given the intermittent nature of viral shedding. IF testing on nuchal skin in combination with RT-PCR has high sensitivity, but brain tissue exam remains the gold standard. False negatives by this method could be due to the predilection of the virus in affecting the brain stem, thalamus, and the cord in earlier stages of the disease, areas that are not usually biopsied [7].

In the 2003 cohort of SOT-associated rabies infection in recipients, CSF exam revealed increased cells, increased protein, and normal glucose. Initial CT scans were normal in all four patients, and some of the subsequent scans showed subtle changes. MRI showed increased fluid-attenuated inversion recovery signal in varying regions of the brain in different patients. EEG showed moderate to severe generalized slowing in delta to theta range. Postmortem macroscopic exam of the brains showed different changes in different patients such as congestion, brain stem flattening, hemorrhages, etc. Histopathological findings were consistent with global hypoxic ischemic injury with the demonstration of Negri bodies [3].

Treatment

A review in 2018 noted that in the 13 cases of rabies virus transmission through solid organs or tissue allotransplantation, there were 30 recipients afflicted, 20 of whom died. The remaining patients received postexposure prophylaxis (PEP) and survived, except a heart recipient who died of an unrelated pulmonary infection. PEP with rabies immunoglobulin administration and vaccination series has shown to be an effective measure in controlling this infection [6]. Some patients died despite administering PEP, likely due to an aggressive virus variant [6, 11]. The penetrance of donor-derived rabies infection appears to be very high. Therefore, all recipients of a suspected donor should be tested and receive PEP.

In immunocompromised hosts, it is advised to give rabies immunoglobulin coupled with five doses of vaccine and to test for rabies virus-neutralizing antibody (RVNA) titers in the serum $\geq$7 days after the vaccine. A concentration of $\geq$0.1 IU/ml is considered adequate in the United States, but the World Health Organization (WHO) considers $\geq$0.5 IU/ml instead. It is unclear if routine serological testing needs to be done given that the recipients still technically harbor the virus by way of infected organs. Also, we need further data on how to manage those patients who never reach either of these targets despite PEP. Some experts advise repeating the whole regimen, while others say administer double-dose vaccines, akin to hepatitis B vaccine nonresponders, but there are no robust data to support this [12–14]. Challenges include differences in serologic responses depending on the host, level of immunosuppression, and monitoring of serial titers as immunity has persisted in some while waned in others. Data regarding postexposure management are available in HIV patients, but there are no prospective trials in transplant recipients [15].

Lymphocytic Choriomeningitis Virus (LCMV)

LCMV is a rodent-borne virus of the *Arenaviridae* family that was first discovered in 1933 as a cause of encephalitis. It can cause a broad spectrum of illness ranging from a mild, self-limiting "flu-like" illness in immunocompetent hosts to an aggressive variant leading to multisystem end-organ dysfunction and death, especially in immunocompromised hosts [12, 16, 17].

Serological surveys in the United States found that up to 5% of individuals have been infected with LCMV [12]. The main and natural reservoir of infection is the domestic mouse. Infections usually occur through contact with lab mice or infected hamsters [17]. Contact with mouse excreta/urine/other secretions via household dust or infected food and drink is a common mode of acquisition in humans [17]. Other than vertical transmission, human-to-human transmission was not reported prior to December 2003 and April 2005 when eight organ transplant recipients from two donors were diagnosed with donor-derived LCMV infection [12].

Since 2003, 5 such clusters have been reported worldwide, and only 5 out of 17 recipients have survived the infection. In some of these cases, the donor was not proven to be infected with the virus and did not demonstrate symptoms of active

infection but could have had contact with a vector sharing the same strain of virus as the recipients [16]. This phenomenon makes it a challenging infection to consider and diagnose in a potential donor prior to transplant. One such case cluster is shown in Figure 1, demonstrating the pace of illness. Fig. 1 Clinical timelines for LCMV infection in a donor-derived cluster. (Reproduced with permission, first published in: Mathur G, Yadav K, Ford B, Schafer IJ, Basavaraju SV, Knust B, Shieh WJ, Hill S, Locke GD, Quinlisk P, Brown S, Gibbons A, Cannon D, Kuehnert M, Nichol ST, Rollin PE, Ströher U, Miller R. High clinical suspicion of donor-derived disease leads to timely recognition and early intervention to treat solid organ transplant-transmitted lymphocytic choriomeningitis virus. Transpl Infect Dis. 2017 Aug;19(4). https://doi.org/10.1111/tid.12707. Epub 2017 May 24)

Signs and Symptoms

LCMV infection in immunocompetent humans is usually sporadic, benign, and usually asymptomatic extrapolating from the seroprevalence. It usually presents in one of three major clinical forms – influenza-like syndrome, aseptic meningitis, or meningoencephalitis. Each of these phases may present individually as self-limited illnesses or in sequence – usually each lasting 1–2 weeks [17].

The incubation period ranges from 5 to 13 days followed by fever, headache, myalgias, and occasionally abdominal pain, diarrhea, and rash. A period of convalescence may be followed by meningitis or, rarely, meningoencephalitis, parotitis, pneumonitis, arthritis, myocarditis, orchitis, or alopecia. Mortality from this infection, as previously stated, is usually less than 1% [12]. The lungs seem to be the primary organ involved, causing pulmonary infiltrates, interstitial edema, and eventual viremia allowing the virus to migrate to other organs including the meninges, choroid plexus, and ventricular ependymal linings. The subsequent high-level viral replication and resulting inflammatory response cause the symptoms of meningitis [18]. This may play out differently in organ transplant recipients given direct inoculation via infected organs.

Deceased organ donors involved in the reported clusters presented for medical care with symptoms unrelated to LCMV infection. On a retrospective analysis, some of them had positive serologies showing exposure to the virus, some only had exposure to infected rodents without any evidence of active or past infection, while some had neither obvious exposure nor evidence of infection. Out of the 17 organ transplant recipients from these 5 clusters, 12 (71%) died. All the recipients presented with a subacute onset of illness 2–3 weeks after transplant with symptoms including fever, abdominal pain, diarrhea, peri-incisional erythema and tenderness, rash, altered mental status, and eventually progression to multisystem organ failure [16].

The marked severity of illness and fatal outcomes in transplant recipients is likely secondary to the extent of immunosuppression, especially affecting T-cell population, coupled with the direct implantation via transplanting infected organs [12].

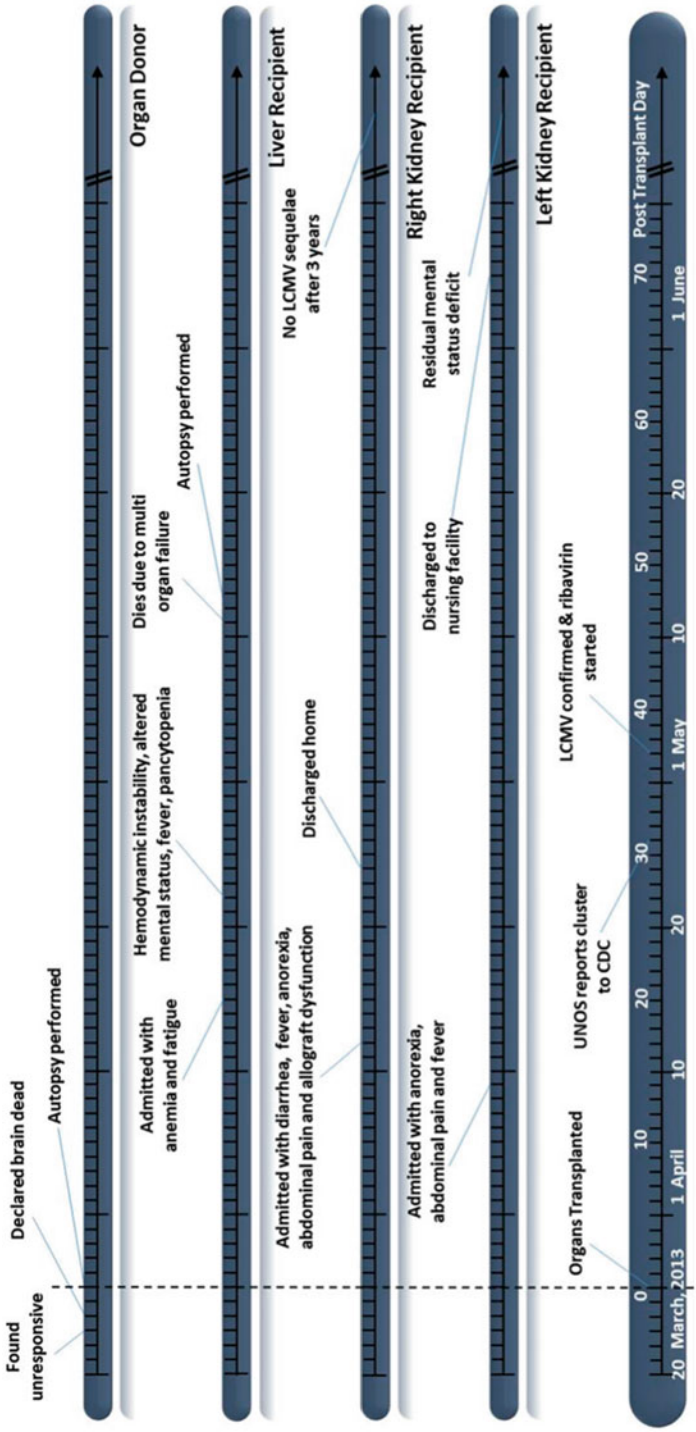


Fig. 1 Clinical timelines for LCMV infection in a donor-derived cluster. (Reproduced with permission, first published in: Mathur G, Yadav K, Ford B, Schafer II, Basavaraju SV, Knust B, Shieh WJ, Hill S, Locke GD, Quinlisk P, Brown S, Gibbons A, Cannon D, Kuehnert M, Nichol ST, Rollin PE, Ströher U, Miller R. High clinical suspicion of donor-derived disease leads to timely recognition and early intervention to treat solid organ transplant-transmitted lymphocytic choriomeningitis virus. Transpl Infect Dis. 2017 Aug;19(4). https://doi.org/10.1111/tid.12707. Epub 2017 May 24)

Diagnosis

Leukopenia, thrombocytopenia, and transaminitis were seen in most of the donor-derived cases [12, 16]. CSF analysis may show lymphocytic pleocytosis with WBCs ranging from 300 to 3000 cells/cc. CSF glucose might be slightly low with an elevated protein [16].

In the United States, the CDC typically runs more specific tests for LCMV. The virus can be isolated in blood or CSF with the use of in vitro cell culture. Serological testing using IF assays, enzyme-linked immunosorbent assay (ELISA), or Western blot may be done [17]. As per the reports on the clusters from 2003 and 2005, histopathological findings included necrotic and occasionally hemorrhagic foci in all infected tissues with a notable absence of inflammatory infiltrates and viral inclusions. LCMV antigens were present in affected tissues. In one case, even though the donor had no evidence of prior infection, he had known exposure to a hamster that harbored LCMV with the same sequence thereby proving causality [12]. This was replicated in one of the outbreaks in Australia in 2008 where there was a strong suspicion for donor-derived disease as all recipients of the donor fell ill [19]. These reports and those from several subsequent cluster analyses included organ donors with no evidence of LCMV infection, neither serologies nor tissue positive for LCMV presence, thus making diagnosis a challenge in deceased organ donors [16]. A high index of suspicion and a close look at all recipients of a donor are required to make these diagnoses.

Treatment

One of the kidney recipients in the 2005 cluster was treated with ribavirin for 37 days and survived. Immunosuppression was also reduced. Therapy was stopped once RT-PCR studies on kidney biopsy samples were negative, and the development of IgM antibodies was noted [12]. However, no trials have been done that prove ribavirin efficacy in treating LCMV. In the SOT-associated LCMV outbreak in 2011, two out of the four afflicted survived without ribavirin [20]. Ribavirin has been used to treat Lassa fever and has in vitro activity against LCMV, but it is unclear if the survival of the transplant recipient was due to ribavirin or was due to a reduction in immunosuppression [12]. The utility of ribavirin and immunoglobulin administration remains unclear.

Favipiravir, currently approved as an anti-influenza drug in Japan, has in vitro activity against several RNA viruses including arenaviruses. A study was conducted in mice that were inoculated with lower-dose LCMV virus to produce a protracted illness and mortality rate of 70–80%, mirroring the target population of organ transplant recipients. Results showed 300 mg/kg/day of favipiravir had superior efficacy if started within 2 days of symptom onset when compared to a 100 mg/kg/day ribavirin group and a placebo group. Survival was not improved if favipiravir was started 6 days after symptom onset. The equivalent dosing in humans would be approximately 24 mg/kg/day, and studies have shown good tolerance with doses as high as 40 mg/kg/day [21].

Emerging Viruses

There have been several instances of other infectious encephalitides transmitted through organ transplants, such as Eastern equine encephalitis and borna disease virus.

Eastern equine encephalitis virus (EEEV) is an arbovirus that cycles between the *Culiseta* mosquito and birds residing in freshwater hardwood swamps with sporadic infections in other vertebrates through bridging vectors like the *Aedes* mosquito. The average incubation period in EEEV infection is 4–10 days. Between 2003 and 2016, a median of 8 cases has been reported in the United States annually, typically in the 20 states along the Atlantic and Gulf Coasts. Although rare, it is one of the most severe arboviral illnesses with a fatality rate close to 30%. Most persons infected are either asymptomatic or suffer from a mild, nonspecific illness. Symptoms may include fever, and <5% progress to have symptoms like headaches, confusion, neurologic deficits, seizures, or coma. Survivors may have lasting neurological sequelae like hemiparesis, seizure disorder, or cognitive dysfunction. The risk of neuroinvasive disease may depend on the route of infection or immunosuppressed state.

The first instance of transplant-associated EEEV encephalitis transmission was reported in the fall of 2017 where one donor transmitted the infection to three recipients. All three presented with signs and symptoms of neuroinvasive infection within a week of transplantation; two of them died, while one survived with neurologic deficits. Similar to other viral encephalitides, the diagnosis of these cases required a high index of suspicion by the infectious diseases physicians involved. It is essential to investigate the recipients of the donor for unusual symptoms to diagnose [22].

Diagnostic testing may reveal peripheral leukocytosis and CSF neutrophilic pleocytosis and hypoglycorrhachia. Brain MRI may be revealing of T2/FLAIR sequence showing enhancement of the basal ganglia, thalami, and brain stem. Serum EEEV IgM usually turns positive within the first week of infection. At times, convalescent testing may have to be performed to confirm serologies. Viremia usually resolves by the time neurological symptoms occur thereby limiting the utility of PCR testing on serum and CSF. Cluster analysis showed that PCR testing on whole blood might be of greater yield when compared to that performed on serum and CSF. EEEV antigens and RNA may also be detected in transplanted tissue by IHC and RT-PCR. Pathological evaluation may demonstrate cytopathic effects with resulting necrosis.

No human vaccine or antiviral therapy exists against EEEV infection. IVIG was used in all three recipients, but no controlled trials have proven efficacy with this therapeutic modality.

A risk of transfusion-associated of infection also exists as blood is not routinely screened for EEEV. Given that most infections are asymptomatic, a high index of suspicion is required to evaluate potential donors for this infection. The lack of an ideal screening test or rapid diagnostic test to detect the virus during the incubation period limits our ability to prevent transmission to organ recipients. Knowledge of

local surveillance data, especially with respect to equine disease prevalence and mosquito pools in the organ donor's region, may be helpful in strengthening screening criteria [22].

As per a report in NEJM in 2018 [23], borna disease virus-1 (BoDV) transmission through solid organ transplantation occurred in Germany, and three recipients were infected from the same donor, of which two died. This virus is known to cause progressive meningoencephalitis in horses and sheep. However, zoonotic transmission to humans through pet squirrels has been reported. The donor had no signs or symptoms of neurological illness on presentation (Table 1). The recipients presented with varied neurological symptoms including facial palsy, anosmia, and acute, flaccid tetraparesis between 80 and 112 days posttransplantation. Neuroimaging revealed encephalitis and cerebral atrophy. Diagnostic metagenomic sequencing performed on a brain biopsy specimen of one of the recipients confirmed the diagnosis. It was further confirmed by the detection of viral antigens in the tissue using RT-PCR and IHC techniques. Identical BoDV-1 sequence was obtained from a brain biopsy specimen of the second recipient. The third recipient's infection was confirmed by antibody testing on serum and CSF. The source and state of infection in the donor could not be ascertained [23]. BoDV is a lethal disease and should be considered as a potential cause of viral encephalitis in recipients when the donor could have had potential direct or indirect contact with BoDV reservoirs.

Prevention of Donor Association Transmission

Screening all organ donors for these rare viruses is resource consuming and impractical given the time constraints required for donor evaluation and the difficulties with next-of-kin medical histories. However, having a standardized case definition coupled with a uniform screening procedure for infectious encephalitis by organ procurement organizations may help in the early detection of potentially lethal transmissible infections. Rapid screening test panels in such targeted cases might be helpful [4].

In the United States, the Organ Procurement and Transplantation Network (OPTN) Disease Transmission Advisory Committee (DTAC) developed guidelines to help OPOs and transplant clinicians decide if a donor could be at increased risk of an undiagnosed central nervous system infection [24]. A recent paper developed a risk calculation to predict donors with nonbacterial central nervous system infections and report the following features in the donor are concerning: male gender, fever, immunocompromised donor, CSF protein >45 mg/dl, altered mental status within 48 h of presentation, psychiatric features during hospitalization, cranial nerve abnormalities, meningeal signs, focal motor weakness, sensory disorders, movement disorders, and Babinski's reflex [25–27].

Caution is recommended when deciding to use organs from such donors. If diagnostic tests are sent on the donor, results will likely not return prior to transplant. Therefore, expedited notification of results must be sent to all receiving transplant

Table 1 Summary of rare viral encephalitides in organ transplantation

Infection	Donor symptoms	Recipient symptoms	Time of presentation posttransplant	Mortality rate	Treatment options
Rabies	Vague symptoms with significant overlap. Including but not limited to fever, nausea, vomiting, abdominal pain, confusion, agitation, autonomic dysfunction, and respiratory failure	Predominant neurological symptoms, signs and symptoms of autonomic dysfunction complicated by respiratory failure, cardia arrhythmias, coma, and death	Majority of the cases presented within 27–42 days posttransplantation. One case who presented 17 months later	20/30 (67%)	Postexposure prophylaxis with Ig and vaccine
	One donor had raccoon exposure. Donor from another cluster did have history of a bat bite. Canine exposure in donors has also been reported				
LCMV	Fever and other vague symptoms eventually progressing to neurological deterioration. Symptoms unrelated to LCMV infection	Fever, abdominal pain, diarrhea, peri-incisional erythema and tenderness, rash, altered mental status, and eventually progressing to multisystem organ failure	2–3 weeks	12/17 (71%)	Ribavirin, favipiravir, IVIG (unclear efficacy of these therapies)
	Some had rodent exposure				
EEEV	No signs or symptoms of neurological disease	Signs and symptoms of neuroinvasive disease including headaches, altered mentation, seizures, coma, etc.	Within 1 week	2/3 (67%)	IVIG
BoDV-1	No signs or symptoms of neurological disease	Varied neurological symptoms including facial palsy, anosmia, and acute, flaccid tetraparesis	80–112 days posttransplantation	2/3 (67%)	Unknown

centers along with national and local governing bodies once results are available [4, 27, 28].

The key to the prevention of rabies virus transmission globally is to address the baseline disease prevalence and improve disease reporting in developing countries of Asia and Africa [6] coupled with effective vaccination strategies for those potentially exposed or at high risk of exposure through animal contact. There are no vaccines that target LCMV, EEEV, or BoDV infection.

Zoonotic transmission of these viruses is a risk posttransplant with possible drastic outcomes in the setting of immunosuppression. Therefore, it is essential for recipients to be aware of the specific preventive measures and limit exposure to exotic animals [12, 29]. Patients should be sure to vaccinate pets for rabies as recommended and avoid handling wild animals such as bats or raccoons. Patients should also be educated about mosquito protection posttransplant.

Prevention of Recipient-Acquired Infection

Quite separate to infection that may be transmitted from donors, transplant recipients are at risk of routine community-acquired viral encephalitis at similar rates to the general community. Under these circumstances precise data on the clinical outcomes is lacking, but high mortality might be expected. Prevention is the best path forward; therefore understanding the natural transmission cycles of the viruses involved is key.

For rabies there are a number of animal hosts in the United States and across the world. Bats, domestic and wild dogs, foxes, coyotes, and raccoons all carry rabies to varying degrees in North America. Bites from any of these animals, or in fact even finding roosting bats inside houses of transplant recipients, should prompt a rapid discussion about rabies postexposure vaccination. For West Nile and Eastern equine encephalitis, mosquitos are the nature vector, as is the case with dengue, Zika, chikungunya, and a number of other infections relevant to transplant. During the summer months, a careful discussion of mosquito avoidance with transplant recipients can help attenuate the risk of illness, especially those traveling. Finally, LCMV is typically associated with rodents, and while complete avoidance is often challenging, patients should be discouraged from keeping such animals as pets in their posttransplant phase.

Challenges and Next Steps

The rare but dangerous possibility of donor-derived transmission of rabies, LCMV, EEEV, and BoDV is fraught with many challenges. The overall risk of transmission of these infections is low given low prevalence, especially in the developed nations [3]. However, the incidence may increase as resource-limited countries perform more transplants [6].

Underreporting is a possibility as many of these infections are difficult to diagnose and require specialized tests that are not commercially available [6]. Overlapping symptoms, difficulty in obtaining and processing samples from donors and recipients along with limited testing ability for rare infections, and geographical spread of transplant centers that might receive organs from one potentially infected donor limit the ability to diagnose rare transplant-related infections [4]. Variable presentations in diseases like rabies, e.g., bat variant, differing from classic bites, vague symptoms in prodromal phase, and rapidity of deterioration once in fulminant neurological phase makes it a difficult diagnosis to make, especially if exposure is not suspected or known. Donors often present with symptoms that could fit with a wide variety of diagnoses, making definitive diagnosis problematic [3].

Screening all donors for these infections is not practical [4]. Antemortem testing exists for most of these infections but requires a high index of clinical suspicion and often involves use of send-out tests or testing by the CDC [3]. Imaging findings are typically vague. Paucity of rapid diagnostics leads to delay in diagnosis and usually is confirmed after the transplant has occurred.

A standardized case definition and targeted screening of donors that are high risk may help in early detection of potentially lethal transmissible infections [4, 25]. In the era of globalization, worldwide travel increases risk for exposures to unusual and rare infections in patients – hence it is of utmost importance to get a good travel, social, and occupational history during donor screening [7]. There is a need for developing risk scores and calculators to identify infectious versus noninfectious causes of encephalitis. Obviously, while doing so, it is important to balance the risk of declining uninfected organs [27].

Rapid diagnostics are changing the overall scene in medicine, especially in the field of infectious diseases. They can help assess for the presence of infection in donors prior to transplantation or soon after transplant and therefore allow early initiation of appropriate management [12]. They can help identify potentially infectious donor organs so that such organs may only be used in extreme or dire cases with prior consent from the patient along with instituting appropriate posttransplant management protocols, thereby trying to reduce mortality [4]. In spite of high fatality rates from these infections, it is essential to diagnose them early to institute appropriate infection control measures and reduce spread [3].

As per a report in 2008 [19], unbiased high-throughput sequencing was used to identify a new variant of arenavirus that was potentially implicated as a cause of encephalitis in organ transplant recipients. Deep sequencing assays and other forms of metagenomic testing can be helpful in identifying rare organisms causing encephalitis especially when conventional tests fail to do so. Over the upcoming years, new and unusual infections are bound to appear in even more atypical fashions especially in the immunocompromised host. Availability, validation, and interpretation of these next-generation testing protocols should be considered high priority if we wish to tackle these complex clinical conundrums [30, 31].

The clusters of transplant-related infectious encephalitides underscore the importance of maintaining a high index of suspicion in potential donors for such infections that could have fatal consequences. Essential data on screening, diagnosis, and

treatment remain limited [32]. In cases of rabies and LCMV, a global registry to document experience with PEP in infected organ recipients would allow a better understanding of these clinical entities [15].

Efficient communication between the OPTN (or similar bodies outside the United States), local organ procurement organizations, hospitals dealing with all recipients, local health officials, and caregiver teams with ancillary staff including lab personnel is key to a swift diagnosis and implementation of management plans [25]. It requires added efforts on behalf of everyone in the organ transplant community including the Department of Health and Human Services, the CDC, hospital networks, physicians, and support staff to maintain the organ supply chain and avoid adverse events [32]. These efforts would go a long way in mitigating, if not eradicating, the risk of transmitting lethal infections via organ transplantation.

Conclusions

Clusters of these infections, though rare, have led to increased awareness about the severity of these illnesses, their high mortality rates, and of the transplant community being somewhat underprepared to deal with them. It is key to maintain a high index of suspicion for these infections in organ donors presenting with unexplained neurological illnesses, apt exposure history, and appropriate epidemiological correlation. Regulating organ transplants from such donors is a possible way of preventing transmission to organ recipients and could help in avoiding further complications. However, as we all know, this is easier said than done given the paucity of vital organs all over the world. Rigorous screening with new-age diagnostics and therapies may help protect these immunocompromised hosts from dire consequences in the future.

Key Points

- A number of highly pathogenic viral infections can lead to clinical encephalitis in transplant recipients. Although each remains rare, the high mortality mandates concern and mitigation.
- Careful donor evaluation can help mitigate the risk of donor-derived viral encephalitis for solid organ recipients, which has a high penetrance among all recipients.
- Few treatments exist once viral infection is established. Ribavirin and favipiravir have been studies in small case series for LCMV, and postexposure prophylaxis should certainly be given to anyone exposed to rabies, irrespective of the route of exposure.
- Teaching transplant recipients safe living techniques, especially vector avoidance, can go a long way to help reducing the risk of many significant viral infections.
- Clinicians should continue to remain vigilant that new viral infections emerge with some frequency, and often transplanted immunosuppressed patients have the

gravest and most severe clinical outcomes. Having an open mind for viral discovery can help identify infections, particularly clusters, more rapidly, especially in an era of expanded microbiologic testing.

References

1. Kotton CN. Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. Clin Infect Dis. 2007;44(6):857–66.
2. Basavaraju SV, Kuehnert MJ, Zaki SR, Sejvar JJ. Encephalitis caused by pathogens transmitted through organ transplants, United States, 2002–2013. Emerg Infect Dis. 2014;20(9):1443–51.
3. Burton EC, Burns DK, Opatowsky MJ, El-Feky WH, Fischbach B, Melton L, et al. Rabies encephalomyelitis: clinical, neuroradiological, and pathological findings in 4 transplant recipients. Arch Neurol. 2005;62(6):873–82.
4. Vora NM, Basavaraju SV, Feldman KA, Paddock CD, Orciari L, Gitterman S, et al. Raccoon rabies virus variant transmission through solid organ transplantation. JAMA. 2013;310(4):398–407.
5. Javadi MA, Fayaz A, Mirdehghan SA, Ainollahi B. Transmission of rabies by corneal graft. Cornea. 1996;15(4):431–3.
6. Lu XX, Zhu WY, Wu GZ. Rabies virus transmission via solid organs or tissue allotransplantation. Infect Dis Poverty. 2018;7(1):82.
7. Bronnert J, Wilde H, Tepsumethanon V, Lumlertdacha B, Hemachudha T. Organ transplantations and rabies transmission. J Travel Med. 2007;14(3):177–80.
8. Dietzschold B, Koprowski H. Rabies transmission from organ transplants in the USA. Lancet. 2004;364(9435):648–9.
9. CDC. Investigation of rabies infections in organ donor and transplant recipients – Alabama, Arkansas, Oklahoma, and Texas, 2004. MMWR Morb Mortal Wkly Rep. 2004;53(26):586–9.
10. CDC. First human death associated with raccoon rabies – Virginia, 2003. MMWR Morb Mortal Wkly Rep. 2003;52(45):1102–3.
11. Zhang J, Lin J, Tian Y, Ma L, Sun W, Zhang L, et al. Transmission of rabies through solid organ transplantation: a notable problem in China. BMC Infect Dis. 2018;18(1):273.
12. Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med. 2006;354(21):2235–49.
13. Hay E, Derazon H, Bukish N, Scharf S, Rishpon S. Postexposure rabies prophylaxis in a patient with lymphoma. JAMA. 2001;285(2):166–7.
14. Rodríguez-Romo R, Morales-Buenrostro LE, Lecuona L, Escalante-Santillán N, Velasco-Villa A, Kuzmin I, et al. Immune response after rabies vaccine in a kidney transplant recipient. Transpl Infect Dis. 2011;13(5):492–5.
15. Vora NM, Orciari LA, Niezgoda M, Selvaggi G, Stosor V, Lyon GM, et al. Clinical management and humoral immune responses to rabies post-exposure prophylaxis among three patients who received solid organs from a donor with rabies. Transpl Infect Dis. 2015;17(3):389–95.
16. Tanveer F, Younas M, Fishbain J. Lymphocytic choriomeningitis virus meningoencephalitis in a renal transplant recipient following exposure to mice. Transpl Infect Dis. 2018;20(6):e13013.
17. Rousseau MC, Saron MF, Brouqui P, Bourgeade A. Lymphocytic choriomeningitis virus in southern France: four case reports and a review of the literature. Eur J Epidemiol. 1997;13(7):817–23.
18. Bonthius DJ. Lymphocytic choriomeningitis virus: an underrecognized cause of neurologic disease in the fetus, child, and adult. Semin Pediatr Neurol. 2012;19(3):89–95.
19. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. N Engl J Med. 2008;358(10):991–8.

20. Macneil A, Ströher U, Farnon E, Campbell S, Cannon D, Paddock CD, et al. Solid organ transplant-associated lymphocytic choriomeningitis, United States, 2011. Emerg Infect Dis. 2012;18(8):1256–62.
21. Hickerson BT, Westover JB, Jung KH, Komeno T, Furuta Y, Gowen BB. Effective treatment of experimental lymphocytic choriomeningitis virus infection: consideration of favipiravir for use with infected organ transplant recipients. J Infect Dis. 2018;218(4):522–7.
22. Pouch SM, Katugaha SB, Shieh WJ, Annambhotla P, Walker WL, Basavaraju SV, et al. Transmission of eastern equine encephalitis virus from an organ donor to three transplant recipients. Clin Infect Dis. 2018;69(3):450–8.
23. Schlottau K, Forth L, Angstwurm K, Höper D, Zecher D, Liesche F, et al. Fatal encephalitic Borna disease virus 1 in solid-organ transplant recipients. N Engl J Med. 2018;379(14):1377–9.
24. Organ Procurement and Transplantation Network. Guidance for recognizing central nervous system infections in potential deceased organ donors. Available at https://optn.transplant.hrsa.gov/resources/guidance/guidance-for-recognizing-central-nervous-system-infections-in-potential-deceased-organ-donors/. Accessed 27 May 2019.
25. Malinis M, Boucher HW, AST Infectious Diseases Community of Practice. Screening of donor and candidate prior to solid organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13548.
26. Wolfe CR, Ison MG, AST Infectious Diseases Community of Practice. Donor-derived infections: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13547.
27. Smalley HK, Anand N, Buczek D, Buczek N, Lin T, Rajore T, et al. Assessment of risk for transplant-transmissible infectious encephalitis among deceased organ donors. Transpl Infect Dis. 2018;20(5):e12933.
28. Organ Procurement and Transplantation Network. Guidance for recognizing central nervous system infections in potential deceased organ donors: what to consider during donor evaluation and organ offers. Available at https://optn.transplant.hrsa.gov/ContentDocuments/Guidance_DTAC_CNS_Infections.pdf (Internet).
29. Avery RK, Michaels MG, AST Infectious Diseases Community of Practice. Strategies for safe living following solid organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13519.
30. Wilson MR, Zimmermann LL, Crawford ED, Sample HA, Soni PR, Baker AN, et al. Acute West Nile virus meningoencephalitis diagnosed via metagenomic deep sequencing of cerebrospinal fluid in a renal transplant patient. Am J Transplant. 2017;17(3):803–8.
31. Chiu CY, Coffey LL, Murkey J, Symmes K, Sample HA, Wilson MR, et al. Diagnosis of fatal human case of St. Louis encephalitis virus infection by metagenomic sequencing, California, 2016. Emerg Infect Dis. 2017;23(10):1964–8.
32. Srinivasan A, Burton EC, Kuehnert MJ, Rupprecht C, Sutker WL, Ksiazek TG, et al. Transmission of rabies virus from an organ donor to four transplant recipients. N Engl J Med. 2005;352(11):1103–11.

Part IV

Fungal Infections Introduction

Candida Infections in Solid Organ Transplantation

44

Nicolas Barros and Michael K. Mansour

Contents

Introduction 1046
Epidemiology, Risk Factors, and Prophylaxis 1047
Lung Transplant 1047
Heart Transplant 1049
Liver Transplant 1051
Kidney 1053
Pancreas 1054
Small Intestine 1055
Donor-Derived *Candida* Infections 1055
Candida Diagnostics 1056
Culture-Based Diagnostic Methods 1057
Non-culture Diagnostic Methods 1058
Polymerase Chain Reaction 1059
T2 Magnetic Resonance (T2MR) 1059
Treatment 1060
Special Considerations: Emergence of *Candida auris* 1064
Therapeutic Drug Monitoring 1064
Conclusion 1066
Future Directions 1066
KeyPoints 1066
References 1067

N. Barros
Division of Infectious Diseases, Indiana University School of Medicine, Indianapolis, IN, USA
e-mail: nbarros@iu.edu

M. K. Mansour (✉)
Transplant Infectious Diseases and Immunocompromised Host Service, Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA

Department of Medicine, Harvard Medical School, Boston, MA, USA
e-mail: mkmansour@mgh.harvard.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_43

Introduction

Recipients of solid organ transplantation (SOT) are at increased risk of fungal infections. Among these pathogens, invasive candidiasis (IC) remains the most common fungal infection in patients with solid organ transplantation except for lung transplant recipients, where invasive *Aspergillus* is the dominant organism [1]. IC is defined as a *Candida* infection from an otherwise sterile space and should be distinguished from oral thrush, esophagitis, and vaginitis.

While IC rates are among the highest in small bowel transplant (SBT) accounting for 85% of all invasive fungal infections (IFI), the rates for other organ transplants vary; IC represents IFI in 68–80% of orthotopic liver transplant (OLT), 23–47% in lung transplant (LT), 38–68% in orthotopic heart transplant (OHT), 49–60% in kidney transplant (KT), and 76% in pancreatic transplant (PT) [1–3]. Unlike hematopoietic stem cell transplants where *Candida glabrata* is the most common species (likely due to widespread fluconazole prophylaxis), *C. albicans* is the most common species in SOT (30–74%) followed by *C. glabrata* (30–38%), *C. parapsilosis* (9–12%), *C. tropicalis* (5–6%), and *C. krusei* (2–5%) [3–7]. However, the incidence of species varies according to transplanted organ and use of prophylaxis [5]. In addition, the prevalence of infections due to different *Candida* species has significant geographical variations [8–11] (see Table 1).

Table 1 Geographic distribution of *Candida* species. (Reference in Parenthesis)

	C. albicans (%)	*C. glabrata* (%)	*C. parapsilosis* (%)	*C. tropicalis* (%)	*C. krusei* (%)
SENTRY study [9]					
Asia-Pacific	46.0	17.9	12.9	14.1	1.8
Europe	52.5	16.0	15.4	7.5	3.0
Latin America	43.9	7.1	24.3	17.0	2.0
North America	42.7	24.3	14.8	8.0	2.9
Total	46.7	18.7	15.9	9.3	2.8
Artemis study [10]					
Asia-Pacific	64.4	12.6	7.4	11.7	1.2
Europe	67.9	11.3	4.2	4.9	3.4
Africa-Middle East	67.1	8.8	6.0	6.6	1.6
Latin America	51.8	7.4	10.0	13.2	1.4
North America	48.9	21.1	13.6	7.3	3.1
Total	63.8	11.6	6.3	7.7	2.5
PATH Alliance [3]					
North America	41.1	38.4	11.3	5.5	2.7
SOT in North America	33.2	38.4	11.3	5.5	2.7
TRANSNET study [2]					
SOT in North America	46.3	24.4	8.1	3.9	3.1

Clinically, of all forms of IC, bloodstream infection with *Candida* or candidemia is the most common presentation across all SOT. In the Transplant-Associated Infection Surveillance Network (TRANSNET) study, candidemia was found in 64% OHT, 54% LT, 32% PT, 44% KT, and 41% OLT [2]. Deep intra-abdominal infections are the second most common presentation, accounting for approximately 14% of all IC [2]. However, intra-abdominal infections are more common following abdominal transplants compared to thoracic, found in about a quarter of abdominal transplants but less than 5% of thoracic transplants [2, 3]. In lung transplant, intrathoracic infections are the second most common presentation of candidiasis (i.e., empyema, tracheobronchitis, and pulmonary abscesses) [2]. Urinary tract infections represent the third most common form of IC accounting for approximately 11% [7].

The median time to occurrence varies between different cohorts, which may be related to epidemiological changes, transplantation technique, prophylaxis protocols, and the expanded types of transplanted organs and tissues. In general, candidemia was diagnosed early between 2 and 6 months following SOT. In a large cohort from the United States consisting of 16,808 patients between 2001 and 2006, the median time to occurrence was 80 days [1]. A multicenter prospective Australian cohort of 1068 patients between 2001 and 2004 showed that 52% of the cases of IC occurred after 6 months [2, 12], although they only analyzed cases of candidemia and most of the patients were renal transplant recipients, which may explain the late onset of IC presentation. All IC cases in LT recipients in that cohort occurred within the first 30 days which may be secondary to prior airway colonization, complexity of transplant, and the increased net state of immunosuppression. In support of early IC presentation in LT, another study by Scheanman et al. confirms that the median time to presentation in LT was indeed early at 25 days [13].

The all-cause mortality range related to IC is between 21% and 34%, although significant variation exists depending on the species, transplanted organ, and comorbidities (e.g., diabetes, COPD, renal insufficiency, CHF) [7, 12, 14]. The mortality is higher for non-*albicans* species (*C. glabrata* 27%, *C. krusei* 31–52%, *C. parapsilosis* 23–35%, and *C. tropicalis* 44%) as compared to *C. albicans* at 22%. In regard to mortality related to organ type, the highest proportion is noted in LT (36%) followed by OLT (30%), OHT (25%), kidney (22%), kidney-pancreas (15%), and pancreas (13%) [2].

Epidemiology, Risk Factors, and Prophylaxis

Lung Transplant

Mortality in lung transplant recipients strongly correlates with allograft rejection and infections. To prevent rejection and improve allograft survival, lung transplant recipients are often managed with high levels of immunosuppression, which increases the risk for invasive fungal pathogens and other opportunistic infections.

Candida infections were historically the most common type of IFI following lung transplantation ranging from 43% to 80% [15–17]. However, the incidence of IC has decreased from 20% in 1980–1986 to 1.8% in 2001–2004 [13]. Despite this decrease in incidence, a cohort of 16 centers in the United States from 2001 to 2006 investigating 1195 lung transplant recipients (TRANSNET) found that the 12-month incidence of IC in lung transplant is the highest at 8.6% in contrast to non-lung SOT where incidences are lower (OLT 4.7%, OHT 4%, pancreas 3.4%, and KT 1.3%). Although *Candida* infections are common in lung transplant, *Aspergillus* remains the most common IFI representing nearly double the *Candida* rate at 44% of all IFI cases in lung transplant recipients [1].

In contrast to the TRANSNET registry, subsequent single-center studies spanning from 2003 until 2014 have demonstrated a higher prevalence of IC ranging from 11.4% to 23%. The authors suggested that their high incidence of IC may have been the result of the lack of prophylaxis and breakthrough infections with nebulized amphotericin B [18, 19]. The median time to occurrence is also highly variable ranging from 6 days to 52 days [7, 19]. However, most results suggest that IC is an early post-lung transplantation event and usually occurs within 30 days [13, 18, 19].

Recently, a prospective study nested within the Swiss Transplant Cohort Study reported all clinically relevant infections in 3541 solid organ transplant recipients. Among 286 lung transplant recipients, 45% developed IC, of which 65% were due to *C. albicans* [3]. In all studies, *C. albicans* represents the most common species isolated (60–70%), followed by *C. glabrata* (13–16%), *C. parapsilosis* (16%), and *C. tropicalis* (11–14%) [2, 18]. However, there are significant regional and center-to-center variations, specifically in the non-*albicans* composition.

In a cohort that spanned almost three decades, Schaenman et al. showed that tracheobronchitis (38%) was the most common *Candida* infection followed by candidemia (28%) [13]. Subsequent studies have shown that candidemia is the most common presentation followed by intrathoracic infections (pleural space and surgical site) which may reflect more modern prophylactic interventions [1, 2, 4, 18, 19]. Multiple infection sites (e.g., candidemia plus intrathoracic infections or intra-abdominal) remain common ranging from 25% to 40% [12, 18]. Pneumonia secondary to *Candida* species is a rare entity and is seen in less than 5% of all IC [13].

The overall mortality for IC in LT recipients is 36.4% [2]. However, there is significant variation within the 90-day mortality according to species (mortality by species, *C. albicans* 22.6%, *C. glabrata* 27.7%, *C. krusei* 31.5%, *C. parapsilosis* 35.2%, and *C. tropicalis* 44%). Organ dysfunction, disseminated infection, and allograft rejection were independently associated with poor outcomes [2]. Although the overall mortality for IC is high, it may represent a marker of overall poor clinical status in addition to potential *Candida* virulence factors. In fact, the *Candida* attributable mortality was estimated to be lower at 15% in one study [13].

Multiple studies to correlate risk factors for *Candida* infection, including pre-transplant diagnosis of cystic fibrosis, pre-transplant *Candida* colonization (presence of fungus in the respiratory secretions in the absence of symptoms, radiologic and endobronchial changes), acute and chronic allograft rejection, cytomegalovirus infection, and hypogammaglobulinemia, found that the only significant risk factors

for IC included the type of lung transplant (single vs. double lung allograft), primary graft dysfunction (PGF), use of airway stents, and allograft rejection [2, 20, 21]. Interestingly, all these variables suggest a more complicated post-transplant clinical course and are likely associated with intensified anti-rejection regimens.

Koo et al. assessed a targeted strategy of adding intravenous echinocandin (micafungin in this study) to nebulized amphotericin B during the first 7–10 days for patients with bilateral lung transplant followed by 3–6 months of further oral antifungal therapy only in individuals with positive microbiologic presence of yeast or mold in their day-of-transplant cultures. Those recipients with positive fungal growth received oral antifungal therapy tailored to their fungal isolate [19]. When comparing their pre- and post-intervention analysis, the authors note a reduction in *Candida* infections of 90% suggesting that a targeted strategy can be effective for those with positive cultures at the time of transplant. The 2015 International Society of Heart and Lung Transplant (ISHLT) guidelines recommend that, depending on local epidemiology, universal prophylaxis *Candida*-active agents should be considered such that nebulized amphotericin B +/− fluconazole or echinocandin should be used in the first 2–4 weeks post-transplant to target *Candida* species [20] (Table 2).

Heart Transplant

The epidemiology of invasive candidiasis in OHT has shifted over recent decades. The incidence of IC has decreased from 6.1% in the 1980s to 2.1% in the early

Table 2 Indications for post-transplant prophylaxis. Recommended prophylaxis by organ. (Adapted from Practice ASTIDCoP [28])

Transplanted organ	Recommended prophylaxis
Lung	Universal prophylaxis with agents that have systemic activity against *Candida* species should be considered in the immediate post-Tx period (i.e., first 2–4 weeks)
	Following this period, universal or preemptive prophylaxis are suitable options depending on the transplant center and should be considered for up to 6 months
Heart	Routine *Candida* prophylaxis is not recommended
Kidney	Routine *Candida* prophylaxis is not recommended
Pancreas	Prophylaxis should be considered if the patient has one or more risk factors including enteric drainage, vascular thrombosis, and post-perfusion pancreatitis. Duration should be individualized according to persistence of risk factors
Small intestine	Universal prophylaxis is recommended for at least 4 weeks or until the anastomosis has healed, and rejection is not present
Liver	Prophylaxis is recommended in recipients with $\geq$2 of the following risk factors: prolonged or repeat operation; re-transplantation; renal failure; high transfusion requirement (transfusion of $\geq$40 units of cellular blood products including platelets, packed red blood cells, and auto transfusion); choledocojejunostomy; and *Candida* colonization in the perioperative period

2000s [22]. In the TRANSNET cohort (2001–2006), Pappas et al. showed that the cumulative incidence at 12 months of an invasive fungal infection in OHT patients was 3.4%. Invasive candidiasis was the most frequent invasive fungal disease and represented half of the infections [1]. Similarly, in a large Italian cohort from the 1985 until 1997, Grossi et al. demonstrate that the incidence was 2.2% [23]. Unlike the TRANSNET study, invasive aspergillosis was the most common IFI, and IC represented only 19% of all invasive fungal infections. It is uncertain if the discrepancy is due to regional variations or due to differences in the surveyed time period. Interestingly, over 50% of the IFI occurred within 30 days of an episode of acute rejection.

A recent report from the Swiss transplant cohort study showed that IC represented 38% of all IFI, of which 86% were secondary to *C. albicans* [3]. A subsequent analysis of the TRANSNET study revealed that candidemia remains the dominant IC clinical presentation at 65%, followed by intrathoracic infections (i.e., deep surgical site infections, empyema) at 8.3% and disseminated disease representing 6.2% of all cases. In the TRANSNET update, *C. albicans* represented 53% of all isolated species, followed by *C. glabrata* (19%), *C. parapsilosis* (8.5%), and *C. krusei* (4.3%).

In a retrospective analysis investigating the development of IFI, Tissot and colleagues showed that renal replacement therapy, extra-corporeal membrane oxygenation, and prior airway colonization with *Aspergillus* species were independent risk factors for the development of IFI [24]. Subsequently, they provided targeted prophylaxis to a high-risk group, which included caspofungin, and showed a significant reduction of IFI as well as improved 30-day mortality. In addition, no cases of IC were identified in patients with targeted prophylaxis as compared to 9% in the control, non-prophylaxis group [24]. Another cohort has shown that increased induction immunosuppression or delayed chest closure are independent risk factors for the development of invasive fungal disease. However, these patients received OKT3 (anti-CD3) and daclizumab (anti-CD25) as induction immunosuppression, which are no longer used for induction rendering this data difficult to extrapolate to contemporary cohorts [25].

Other induction agents have been investigated for increased risk of IFI. Echenique et al. showed that the use of anti-thymocyte globulin, antibody-mediated rejection, and renal replacement therapy were independent risk factors associated with IFI [26]. None of the studies provided risk factors specific for the development of IC.

The use of IFI chemoprophylaxis has been studied in OHT with some success. Muñoz et al. identified the protective effects of itraconazole given as universal prophylaxis for invasive aspergillosis in OHT. Although they showed a significant prolonged 1-year survival, this study compared all heart transplant recipients from 1988 through 1995 (without prophylaxis) to 1995 until 2002 (with prophylaxis). The authors conclude that the use of itraconazole in OHT recipients decreased IFI from 11% to 3%. That said, during the study periods, significant improvements in perioperative management and immunosuppressive medications were made in parallel rendering the individual impact of itraconazole on survival difficult to ascertain. Interestingly, no cases of invasive candidiasis were noted in patients with prophylaxis [27].

Currently, neither the 2015 guidelines from the international society of heart and lung transplantation nor the 2019 guidelines from the American Society of Transplantation Infectious Diseases Community of Practice have specific recommendations for either targeted or universal prophylaxis against invasive candidiasis following heart transplantation [20, 28].

Liver Transplant

The advances in surgical technique, medical management, and more modern immunosuppressive strategies have led to a decrease in the incidence of invasive fungal infections over the past three decades in OLT. Before the advent of prophylactic strategies, the risk for invasive fungal infections in liver transplant recipients ranged from 7% to 42% [29, 30]. While a wide range of fungal pathogens were found post-liver transplant, the vast majority of IFI in liver transplant recipients are secondary to invasive candidiasis (80%), possibly due to *Candida* as a normal commensal in the gastrointestinal tract coupled with the technical necessity to surgically manipulate the bowel. During the first 6 months following liver transplant, IC represents 85% of all IFI but then drops to 40% in the subsequent 6 months following transplant. *C. albicans* remains the dominant species isolated in patients with invasive candidiasis ranging from 45% to 65% [31–33]. There are regional variations on prevalence of non-*albicans* species. In some reports, *C. parapsilosis* is responsible for 19–28% of IC [31, 32, 34].

Most of the cases occur within the first month with a median time to occurrence of 10–21 days, though older reports described later times to infection [31, 35, 36]. The mortality related to IC remains high, ranging from 25% to 70% compared to liver transplant recipients without *Candida* infection (2.8–6.4%) [32, 37–39].

A wide array of risk factors have been described for IC in OLT, including hospitalization prior to liver transplantation, renal failure with or without requirement for dialysis (creatinine >2), split liver grafts, living donation, transplant due to acute fulminant hepatic failure, high blood product transfusions, abdominal reoperation including re-transplantation, bile leaks, thymoglobulin, colonization with *Candida* species, and choledocojejunostomy anastomosis [31, 32, 34–36, 38, 40–43].

Several studies have correlated severity of pre-transplant liver disease status with *Candida* infection following OLT. In a study by Bassetti et al., 83% of patients with candidemia had a pre-transplant MELD score >15 [33]. In addition, Saliba et al. showed that pre-transplant MELD score of 20–30 or ≥30 was associated with a 2.1-fold or 3.1-fold increase in relative risk of invasive candidiasis, respectively [41].

A simple predictive model for risk stratification showed that the risk of invasive fungal infection ranged from 1% in patients with no predictors to 67% in patients with two or more predictors (creatinine of >3.0 mg/dL, operative time of > or = 11 h, re-transplantation, and early colonization) [44]. Based on these observations, the American Society of Transplantation Infectious Diseases Community of Practice has recognized patients as high risk for development of invasive fungal disease if they

have ≥2 risk factors including prolonged or repeat operation, re-transplantation, renal failure, high transfusion requirement (≥40 units of cellular blood products including platelets, packed red blood cells, and autotransfusion), choledocojejunostomy, and *Candida* colonization in the perioperative period (isolation of *Candida* in at least two non-sterile sites (e.g., urine, sputum) in the absence of signs or symptoms of invasive disease in the 4 weeks preceding transplantation) [28].

Due to the high incidence of invasive candidiasis following liver transplant, multiple trials have addressed the use of prophylactic antifungal therapy during the immediate post-transplant period. Earlier trials of universal prophylaxis with liposomal amphotericin B showed that it was more effective than placebo with IC rates close to 0% versus 16%, respectively. In addition, this intervention was cost-effective [45]. A recent study of a simplified targeted prophylaxis with amphotericin B lipid complex or liposomal amphotericin B on high-risk individuals was safe and applicable and was associated with low IFI incidence in both the high-risk group (5.4%) and low-risk group (0%) [38].

Given their safety profile, multiple triazoles (e.g., fluconazole, itraconazole) have been studied for prophylaxis. In a double-blind randomized control trial of universal prophylaxis with itraconazole vs. placebo, Sharpe et al. showed that itraconazole was well tolerated and reduced the rate of IFIs in patients undergoing OLT (4% with itraconazole vs. 24% with placebo) [46]. In another study, the use of fluconazole led to a significant decrease of the risk of IC. However, the incidence remained higher than expected, which was attributed to using lower doses of fluconazole (100–200 mg once daily) [31]. Compounding this lower dosage may have led to the surge of fluconazole-resistant organisms including *C. glabrata* [34, 47]. Although fewer data on prophylactic itraconazole is available, several studies suggested similar efficacy when compared to fluconazole [46, 48, 49].

While prophylaxis in high-risk individuals has been shown to be effective in reducing the risk of invasive candidiasis, the use of universal prophylaxis with fluconazole on a large cohort of liver transplant recipients did not show a reduction in the risk of invasive candidiasis, suggesting that prophylaxis may need to be rationally applied in a risk-stratified manner separating those with high- and low-risk profiles [39].

Echinocandins (e.g., anidulafungin, micafungin) are effective in the reduction of IFI incidence when used as targeted prophylaxis in high-risk individuals [38]. In addition, echinocandins have similar efficacy in reducing the risk of invasive candidiasis in high-risk individuals when compared to amphotericin B (liposomal amphotericin B and amphotericin B lipid complex) and triazoles [40].

There has been controversy about the use of antifungal prophylaxis and the potential to produce selective pressure, leading to antifungal resistance or selective population pressure. Raghuram et al. found that only 43% of their *C. albicans* and 33% of *C. glabrata* isolates were fluconazole sensitive. However, a low dose of fluconazole was prescribed in that trial (100 and 200 mg once daily) which may have been a factor for resistance selection [31]. In fact, a meta-analysis by Playford et al. found that fluconazole prophylaxis did not increase invasive fungal infections or

colonization with fluconazole-resistant fungi [49]. Echinocandin prophylaxis has also been associated with an increased risk of IC due to *C. parapsilosis* and echinocandin resistance [53].

Based on all these studies, the American Society of Transplantation Infectious Diseases Community of Practice recommends that antifungal prophylaxis should be given to all adult liver transplant recipients at high risk for development of IC ($\geq$2 of the following risk factors: prolonged or repeat operation, re-transplantation, renal failure, high transfusion requirement [transfusion of $\geq$40 units of cellular blood products including platelets, packed red blood cells, and autotransfusion], choledocojejunostomy, and *Candida* colonization in the perioperative period) [28] (Table 2).

The duration of prophylaxis is poorly defined with significant duration variability among the studies. Reported durations extend from 5 days up to 8 weeks. Most studies have used a variety of endpoints including empiric durations of 4 weeks and use of prophylaxis until discharged or when identified risk factors have resolved [38, 40, 42, 45–47, 52, 54, 55].

Patients on the wait list are at increased risk of fungal infections which in turn can negatively affect the outcomes of liver transplant candidates and recipients, causing disease progression and increased mortality [56]. Patients with severe alcoholic hepatitis admitted to the intensive care unit (ICU) have an increased susceptibility to invasive mycoses associated with high mortality. Lahmer et al. showed that 25% of patients admitted to the ICU due to alcoholic hepatitis developed invasive candidiasis with two thirds succumbing prior to transplant [57]. In a sub-analysis report of the Fungal Infection Risk Evaluation (FIRE) study, Theocharidou et al. showed that the incidence of IFI in cirrhotic patient is higher compared to other critically ill patients in a medical ICU (1% vs. 0.4%) but in-hospital mortality was similar in the two groups [58]. Risk factors for development of IFI in patients with cirrhosis include the use of prophylactic antibiotics, high MELD score, and hepatorenal syndrome. Fungal infections are an emerging problem in cirrhosis. In addition, there is a shift toward increased prevalence of *C. glabrata* and *C. parapsilosis*. This trend in turn may affect the success of antifungal prophylaxis and empiric therapy following transplant. Delayed diagnosis due to a low index of suspicion is common which may contribute to a high mortality rate [59, 60]. Despite these studies, there are no current guidelines on how to risk stratify patients, and most authors do not recommend antifungal prophylaxis in the pre-transplant setting [59].

Kidney

Kidney transplant remains the most common type of transplant performed in the United States and worldwide (organ procurement and transplantation network, 2019) [61]. Fortunately, the incidence of candidiasis and invasive fungal infections remains low. A large cohort of 8672 patients from March 2001 through March 2006 in 15 different transplant centers in the United States showed that the incidence of IFI

in renal transplant recipients was 1.3% and half (49%) of those cases were secondary to IC [1]. More contemporary cohorts have shown similar incidences of IFI ranging from 1.3% up to 5% with IC representing 29–55% of all fungal infections [3, 62–68].

However, there are significant regional variations as described by Santos et al. In Iran the incidence of IFI was reported at 0.87%, while in Turkey, Kuwait, and India, it was 4%, 3.5%, and 14%, respectively [63]. In North America, the incidence of IFI is 1.3% while in Spain is 1.7% [1, 69]. Specifically, IC represents about 49% of all IFI [1]. Over the past two decades, *C. albicans* had been the most common isolated species, and more contemporary cohorts suggest that *C. glabrata* is rapidly rising and may soon represent the most common invasive *Candida* species in KT [2, 67, 70–72].

The most common presentation of invasive candidiasis are bloodstream infections followed by intra-abdominal infections (44% and 11%, respectively). Candiduria is common following KT but is rarely of clinical significance with a median time to occurrence of 25 days, likely to be diabetic, female patients who have experienced significant prior antimicrobial exposure. Antifungal therapy in patients with asymptomatic candiduria has been shown to have no impact on the risk of recurrence, urinary clearance, or the development of severe complications [2, 71–73]. Given the low incidence of infection, and the lack of impact of treatment of asymptomatic candiduria, both the Infectious Diseases Society of America (IDSA) and the American Society of Transplantation Infectious Diseases Community of Practice do not recommend antifungal therapy for asymptomatic candiduria [28, 74] (Table 2).

Pancreas

The incidence of IC following pancreas transplantation ranges from 2.7% to 20% [1, 75–79]. There are no differences in the incidence of invasive candidiasis between different types of pancreas transplants (simultaneous pancreas-kidney, pancreas transplant alone, pancreas after kidney) [80]. Up to two thirds of the cases occur during the first 6 months [78].

Based on a single-center study by Benedetti et al., both the IDSA guidelines and the American Society of Transplantation Infectious Diseases Community of Practice recommend systemic perioperative antifungal prophylaxis for enteric-drained pancreas transplant recipients, vascular thrombosis, and post-perfusion pancreatitis [28, 74] (Table 2). However, this study presented multiple limitations, including large differences in the study populations and a lack of multivariate analysis. In addition, the group that received fluconazole continued to have a high incidence of fungal infections compared to other studies [79]. In subsequent studies of universal antifungal prophylaxis, no difference in intra-abdominal fungal infections has been found between bladder and enteric-drained pancreas allografts [81, 82].

In a study of 154 enteric-drained pancreas transplants, Bartoletti et al. showed that 1.9% of the patients developed invasive *Candida* infections [76]. While all the

patients were scheduled to receive antifungal prophylaxis, more than 10% failed to follow the protocol and did not receive prophylaxis. The lack of antifungal prophylaxis did not affect the incidence of IC [76]. Similar to Bartoletti, in a recent single-center study, Shaik et al. showed that enteric-drained pancreas transplant recipients who did not receive systemic antifungal prophylaxis had similar infectious complications, including an incidence of invasive candidiasis of 3.6%, which is similar to previously reported rates [75].

Small Intestine

Recipients of small bowel are at extremely high risk for the development of invasive candidiasis. The rate of invasive fungal infections ranges from 25% up to 59%, of which 80–100% represent invasive candidiasis. Recipients of small bowel alone are at higher risk, when compared to small bowel and liver and multivisceral transplants [83, 84].

The Prospective Antifungal Therapy (PATH) Alliance is a comprehensive multicenter, prospective, observational registry that collects detailed clinical data on patients with IFIs, with special emphasis on fungal epidemiology, diagnosis, treatment, and associated patient outcomes [6]. While both the TRANSNET and the PATH Alliance registries showed that *C. albicans* represented almost half of all *Candida* isolates, studies including only small bowel transplant recipients show that *C. albicans* infection can range from 22% to 53% [83–85]. In the largest cohort, a mix of non-*albicans* species represented two thirds of all isolates [84]. This observation may be secondary to the use of azoles as prophylaxis. Candidemia is the most common presentation (14–66%), followed by intra-abdominal infections (28–37.5%) and *Candida* isolated from the urinary tract (2.5–40%) [83, 85–87]. Patients at high risk are those with graft rejection or dysfunction, enhanced immunosuppression, anastomotic disruption, abdominal re-operation, or multivisceral transplantation. Despite the lack of clinical trials, most experts recommend continuing prophylaxis with fluconazole as the preferred agent for at least 4 weeks and until the anastomosis has completely healed and there are no signs of rejection [28] (Table 2). Studies investigating use of selective oral decontamination of the gastrointestinal tract with antimicrobial agents show that this strategy has no impact on the rate of bloodstream infections or abdominal fungal infections [88].

Donor-Derived *Candida* Infections

Candida species are one of the most common fungal pathogens transmitted from donors to recipients and are associated with frequent graft dysfunction, as well as significant morbidity and mortality [89]. In kidney transplants, it is estimated that 1 case of donor-derived invasive candidiasis will occur per 1000 grafts with a median time to occurrence of 25 days [90].

Many of these infections are presumed to be secondary to preservation fluid contamination, which can occur prior to or at the time of organ procurement. The rates of preservation fluid contamination range from 9.5% up to 37%, although of those specifically due to *Candida* species range from 0.6% up to 4.1% [91–96]. *C. albicans* remains the most common species identified ranging from 25% up to 64% of all cases [93, 95, 96].

The risk factors for contamination of the preservation fluid include intestinal perforation at time of procurement, multiorgan procurement, and en bloc transplantation [91]. While the contamination of preservation solution in kidney transplants does not increase the risk of allograft pyelonephritis, multiple reports of both kidney and liver transplant indicate an increased risk for vascular complications including arteritis and aneurysms, graft site abscess, surgical site infections, and graft loss [90, 95, 97–99].

Despite the high rates of preservation fluid contamination, there are few studies on how to manage these infections. A study by Levesque et al. showed that treatment of liver transplant recipients in whom *Candida* sp. was isolated from the preservation fluid only reduced the rate of complications from 33% to 27% [95]. However, a study by Oriol et al. showed that pre-emptive therapy with high-risk preservation fluid contamination (defined as isolation of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacte*r spp. and/or *Candida* species) led to a decrease in the rate of infections [96]. In the cohort with contaminated high-risk preservation fluid with pre-emptive therapy, no complications were found versus a rate of 8.5% complications in the contaminated high-risk group without pre-emptive therapy. This suggests that pre-emptive therapy in solid organ recipients with high-risk culture-positive preservation fluid may lead to a decrease in subsequent preservation fluid-related infections. Donor candidemia has also been implicated in donor-derived infections [68]. In addition, a recent study has shown the transmission of *C. auris* in a lung transplant recipient whose donors' bronchial tree was colonized with *C. auris* [100]. Hence, the presence of *Candida* in donor cultures should prompt consideration for treatment.

Candida Diagnostics

The gold standard for diagnosis of invasive candidiasis relies on the isolation of *Candida* species using a culture-based method. This goal can be achieved by either blood cultures or collecting deep tissue specimens. Other techniques include the detection of components of the *Candida* cell wall including mannan/anti-mannan or 1-3-β-D-glucan. In addition, new molecular strategies allow for the detection of *Candida* nucleic acid using direct from blood PCR. The sensitivity and specificity of these tests vary depending on the pathogen load (e.g., transient candidemia vs. persistent candidemia vs. no candidemia) and prior exposure to therapy and most importantly the net state of immunosuppression [74, 101] (Table 3).

Table 3 Diagnostic test performance for invasive candidiasis. Performance of diagnostic tests for invasive candidiasis in blood samples. Mn, *Candida* mannan antigen; Anti-Mn Ab, Anti-*Candida* mannan antibody; PCR, Polymerase chain reaction; T2MR, T2 magnetic resonance. (References in parenthesis)

Test	Sensitivity (%)	Specificity (%)	Comments
Culture [104]	21–71	NA	In the presence of candidemia, the sensitivity is higher than in patients with invasive candidiasis without candidemia
l-3-β-D-glucan [109–113]	58–85	80–98	Performance depends on cutoff value. Has a low positive predictive value
Mannan [114–118]			
per sample	17–61	92–99	
per patient	53–62	91–94	
Anti-Mn Ab [114–118]			
per sample	23–39	87–100	
per patient	54–65	79–87	
Mn/Anti-Mn Ab [114–118]			
per sample	55–68	94–98	
per patient	79–87	82–90	
PCR [119]	88–98	88–95	
T2MR [121–123]	86–94	99	Sensitivity and specificity vary by species

Culture-Based Diagnostic Methods

The sensitivity of blood cultures in invasive candidiasis ranges between 21% and 71% with an overall sensitivity of about 50% [101]. Blood cultures are sensitive for the detection of viable *Candida*, and the limit of detection is $\leq$1 colony-forming unit/mL [102]. Although the sensitivity of blood cultures for *Candida* bloodstream infections with or without deep-seated infections is about 75%, the sensitivity is much lower in cases of low-level candidemia, intermittent candidemia, deep-seated candidiasis that persists after sterilization of the bloodstream, or deep-seated candidiasis without candidemia [101, 103]. Furthermore, the sensitivity of tissue cultures from deep-seated infections is approximately 50% which may be related to sampling error, small sample volumes, or low burden of infection [104]. While most of the studies include immunocompromised populations, there are no specific results for the performance of blood and tissue cultures for the diagnosis of invasive candidiasis in the solid organ transplant population.

Blood and tissue cultures have slow turnaround time (median time to positivity of 2–3 days) with 98% of positive cultures detected by the first week and 99% by the second week [105]. Given that the time to initiation of antifungal therapy impacts the morbidity and mortality in patients with candidemia, more rapid diagnostic tools are in need [106–108]. However, cultures have the advantage of providing identification to a species level as well as providing antifungal susceptibility testing.

Non-culture Diagnostic Methods

1-3-β-D-Glucan, Mannan, and Anti-mannan Antibodies

The lack of sensitivity of blood cultures in the diagnosis of invasive candidiasis, especially deep-seated infections without candidemia, has led to the development of multiple tests to detect components of the fungal cell wall. 1-3-β-D-Glucan, *Candida* mannan, and circulating IgG anti-*Candida* mannan antibodies have been tested during the past decades with variable results.

1-3-β-D-Glucan can be detected by multiple commercial platforms as a surrogate marker of invasive candidiasis with or without candidemia. 1-3-β-D-Glucan is a cell wall component of multiple pathogenic fungi, including as *Aspergillus* species, *Pneumocystis jirovecii*, and many others with few exceptions including *Cryptococcus* and species from the *Mucorales* group. False positive tests are associated with infusion of albumin, intravenous immunoglobulins, hemodialysis with cellulose membranes, gauze packing of serosal surfaces, and infections with gram-negative bacteria, including *Pseudomonas aeruginosa* [109, 110].

There have been ample reports on the performance of 1-3-β-D-glucan for invasive fungal infections [109, 111, 112]. In two recent meta-analyses, the sensitivity of a positive result was 75–80%, and the specificity was ~80% [113, 114]. Studies of active surveillance for invasive candidiasis in high-risk surgical patients in the intensive care unit showed that the sensitivity 1-3-β-D-glucan increased when the test was repeated twice weekly [112, 115]. Furthermore, two consecutive positive results within the same week increased the specificity of the test [116].

Koo et al. showed that higher values of 1-3-β-D-glucan are associated with higher positive likelihood ratios. Furthermore, sensitivity and specificity of the test were not affected by the prior use of antifungal therapy [109].

The use of circulating *Candida* antigens, including mannan antigen (Mn), and anti-mannan antibodies (A-Mn-Ab) has been reported in several studies. Prior studies have shown that positive Mn was preferentially observed in the absence of A-Mn antibodies, while high levels of A-Mn antibodies are usually not seen with circulating Mn [117]. Mikulska et al. showed that the pooled sensitivity for Mn, A-Mn-Ab, and Mn/A-Mn-Ab was 58%, 59%, and 83%, respectively, and had a pooled specificity of 93%, 83%, and 86%, respectively [118]. Interestingly, 75% of the patients with candidemia had a positive Mn and A-Mn-Ab test prior to the positive fungal blood culture which increased up to 86% on the day of positive blood cultures. Furthermore, 86% of patients with hepatosplenic candidiasis had positive Mn or A-Mn-Ab prior to the development of characteristic radiological features [118].

The presence of circulating Mn varies depending on the type of IC presentation. Previous studies show that Mn was detected in only 7% of patients with transient or catheter-associated candidemia compared with 76% with persistent candidemia or IC [119, 120]. In addition, the test is more sensitive with *C. albicans, C. glabrata*, and *C. tropicalis* (sensitivity of 80–100%) compared to *C. krusei* and *C. parapsilosis* (sensitivity of 40–50%) [121].

Polymerase Chain Reaction

PCR-based methods to detect *Candida* spp. from positive blood cultures are now commercially available. Avni et al. reported a meta-analysis of 54 studies, where they show that the pool sensitivity for proven/probable IC is 95% (88–98%) and specificity of 92% (88–95%), with a positive likelihood ratio of 14 and a negative likelihood ratio of 0.05 [122]. However, there is a remarkable heterogeneity in these studies. The sensitivity of PCR varies according to the type of IC syndrome. While the sensitivity in candidemia is only 50–62%, the sensitivity for deep-seated infections is 83–95%. A possible explanation is related to the intermittent release of circulating DNA with line-related infections versus more constant circulating DNA as a result of deep-seated infections [103]. The inoculum of *Candida* CFU per mL affects the sensitivity of PCR as it can reliably detect 1 CFU per mL in 5% of the cases versus 83% for 10 CFU per mL and 100% for 100 CFU per mL [123]. Given that it does not rely on the detection of live organisms, the test is not affected by the use of antifungal therapy. When paired with blood cultures, the sensitivity of PCR (PCR plus blood culture) rose to 98% suggesting that this test may be an important complementary tool for the relatively insensitive blood cultures [103].

T2 Magnetic Resonance (T2MR)

T2MR combines the nuclear magnetic resonance and PCR molecular assay to directly detect and identify the species of *Candida* spp. directly from whole blood. The T2MR system can detect *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C tropicalis*, and *C. krusei* [124].

In the Direct study, the pooled sensitivity of a positive T2MR was 91.1 (86–94.2%) and a specificity of 99.4% (99.1–99.6%) [125]. Both the sensitivity and specificity varied by species such that *C. albicans/C. tropicalis* has a sensitivity of 92.3% and specificity of 98.9%; *C. parapsilosis*, sensitivity 94.2% and specificity 99.3%; and *C. krusei/C. glabrata*, sensitivity 88.1% and specificity 99.9%. In a population with a prevalence of IC of 5–10%, the positive predictive value ranged from 71.6% to 84.2%, and the negative predictive value ranges from 99.5% to 99.0% [125]. In contrast, in the general hospital setting, the prevalence of candidemia is significantly lower, which would limit the positive and negative predictive values.

In a recent study in the ICU, the use of negative T2MR test allowed for the early discontinuation of antifungal agents. In patients with negative T2MR tests, only 2%

developed proven candidemia with a median time of 21 days (range, 9–30 days) which raises the possibility of a de novo infection rather than a false negative. The short turnaround time and the high negative predictive value suggest that T2MR may be a valuable adjunctive test for antifungal stewardship [126]. There is limited data on the performance of T2MR in the diagnosis of deep-seated infections without candidemia.

Treatment

Empiric therapy for invasive candidiasis should be initiated as soon as the diagnosis is suspected, as early treatment initiation decreases mortality [106–108, 127, 128]. The choice of therapeutic agent should take into consideration the epidemiology of *Candida* species in the local medical center, prior exposure to antifungal agents, and the tissue penetration of the drug. In addition, removal of central line catheters and drainage of the potential source have direct impact on survival [74, 129, 130].

There are four major classes of antifungal medications available for the treatment of invasive candidiasis, with several new classes in development. These include triazoles, polyenes, echinocandins, and flucytosine. It is important to note that the recommendations on the treatment of IC are largely derived from studies on patients with candidemia and other common forms of IC [131].

Early studies showed that fluconazole was not inferior to amphotericin B in the management of candidemia in non-neutropenic patient [132]. Although there was a trend toward more rapid clearance from bloodstream, the combination of fluconazole plus amphotericin B deoxycholate was not superior to fluconazole alone [133]. Subsequently, Kullberg et al. showed that voriconazole was as effective as a regimen of amphotericin B in the treatment of candidemia [134]. Other studies have shown that echinocandins, including caspofungin, anidulafungin, and micafungin, are as effective as amphotericin B deoxycholate and were associated with significantly lower adverse effects [135, 136]. There are no significant differences between echinocandins, and higher doses have not been shown to be superior to standard dosing, except in the management of *Candida* esophagitis and infective endocarditis [137, 138].

In patients with IC due to *C. albicans*, anidulafungin was more effective than fluconazole, with more rapid clearance of positive blood cultures [138]. A meta-analysis by Andes et al. showed that therapy with an echinocandin (e.g., micafungin, anidulafungin, caspofungin) had significantly better survival rates than either a polyene (e.g., amphotericin B) or a triazole (e.g., fluconazole, voriconazole) [139].

Given these data, echinocandins are recommended as the first line of therapy for candidemia by the Infectious Diseases Society of America except for tissue with low penetration, including eyes, central nervous system, and urinary tract. Initial echinocandin treatment can then be followed by step-down to oral triazole therapy depending on *Candida* isolated and susceptibility to complete the therapy [28, 74]. Recommendations for the agent of choice, dosing, and length of therapy are detailed in Table 4.

Table 4 Treatment of invasive candidiasis. (Adapted from Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America and Practice ASTIDCo. Candida infections in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Disease Community of Practice)

Type of infection	Primary	Alternative	Duration	Comments
Candidemia and invasive candidiasis	Echinocandin: Micafungin 100 mg/day Caspofungin 70 mg loading then 50 mg/day Anidulafungin 200 mg loading then 100 mg/day	Azole: Fluconazole 12 mg/kg loading dose and then 6 mg/kg IV/PO daily if the patient is not critically ill and fluconazole-resistance is unlikely Lipid formulation amphotericin B (AmB) (3–5 mg/kg daily) is a reasonable alternative if other antifungals are not an option (availability or resistance)	If there is no obvious metastatic complication, therapy should be continued for 2 weeks from documented clearance of bloodstream infection Transition to an azole is recommended after 5–7 days if the patient is stable and the isolates are susceptible to the selected azole	Central venous catheters (CVCs) should be removed as early as possible Dilated ophthalmological examination should be performed in all patients within the first week, except in neutropenic patients in whom the exam should be delayed until recovery from neutropenia For invasive candidiasis, duration of therapy may be at least 2 weeks and resolution of symptoms attributable to invasive candidiasis Source control, with drainage and/or debridement should be included in intra-abdominal candidiasis or abscess/ collections.
Chronic disseminated candidiasis	Lipid formulation amphotericin B (AmB) (3–5 mg/kg daily) Echinocandin: Micafungin 100 mg/day Caspofungin 70 mg loading then 50 mg/day Anidulafungin 200 mg loading then 100 mg/day	Azole: Fluconazole 12 mg/kg loading dose and then 6 mg/kg IV/PO daily if fluconazole-resistance is unlikely	3–6 months and resolution or calcification of radiologic lesions	Transition to an azole can be considered if the patient is stable and the isolate is susceptible or resistance to selected azole is unlikely

(continued)

Table 4 (continued)

Type of infection	Primary	Alternative	Duration	Comments
Endocarditis and implantable cardiac devices	Lipid formulation amphotericin B (AmB) (3–5 mg/kg daily) with or without Flucytosine 25 mg/kg PO 4 times daily High dose echinocandin: Micafungin 150 mg/day Caspofungin 150 mg/day Anidulafungin 200 mg/day			Transition to an azole can be considered if the patient is stable and the isolate is susceptible to selected azole Valve replacement is recommended If valve is not replaced or patient has prosthetic valve endocarditis, chronic suppressive antifungal therapy with a susceptible azole is recommended For pacemakers and implantable cardiac defibrillators, the entire device should be removed and therapy extended for 4–6 weeks after removal
Osteoarticular and septic arthritis	Lipid formulation amphotericin B (AmB) (3–5 mg/kg daily) Echinocandin: Micafungin 100 mg/day Caspofungin 70 mg loading then 50 mg/day Anidulafungin 200 mg loading then 100 mg/day	Azole: Fluconazole 12 mg/kg loading dose and then 6 mg/kg IV/PO daily if fluconazole-resistance is unlikely	6–12 months for osteoarticular infections. At least 4 weeks for septic arthritis	In osteoarticular infections, transition to an azole can be considered after 2 weeks of IV therapy with either a AmB or echinocandins if the isolate is susceptible to selected azole In septic arthritis, surgical drainage is indicated Device removal is recommended in prosthetic joint infections. If device is not removed, chronic suppressive antifungal therapy with a susceptible azole is recommended

Endophthalmitis	Azole: Fluconazole 12 mg/kg loading dose and then 6 mg/kg IV/PO daily Voriconazole 6 mg/kg loading dose and then 4 mg/kg IV/PO twice daily Lipid formulation amphotericin B (AmB) (3–5 mg/kg daily)	Intravitreal lipid formulation amphotericin B 5–10 mcg Voriconazole 100 mcg	At least 4–6 weeks after surgery	Vitrectomy is usually performed when vitreitis is present
Pyelonephritis	Azole: Fluconazole 3–6 mg/kg IV/PO daily Amphotericin B deoxycholate 0.3–0.6 mg/kg IV D with or without flucytosine 25 mg/kg PO 4 times	Flucytosine 25 mg/kg PO 4 times daily	7–14 days	Nephrostomy tubes or stents in place should be removed or replaced if feasible Elimination of urinary tract obstructions is indicated
Esophageal	Azole: Fluconazole 3–6 mg/kg IV/PO daily Itraconazole solution 200 mg PO D Voriconazole 3 mg/kg PO twice daily	Echinocandin: Micafungin 100 mg/day Caspofungin 70 mg loading then 50 mg/day Anidulafungin 200 mg loading then 100 mg/day	14–21 days	Mild – moderate oropharyngeal infection can be treated with Clotrimazole troche or Nystatin

Special Considerations: Emergence of *Candida auris*

C. auris is an emerging pathogenic fungus that has been reported from at least 32 countries on 6 continents during 2009–2019 [140]. *C. auris* was isolated in 2009 from the outer ear of a Japanese patient [141]. Since that time, there has been a simultaneous appearance around the world that segregates in four major clades [142]. As of now, it is unclear how these clades of *C. auris* appeared in synchronized fashion and the significance of this phenomenon. This yeast is found predominantly on the skin, and there is good evidence that it does not live as part of the gastrointestinal tract and healthy microbiome. *C. auris* is able to persist in the environment for extended periods of time and poses a challenge in the decontamination with conventional cleaners resulting in persistence and multiple reported hospital and ICU outbreaks [143, 144]. *C. auris* presents with reduced susceptibility to fluconazole in over 90% of cases and with reduced susceptibility to voriconazole, itraconazole, posaconazole, and isavuconazole. Varying susceptibility to amphotericin B (35–43% resistance) and resistance to echinocandins (7%) have been reported. Furthermore, 41% of the isolates were resistant to two classes of antifungals, and 4% were resistant to all three classes of antifungal agents (triazoles, echinocandins, and polyenes). Flucytosine has broad antifungal activity but has to be given in combination due to rapid emergence of resistance [74]. The combination of flucytosine with either amphotericin B or echinocandins (i.e., micafungin, caspofungin, and anidulafungin) resulted in in vitro inhibition of most *C. auris* isolates [145]. There are no concrete therapeutic guidelines for the management of *C. auris* infections. The consequences of an active *C. auris* infection are high, with some studies reporting up to a 50% mortality rate [146]. Hence, case-by-case options should be followed in consultation with infectious diseases specialists [147].

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is indicated when the standard dosing of any given drug results in marked variability in drug blood concentrations and when relationships between drug exposure (plasma drug level) and either efficacy or toxicity have been established. The plasma drug levels of certain medications may vary due to inconsistent absorption, metabolism, elimination, or interaction with concomitant medications [148–150].

Fluconazole has a predictable pharmacokinetics (PK) with a direct correlation between dosing and blood levels. Although a recent report of 27 liver transplant recipients showed increased treatment success in patients with trough plasma levels >11 mcg/mL, most studies have not measured clinical impact with fluconazole TDM [151–154]. For that reason, current guidelines do not support the use of TDM for fluconazole [28, 74].

Itraconazole has intrinsic suboptimal absorption. In addition, the drug-drug interactions lead to significant PK variability despite the recommended therapeutic

dosing ranges. The suboptimal absorption is particularly poor under circumstances of impaired GI integrity, reduced acidic environment which is required for capsules, and oral intolerance leading to poor compliance, although improved with newer formulations. For itraconazole, when measured by high-pressure liquid chromatography (HPLC), both itraconazole and its bioactive hydroxy-itraconazole metabolite are reported, the sum of which should be considered in assessing drug levels [74, 149]. TDM is recommended for long-term use of itraconazole.

While the absorption of voriconazole is usually reliable, there are large variabilities in PK between individuals. There is ample evidence that TDM is associated with better treatment success and less drug discontinuation due to adverse events [155, 156].

Like the triazoles voriconazole and itraconazole, the PK of posaconazole has marked interpatient variability, and there is increased efficacy when TDM is applied [157]. Isavuconazole is the newest triazole available for management of invasive fungal infections. The utility of TDM of isavuconazole has been unclear, and currently there is insufficient data to support TDM for this agent.

Circulating levels of flucytosine have a strong relationship with both toxicity and efficacy. The strongest of these associations has been bone marrow toxicity with levels grated than 100 mcg/mL [148–150]. For these reasons, there is strong evidence to support TDM when using flucytosine.

Altogether, TDM for itraconazole, voriconazole, posaconazole, and flucytosine are recommended for optimizing the therapy of mucosal and invasive candidiasis [28, 74]. Given reliable pharmacokinetics, neither echinocandins nor amphotericin B nor fluconazole require TDM (see Table 5).

Table 5 Therapeutic drug monitoring parameters based on high performance liquid chromatography. [a] Itraconazole levels are a result of adding both itraconazole and hydroxy-itraconazole (active metabolite). Echinocandins and polyenes do not require therapeutic drug monitoring. (References [28, 77])

	Efficacy		Safety	Levels obtained	
Triazole	Prophylaxis	Treatment	Toxicity	Related to dose	Day
Fluconazole	Not indicated				
Itraconazole[a]	≥0.5 mcg/mL	1.0–2.0 mcg/mL	No data	30 min pre-dose	7–14 days
Voriconazole	≥0.5 mcg/mL	1.5–5.5 mcg/mL	<6 mcg/mL	30 min pre-dose	5–7 days
Posaconazole	≥0.7 mcg/mL	>1.0 mcg/mL	No data	30 min pre-dose	5–7 days
Isavuconazole	Optimal drug concentrations not established				
Antimetabolite					
5-Flucytosine		30–80 mcg/mL	<100 mcg/mL	2 h post-dose	3–5 days

Conclusion

Invasive candidiasis (IC) remains the most common invasive fungal infection (IFI) in patients with solid organ transplant with an unacceptable high morbidity and mortality. *C. albicans* is the most common isolated species though non-*albicans* species are increasing.

While isolation of the *Candida* species in a culture media remains as the goal standard, newer techniques including the detection of cell wall components and PCR are now available with higher sensitivities and more acceptable turnaround times. However, these newer techniques have varying sensitivity and specificity depending on the pathogen load and prior exposure to therapy.

Early initiation of therapy significantly improves survival, but given the limitations of the diagnostic tests, a high degree of clinical suspicion is required. Initial therapy usually involves an echinocandin, though in selected scenarios either amphotericin B or azoles are other options.

Finally, future research efforts in personalized microbiome analysis and host immune susceptibility profiling would allow for pre-emptive prophylactic strategies in selected, risk-stratified patients. Given the emergence of multidrug-resistant *Candida* species such as *Candida auris*, newer medications are in dire need to ensure safe outcomes for future transplant patients.

Future Directions

Invasive candidiasis continues to have a high morbidity and mortality. Early initiation of therapy has been demonstrated to have a positive impact in survival. Due to low sensitivity and long turnaround time of current diagnostic tests, clinicians are forced to have a high index of clinical suspicion. Future research should focus on better diagnostics tests with short turnaround times. In addition, microbiome dysbiosis analysis and host susceptibility profiling may be useful in risk stratification of patients and could potentially aid in defining pre-emptive prophylactic strategies for high-risk SOT recipients. Finally, the emergence of multidrug-resistant *Candida* species, including *Candida auris*, poses new threats to SOT patients and calls for research into newer therapeutic options.

KeyPoints

1. *Candida* species remain a common infectious complication following solid organ transplantation.
2. *Candida albicans* is the most common species isolated.
3. Non-albicans *Candida* species, including *C. glabrata*, are on the rise and demonstrate multidrug resistance.
4. A fungal pandemic caused by drug-resistant *C. auris* requires careful consideration in transplant programs.

5. Culture remains the gold standard for diagnosis, although nucleic acid-based tests are more efficient.
6. Therapeutic drug monitoring of specific antifungal agents permits a safer and more effective outcome.

References

1. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.
2. Andes DR, Safdar N, Baddley JW, Alexander B, Brumble L, Freifeld A, et al. The epidemiology and outcomes of invasive Candida infections among organ transplant recipients in the United States: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Transpl Infect Dis. 2016;18(6):921–31.
3. van Delden C, Stampf S, Hirsch HH, Manuel O, Meylan P, Cusini A, et al. Burden and timeline of infectious diseases in the first year after solid organ transplantation in the Swiss Transplant Cohort Study. Clin Infect Dis. 2020. https://doi.org/10.1093/cid/ciz1113.
4. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, et al. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance(R)) registry, 2004–2008. Diagn Microbiol Infect Dis. 2012; 74(4):323–31.
5. Lockhart SR, Wagner D, Iqbal N, Pappas PG, Andes DR, Kauffman CA, et al. Comparison of in vitro susceptibility characteristics of Candida species from cases of invasive candidiasis in solid organ and stem cell transplant recipients: Transplant-Associated Infections Surveillance Network (TRANSNET), 2001 to 2006. J Clin Microbiol. 2011;49(7):2404–10.
6. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis. 2009;48(12):1695–703.
7. Neofytos D, Fishman JA, Horn D, Anaissie E, Chang CH, Olyaei A, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2010;12(3):220–9.
8. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans *Candida* spp among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis. 2010;14(11):e954–66.
9. Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, et al. Epidemiology and outcomes of invasive candidiasis due to non-albicans species of Candida in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004–2008. PLoS One. 2014;9(7):e101510.
10. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty years of the SENTRY Antifungal Surveillance Program: results for *Candida* species from 1997–2016. Open Forum Infect Dis. 2019;6(Suppl 1):S79–94.
11. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol. 2010;48(4):1366–77.
12. van Hal SJ, Marriott DJ, Chen SC, Nguyen Q, Sorrell TC, Ellis DH, et al. Candidemia following solid organ transplantation in the era of antifungal prophylaxis: the Australian experience. Transpl Infect Dis. 2009;11(2):122–7.
13. Schaenman JM, Rosso F, Austin JM, Baron EJ, Gamberg P, Miller J, et al. Trends in invasive disease due to *Candida* species following heart and lung transplantation. Transpl Infect Dis. 2009;11(2):112–21.

14. Gavalda J, Meije Y, Fortun J, Roilides E, Saliba F, Lortholary O, et al. Invasive fungal infections in solid organ transplant recipients. Clin Microbiol Infect. 2014;20(Suppl 7):27–48.
15. Dauber JH, Paradis IL, Dummer JS. Infectious complications in pulmonary allograft recipients. Clin Chest Med. 1990;11(2):291–308.
16. Maurer JR, Tullis DE, Grossman RF, Vellend H, Winton TL, Patterson GA. Infectious complications following isolated lung transplantation. Chest. 1992;101(4):1056–9.
17. Kramer MR, Marshall SE, Starnes VA, Gamberg P, Amitai Z, Theodore J. Infectious complications in heart-lung transplantation. Analysis of 200 episodes. Arch Intern Med. 1993;153(17):2010–6.
18. Baker AW, Maziarz EK, Arnold CJ, Johnson MD, Workman AD, Reynolds JM, et al. Invasive fungal infection after lung transplantation: epidemiology in the setting of antifungal prophylaxis. Clin Infect Dis. 2019;70(1):30–39.
19. Koo S, Kubiak DW, Issa NC, Dietzek A, Boukedes S, Camp PC, et al. A targeted peritransplant antifungal strategy for the prevention of invasive fungal disease after lung transplantation: a sequential cohort analysis. Transplantation. 2012;94(3):281–6.
20. Husain S, Sole A, Alexander BD, Aslam S, Avery R, Benden C, et al. The 2015 International Society for Heart and Lung Transplantation guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients: executive summary. J Heart Lung Transplant. 2016;35(3):261–82.
21. Husain S, Mooney ML, Danziger-Isakov L, Mattner F, Singh N, Avery R, et al. A 2010 working formulation for the standardization of definitions of infections in cardiothoracic transplant recipients. J Heart Lung Transplant. 2011;30(4):361–74.
22. Montoya JG, Giraldo LF, Efron B, Stinson EB, Gamberg P, Hunt S, et al. Infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center. Clin Infect Dis. 2001;33(5):629–40.
23. Grossi P, Farina C, Fiocchi R, Dalla Gasperina D. Prevalence and outcome of invasive fungal infections in 1,963 thoracic organ transplant recipients: a multicenter retrospective study. Italian Study Group of Fungal Infections in Thoracic Organ Transplant Recipients. Transplantation. 2000;70(1):112–6.
24. Tissot F, Pascual M, Hullin R, Yerly P, Tozzi P, Meylan P, et al. Impact of targeted antifungal prophylaxis in heart transplant recipients at high risk for early invasive fungal infection. Transplantation. 2014;97(11):1192–7.
25. Rabin AS, Givertz MM, Couper GS, Shea MM, Peixoto D, Yokoe DS, et al. Risk factors for invasive fungal disease in heart transplant recipients. J Heart Lung Transplant. 2015;34(2):227–32.
26. Echenique IA, Angarone MP, Gordon RA, et al. Invasive fungal infection after heart transplantation: A 7-year, single-center experience. Transpl Infect Dis. 2017;19(1). https://doi.org/10.1111/tid.12650.
27. Munoz P, Rodriguez C, Bouza E, Palomo J, Yanez JF, Dominguez MJ, et al. Risk factors of invasive aspergillosis after heart transplantation: protective role of oral itraconazole prophylaxis. Am J Transplant. 2004;4(4):636–43.
28. Aslam S, Rotstein C, Practice ASTIDCo. Candida infections in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13623.
29. Paya CV. Prevention of fungal and hepatitis virus infections in liver transplantation. Clin Infect Dis. 2001;33(Suppl 1):S47–52.
30. Singh N, Wagener MM, Cacciarelli TV, Levitsky J. Antifungal management practices in liver transplant recipients. Am J Transplant. 2008;8(2):426–31.
31. Raghuram A, Restrepo A, Safadjou S, Cooley J, Orloff M, Hardy D, et al. Invasive fungal infections following liver transplantation: incidence, risk factors, survival, and impact of fluconazole-resistant *Candida parapsilosis* (2003–2007). Liver Transpl. 2012;18(9):1100–9.
32. Husain S, Tollemar J, Dominguez EA, Baumgarten K, Humar A, Paterson DL, et al. Changes in the spectrum and risk factors for invasive candidiasis in liver transplant recipients: prospective, multicenter, case-controlled study. Transplantation. 2003;75(12):2023–9.

33. Bassetti M, Peghin M, Carnelutti A, Righi E, Merelli M, Ansaldi F, et al. Invasive Candida infections in liver transplant recipients: clinical features and risk factors for mortality. Transplant Direct. 2017;3(5):e156.
34. Eschenauer GA, Kwak EJ, Humar A, Potoski BA, Clarke LG, Shields RK, et al. Targeted versus universal antifungal prophylaxis among liver transplant recipients. Am J Transplant. 2015;15(1):180–9.
35. Hadley S, Samore MH, Lewis WD, Jenkins RL, Karchmer AW, Hammer SM. Major infectious complications after orthotopic liver transplantation and comparison of outcomes in patients receiving cyclosporine or FK506 as primary immunosuppression. Transplantation. 1995;59(6):851–9.
36. Rubin RH. Prevention of infection in the liver transplant recipient. Liver Transpl Surg. 1996;2(5 Suppl 1):89–98.
37. Playford EG, Webster AC, Sorrell TC, Craig JC. Systematic review and meta-analysis of antifungal agents for preventing fungal infections in liver transplant recipients. Eur J Clin Microbiol Infect Dis. 2006;25(9):549–61.
38. Lavezzo B, Patrono D, Tandoi F, Martini S, Fop F, Ballerini V, et al. A simplified regimen of targeted antifungal prophylaxis in liver transplant recipients: a single-center experience. Transpl Infect Dis. 2018;20(2):e12859.
39. San-Juan R, Aguado JM, Lumbreras C, Fortun J, Len O, Munoz P, et al. Universal prophylaxis with fluconazole for the prevention of early invasive fungal infection in low-risk liver transplant recipients. Transplantation. 2011;92(3):346–50.
40. Hadley S, Huckabee C, Pappas PG, Daly J, Rabkin J, Kauffman CA, et al. Outcomes of antifungal prophylaxis in high-risk liver transplant recipients. Transpl Infect Dis. 2009;11(1): 40–8.
41. Saliba F, Delvart V, Ichai P, Kassis N, Botterel F, Mihaila L, et al. Fungal infections after liver transplantation: outcomes and risk factors revisited in the MELD era. Clin Transpl. 2013; 27(4):E454–61.
42. Winston DJ, Limaye AP, Pelletier S, Safdar N, Morris MI, Meneses K, et al. Randomized, double-blind trial of anidulafungin versus fluconazole for prophylaxis of invasive fungal infections in high-risk liver transplant recipients. Am J Transplant. 2014;14(12):2758–64.
43. Karchmer AW, Samore MH, Hadley S, Collins LA, Jenkins RL, Lewis WD. Fungal infections complicating orthotopic liver transplantation. Trans Am Clin Climatol Assoc. 1995;106: 38–47; discussion 47–8
44. Collins LA, Samore MH, Roberts MS, Luzzati R, Jenkins RL, Lewis WD, et al. Risk factors for invasive fungal infections complicating orthotopic liver transplantation. J Infect Dis. 1994;170(3):644–52.
45. Tollemar J, Hockerstedt K, Ericzon BG, Jalanko H, Ringden O. Liposomal amphotericin B prevents invasive fungal infections in liver transplant recipients. A randomized, placebo-controlled study. Transplantation. 1995;59(1):45–50.
46. Sharpe MD, Ghent C, Grant D, Horbay GL, McDougal J, David Colby W. Efficacy and safety of itraconazole prophylaxis for fungal infections after orthotopic liver transplantation: a prospective, randomized, double-blind study. Transplantation. 2003;76(6):977–83.
47. Biancofiore G, Bindi ML, Baldassarri R, Romanelli AM, Catalano G, Filipponi F, et al. Antifungal prophylaxis in liver transplant recipients: a randomized placebo-controlled study. Transpl Int. 2002;15(7):341–7.
48. Winston DJ, Busuttil RW. Randomized controlled trial of oral itraconazole solution versus intravenous/oral fluconazole for prevention of fungal infections in liver transplant recipients. Transplantation. 2002;74(5):688–95.
49. Playford EG, Webster AC, Sorell TC, Craig JC. Antifungal agents for preventing fungal infections in solid organ transplant recipients. Cochrane Database Syst Rev. 2004;(3): CD004291.
50. Saliba F, Pascher A, Cointault O, Laterre PF, Cervera C, De Waele JJ, et al. Randomized trial of micafungin for the prevention of invasive fungal infection in high-risk liver transplant recipients. Clin Infect Dis. 2015;60(7):997–1006.

51. Fortun J, Muriel A, Martin-Davila P, Montejo M, Len O, Torre-Cisneros J, et al. Caspofungin versus fluconazole as prophylaxis of invasive fungal infection in high-risk liver transplantation recipients: a propensity score analysis. Liver Transpl. 2016;22(4):427–35.
52. Kang WH, Song GW, Lee SG, Suh KS, Lee KW, Yi NJ, et al. A multicenter, randomized, open-label study to compare micafungin with fluconazole in the prophylaxis of invasive fungal infections in living-donor liver transplant recipients. J Gastrointest Surg. 2019;24:832.
53. Hogen R, Dhanireddy KK. Invasive fungal infections following liver transplantation. Curr Opin Organ Transplant. 2017;22(4):356–63.
54. Winston DJ, Pakrasi A, Busuttil RW. Prophylactic fluconazole in liver transplant recipients. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1999;131(10):729–37.
55. Sun HY, Cacciarelli TV, Singh N. Micafungin versus amphotericin B lipid complex for the prevention of invasive fungal infections in high-risk liver transplant recipients. Transplantation. 2013;96(6):573–8.
56. Righi E. Management of bacterial and fungal infections in end stage liver disease and liver transplantation: current options and future directions. World J Gastroenterol. 2018;24(38): 4311–29.
57. Lahmer T, Messer M, Schwerdtfeger C, Rasch S, Lee M, Saugel B, et al. Invasive mycosis in medical intensive care unit patients with severe alcoholic hepatitis. Mycopathologia. 2014;177(3–4):193–7.
58. Theocharidou E, Agarwal B, Jeffrey G, Jalan R, Harrison D, Burroughs AK, et al. Early invasive fungal infections and colonization in patients with cirrhosis admitted to the intensive care unit. Clin Microbiol Infect. 2016;22(2):189.e1–7.
59. Hassan EA, Abd El-Rehim AS, Hassany SM, Ahmed AO, Elsherbiny NM, Mohammed MH. Fungal infection in patients with end-stage liver disease: low frequency or low index of suspicion. Int J Infect Dis. 2014;23:69–74.
60. Bassetti M, Peghin M, Carnelutti A, Righi E, Merelli M, Ansaldi F, et al. Clinical characteristics and predictors of mortality in cirrhotic patients with candidemia and intra-abdominal candidiasis: a multicenter study. Intensive Care Med. 2017;43(4):509–18.
61. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Castro S, et al. OPTN/SRTR 2018 annual data report: kidney. Am J Transplant. 2020;20(Suppl s1):20–130.
62. Patel MH, Patel RD, Vanikar AV, Kanodia KV, Suthar KS, Nigam LK, et al. Invasive fungal infections in renal transplant patients: a single center study. Ren Fail. 2017;39(1):294–8.
63. Santos T, Aguiar B, Santos L, Romaozinho C, Tome R, Macario F, et al. Invasive fungal infections after kidney transplantation: a single-center experience. Transplant Proc. 2015;47(4):971–5.
64. Agrawal V, Gupta RK, Jain M. Invasive fungal infections in renal allograft recipients. Indian J Pathol Microbiol. 2005;48(4):448–52.
65. Badiee P, Alborzi A. Invasive fungal infections in renal transplant recipients. Exp Clin Transplant. 2011;9(6):355–62.
66. Moreno A, Cervera C, Gavalda J, Rovira M, de la Camara R, Jarque I, et al. Bloodstream infections among transplant recipients: results of a nationwide surveillance in Spain. Am J Transplant. 2007;7(11):2579–86.
67. Nampoory MR, Khan ZU, Johny KV, Constandi JN, Gupta RK, Al-Muzairi I, et al. Invasive fungal infections in renal transplant recipients. J Infect. 1996;33(2):95–101.
68. Altiparmak MR, Apaydin S, Trablus S, Serdengecti K, Ataman R, Ozturk R, et al. Systemic fungal infections after renal transplantation. Scand J Infect Dis. 2002;34(4):284–8.
69. Bodro M, Sabe N, Gomila A, Ayats J, Baliellas C, Roca J, et al. Risk factors, clinical characteristics, and outcomes of invasive fungal infections in solid organ transplant recipients. Transplant Proc. 2012;44(9):2682–5.
70. Lazareth H, Burbach M, Gosset C, Lefaucheur C, Pashootan P, Zagdanski AM, et al. Renal arterial mycotic aneurysm after kidney transplantation. Urology. 2017;106:e7–8.
71. Delgado J, Calvo N, Gomis A, Perez-Flores I, Rodriguez A, Ridao N, et al. Candiduria in renal transplant recipients: incidence, clinical repercussion, and treatment indication. Transplant Proc. 2010;42(8):2944–6.

72. Safdar N, Slattery WR, Knasinski V, Gangnon RE, Li Z, Pirsch JD, et al. Predictors and outcomes of candiduria in renal transplant recipients. Clin Infect Dis. 2005;40(10):1413–21.
73. Denis B, Chopin D, Piron P, Resche-Rigon M, Bretagne S, Gits-Muselli M, et al. Candiduria in kidney transplant recipients: is antifungal therapy useful? Mycoses. 2018;61(5):298–304.
74. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of Candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1–50.
75. Shaikh SA, Zimmerman A, Nolan A, Cooper M, Abrams PL. The incidence of fungal infections in pancreas transplant recipients in the absence of systemic antifungal prophylaxis. Clin Transpl. 2019;33:e13691.
76. Bartoletti M, Cervera C, Hoyo I, Linares L, Sanclemente G, Bosch J, et al. Incidence and outcome of early Candida peritonitis after liver and pancreas transplantation. Mycoses. 2013;56(2):162–7.
77. Berger N, Wirmsberger R, Kafka R, Margreiter C, Ebenbichler C, Stelzmueller I, et al. Infectious complications following 72 consecutive enteric-drained pancreas transplants. Transpl Int. 2006;19(7):549–57.
78. Lumbreras C, Fernandez I, Velosa J, Munn S, Sterioff S, Paya CV. Infectious complications following pancreatic transplantation: incidence, microbiological and clinical characteristics, and outcome. Clin Infect Dis. 1995;20(3):514–20.
79. Benedetti E, Gruessner AC, Troppmann C, Papalois BE, Sutherland DE, Dunn DL, et al. Intra-abdominal fungal infections after pancreatic transplantation: incidence, treatment, and outcome. J Am Coll Surg. 1996;183(4):307–16.
80. Rostambeigi N, Kudva YC, John S, Mailankody S, Pedersen RA, Dean PG, et al. Epidemiology of infections requiring hospitalization during long-term follow-up of pancreas transplantation. Transplantation. 2010;89(9):1126–33.
81. Pirsch JD, Odorico JS, D'Alessandro AM, Knechtle SJ, Becker BN, Sollinger HW. Posttransplant infection in enteric versus bladder-drained simultaneous pancreas-kidney transplant recipients. Transplantation. 1998;66(12):1746–50.
82. Michalak G, Kwiatkowski A, Bieniasz M, Meszaros J, Czerwinski J, Wszola M, et al. Infectious complications after simultaneous pancreas-kidney transplantation. Transplant Proc. 2005;37(8):3560–3.
83. Clouse JW, Kubal CA, Fridell JA, Mangus RS. One-year incidence of infection in pediatric intestine transplantation. Pediatr Infect Dis J. 2019;38(3):219–23.
84. Florescu DF, Sandkovsky U. Fungal infections in intestinal and multivisceral transplant recipients. Curr Opin Organ Transplant. 2015;20(3):295–302.
85. Florescu DF, Qiu F, Mercer DF, Langnas AN, Shafer LR, Kalil AC. Risk factors for systemic Candida infections in pediatric small bowel transplant recipients. Pediatr Infect Dis J. 2012;31(2):120–3.
86. Florescu DF, Islam KM, Grant W, Mercer DF, Langnas A, Botha J, et al. Incidence and outcome of fungal infections in pediatric small bowel transplant recipients. Transpl Infect Dis. 2010;12(6):497–504.
87. Florescu DF, Qiu F, Langnas AN, Mercer DF, Chambers H, Hill LA, et al. Bloodstream infections during the first year after pediatric small bowel transplantation. Pediatr Infect Dis J. 2012;31(7):700–4.
88. Galloway D, Danziger-Isakov L, Goldschmidt M, Hemmelgarn T, Courter J, Nathan JD, et al. Incidence of bloodstream infections in small bowel transplant recipients receiving selective decontamination of the digestive tract: a single-center experience. Pediatr Transplant. 2015;19(7):722–9.
89. Singh N, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ, et al. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, infectious diseases community of practice. Am J Transplant. 2012;12(9):2414–28.
90. Albano L, Bretagne S, Mamzer-Bruneel MF, Kacso I, Desnos-Ollivier M, Guerrini P, et al. Evidence that graft-site candidiasis after kidney transplantation is acquired during organ recovery: a multicenter study in France. Clin Infect Dis. 2009;48(2):194–202.

91. Corbel A, Ladriere M, Le Berre N, Durin L, Rousseau H, Frimat L, et al. Microbiological epidemiology of preservation fluids in transplanted kidney: a nationwide retrospective observational study. Clin Microbiol Infect. 2019;26:475.
92. Janny S, Bert F, Dondero F, Durand F, Guerrini P, Merckx P, et al. Microbiological findings of culture-positive preservation fluid in liver transplantation. Transpl Infect Dis. 2011;13(1): 9–14.
93. Oriol I, Sabe N, Tebe C, Veroux M, Boin I, Carratala J. Clinical impact of culture-positive preservation fluid on solid organ transplantation: a systematic review and meta-analysis. Transplant Rev (Orlando). 2018;32(2):85–91.
94. Botterel F, Foulet F, Legrand P, Soria AM, Farrugia C, Grimbert P, et al. Yeast contamination of kidney, liver and cardiac preservation solutions before graft: need for standardisation of microbial evaluation. J Hosp Infect. 2010;76(1):52–5.
95. Levesque E, Paugam-Burtz C, Saliba F, Khoy-Ear L, Merle JC, Jung B, et al. Fungal complications after Candida preservation fluid contamination in liver transplant recipients. Transpl Int. 2015;28(11):1308–16.
96. Oriol I, Sabe N, Camara J, Berbel D, Ballesteros MA, Escudero R, et al. The impact of culturing the organ preservation fluid on solid organ transplantation: a prospective multicenter cohort study. Open Forum Infect Dis. 2019;6(6):ofz180.
97. Encatassamy F, Valentin AS, Capsec J, Buchler M, Bruyere F. Colonization of preservation solution in kidney transplantation: clinical impact and risk of secondary acute graft pyelonephritis. Prog Urol. 2018;28(6):322–8.
98. Levesque E, Suet G, Merle JC, Compagnon P, Amathieu R, Feray C, et al. Candida vascular complication in a liver transplant recipient due to yeast contamination of preservation solution. Transpl Infect Dis. 2014;16(5):827–9.
99. Addeo P, Saouli AC, Woehl-Jaegle ML, Ellero B, Oussoultzoglou E, Marcellin L, et al. *Candida albicans* arteritis transmitted by preservation fluid after liver transplantation. Ann Transplant. 2014;19:64–7.
100. Azar MM, Turbett SE, Fishman JA, Pierce VM. Donor-derived transmission of *Candida auris* during lung transplantation. Clin Infect Dis. 2017;65(6):1040–2.
101. Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56(9):1284–92.
102. Pfeiffer CD, Samsa GP, Schell WA, Reller LB, Perfect JR, Alexander BD. Quantitation of Candida CFU in initial positive blood cultures. J Clin Microbiol. 2011;49(8):2879–83.
103. Nguyen MH, Wissel MC, Shields RK, Salomoni MA, Hao B, Press EG, et al. Performance of Candida real-time polymerase chain reaction, beta-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis. 2012;54(9):1240–8.
104. Clancy CJ, Nguyen MH. Diagnosing invasive Candidiasis. J Clin Microbiol. 2018;56(5): e01909–17.
105. Morris AJ, Byrne TC, Madden JF, Reller LB. Duration of incubation of fungal cultures. J Clin Microbiol. 1996;34(6):1583–5.
106. Ostrosky-Zeichner L, Kullberg BJ, Bow EJ, Hadley S, Leon C, Nucci M, et al. Early treatment of candidemia in adults: a review. Med Mycol. 2011;49(2):113–20.
107. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother. 2005;49(9):3640–5.
108. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis. 2006;43(1):25–31.
109. Koo S, Bryar JM, Page JH, Baden LR, Marty FM. Diagnostic performance of the (1–>3)-beta-D-glucan assay for invasive fungal disease. Clin Infect Dis. 2009;49(11):1650–9.
110. Pini P, Bettua C, Orsi CF, Venturelli C, Forghieri F, Bigliardi S, et al. Evaluation of serum (1->3)-beta-D-glucan clinical performance: kinetic assessment, comparison with galactomannan and evaluation of confounding factors. Infection. 2016;44(2):223–33.

111. Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL. Evaluation of a (1->3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J Clin Microbiol. 2005;43(12):5957–62.
112. Tissot F, Lamoth F, Hauser PM, Orasch C, Fluckiger U, Siegemund M, et al. beta-glucan antigenemia anticipates diagnosis of blood culture-negative intraabdominal candidiasis. Am J Respir Crit Care Med. 2013;188(9):1100–9.
113. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin Infect Dis. 2011;52(6):750–70.
114. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, et al. Diagnostic accuracy of serum 1,3-beta-D-glucan for *Pneumocystis jirovecii* pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol. 2012;50(1):7–15.
115. Hanson KE, Pfeiffer CD, Lease ED, Balch AH, Zaas AK, Perfect JR, et al. beta-D-glucan surveillance with preemptive anidulafungin for invasive candidiasis in intensive care unit patients: a randomized pilot study. PLoS One. 2012;7(8):e42282.
116. Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, et al. beta-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). Clin Infect Dis. 2012;54(5):633–43.
117. Sendid B, Tabouret M, Poirot JL, Mathieu D, Fruit J, Poulain D. New enzyme immunoassays for sensitive detection of circulating *Candida albicans* mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. J Clin Microbiol. 1999;37(5):1510–7.
118. Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C. Third European Conference on Infections in Leukemia G. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Crit Care. 2010;14(6):R222.
119. Girmenia C, Martino P, De Bernardis F, Cassone A. Assessment of detection of Candida mannoproteinemia as a method to differentiate central venous catheter-related candidemia from invasive disease. J Clin Microbiol. 1997;35(4):903–6.
120. Girmenia C, Micozzi A, Cartoni C, De Bernardis F, Cassone A, Martino P. Detection of Candida mannoproteinemia in patients with neutropenic enterocolitis. Eur J Clin Microbiol Infect Dis. 1999;18(1):55–8.
121. Prella M, Bille J, Pugnale M, Duvoisin B, Cavassini M, Calandra T, et al. Early diagnosis of invasive candidiasis with mannan antigenemia and antimannan antibodies. Diagn Microbiol Infect Dis. 2005;51(2):95–101.
122. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. J Clin Microbiol. 2011;49(2):665–70.
123. Schell WA, Benton JL, Smith PB, Poore M, Rouse JL, Boles DJ, et al. Evaluation of a digital microfluidic real-time PCR platform to detect DNA of *Candida albicans* in blood. Eur J Clin Microbiol Infect Dis. 2012;31(9):2237–45.
124. Zacharioudakis IM, Zervou FN, Mylonakis E. Use of T2MR in invasive candidiasis with and without candidemia. Future Microbiol. 2018;13:1165–73.
125. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis. 2015;60(6):892–9.
126. Gill CM, Kenney RM, Hencken L, Mlynarek ME, Alangaden GJ, Samuel LP, et al. T2 Candida versus beta-D-glucan to facilitate antifungal discontinuation in the intensive care unit. Diagn Microbiol Infect Dis. 2019;95:162.
127. Kollef M, Micek S, Hampton N, Doherty JA, Kumar A. Septic shock attributed to Candida infection: importance of empiric therapy and source control. Clin Infect Dis. 2012;54(12):1739–46.

128. Grim SA, Berger K, Teng C, Gupta S, Layden JE, Janda WM, et al. Timing of susceptibility-based antifungal drug administration in patients with Candida bloodstream infection: correlation with outcomes. J Antimicrob Chemother. 2012;67(3):707–14.
129. Kullberg BJ, Arendrup MC. Invasive Candidiasis. N Engl J Med. 2015;373(15):1445–56.
130. Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE Jr, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(5):503–35.
131. Nami S, Aghebati-Maleki A, Morovati H, Aghebati-Maleki L. Current antifungal drugs and immunotherapeutic approaches as promising strategies to treatment of fungal diseases. Biomed Pharmacother. 2019;110:857–68.
132. Rex JH, Bennett JE, Sugar AM, Pappas PG, van der Horst CM, Edwards JE, et al. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. Candidemia Study Group and the National Institute. N Engl J Med. 1994;331(20):1325–30.
133. Rex JH, Pappas PG, Karchmer AW, Sobel J, Edwards JE, Hadley S, et al. A randomized and blinded multicenter trial of high-dose fluconazole plus placebo versus fluconazole plus amphotericin B as therapy for candidemia and its consequences in nonneutropenic subjects. Clin Infect Dis. 2003;36(10):1221–8.
134. Kullberg BJ, Sobel JD, Ruhnke M, Pappas PG, Viscoli C, Rex JH, et al. Voriconazole versus a regimen of amphotericin B followed by fluconazole for candidaemia in non-neutropenic patients: a randomised non-inferiority trial. Lancet. 2005;366(9495):1435–42.
135. Mora-Duarte J, Betts R, Rotstein C, Colombo AL, Thompson-Moya L, Smietana J, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med. 2002;347(25):2020–9.
136. Kuse ER, Chetchotisakd P, da Cunha CA, Ruhnke M, Barrios C, Raghunadharao D, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. Lancet. 2007;369(9572):1519–27.
137. Pappas PG, Rotstein CM, Betts RF, Nucci M, Talwar D, De Waele JJ, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. Clin Infect Dis. 2007;45(7):883–93.
138. Betts RF, Nucci M, Talwar D, Gareca M, Queiroz-Telles F, Bedimo RJ, et al. A multicenter, double-blind trial of a high-dose caspofungin treatment regimen versus a standard caspofungin treatment regimen for adult patients with invasive candidiasis. Clin Infect Dis. 2009;48(12):1676–84.
139. Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clin Infect Dis. 2012;54(8):1110–22.
140. https://www.cdc.gov/fungal/candida-auris/index.html. *Candida auris*.
141. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol Immunol. 2009;53(1):41–4.
142. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis. 2017;64(2):134–40.
143. Vallabhaneni S, Kallen A, Tsay S, Chow N, Welsh R, Kerins J, et al. Investigation of the first seven reported cases of *Candida auris*, a globally emerging invasive, multidrug-resistant fungus-United States, May 2013–August 2016. Am J Transplant. 2017;17(1):296–9.
144. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, et al. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. Antimicrob Resist Infect Control. 2016;5:35.
145. O'Brien B, Chaturvedi S, Chaturvedi V. In vitro evaluation of antifungal drug combinations against multidrug-resistant *Candida auris* isolates from New York Outbreak. Antimicrob Agents Chemother. 2020;64:e02195.

146. Chowdhary A, Sharma C, Duggal S, Agarwal K, Prakash A, Singh PK, et al. New clonal strain of *Candida auris*, Delhi, India. Emerg Infect Dis. 2013;19(10):1670–3.
147. Lone SA, Ahmad A. *Candida auris*-the growing menace to global health. Mycoses. 2019;62(8):620–37.
148. Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. Antimicrob Agents Chemother. 2009;53(1):24–34.
149. Laverdiere M, Bow EJ, Rotstein C, Autmizguine J, Broady R, Garber G, et al. Therapeutic drug monitoring for triazoles: a needs assessment review and recommendations from a Canadian perspective. Can J Infect Dis Med Microbiol. 2014;25(6):327–43.
150. Ashbee HR, Barnes RA, Johnson EM, Richardson MD, Gorton R, Hope WW. Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology. J Antimicrob Chemother. 2014;69(5):1162–76.
151. Righi E, Carnelutti A, Baccarani U, Sartor A, Cojutti P, Bassetti M, et al. Treatment of Candida infections with fluconazole in adult liver transplant recipients: is TDM-guided dosing adaptation helpful? Transpl Infect Dis. 2019;21(4):e13113.
152. Cojutti PG, Lugano M, Righi E, Della Rocca G, Bassetti M, Hope W, et al. Population pharmacokinetics of fluconazole in liver transplantation: implications for target attainment for infections with *Candida albicans* and non-albicans spp. Eur J Clin Pharmacol. 2018;74(11):1449–59.
153. Buijk SL, Gyssens IC, Mouton JW, Verbrugh HA, Touw DJ, Bruining HA. Pharmacokinetics of sequential intravenous and enteral fluconazole in critically ill surgical patients with invasive mycoses and compromised gastro-intestinal function. Intensive Care Med. 2001;27(1):115–21.
154. Csajka C, Decosterd LA, Buclin T, Pagani JL, Fattinger K, Bille J, et al. Population pharmacokinetics of fluconazole given for secondary prevention of oropharyngeal candidiasis in HIV-positive patients. Eur J Clin Pharmacol. 2001;57(10):723–7.
155. Park WB, Kim NH, Kim KH, Lee SH, Nam WS, Yoon SH, et al. The effect of therapeutic drug monitoring on safety and efficacy of voriconazole in invasive fungal infections: a randomized controlled trial. Clin Infect Dis. 2012;55(8):1080–7.
156. Pascual A, Calandra T, Bolay S, Buclin T, Bille J, Marchetti O. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis. 2008;46(2):201–11.
157. Dekkers BGJ, Bakker M, van der Elst KCM, Sturkenboom MGG, Veringa A, Span LFR, et al. Therapeutic drug monitoring of posaconazole: an update. Curr Fungal Infect Rep. 2016;10:51–61.

Aspergillus in Solid Organ and Stem Cell Transplant Patients: Emerging Options for Diagnosis and Management

45

Armelle Pérez-Cortés Villalobos and Shahid Husain

Contents

Introduction 1078
Cryptic Emergence of Resistant *Aspergillus* Species and Azole-Resistant Strains 1080
Diagnosis of Aspergillosis 1081
Clinical Manifestations 1082
Bronchoscopy 1082
Microbiology 1083
Immunohistochemistry for *Aspergillus* 1084
Serological and Molecular Tests 1084
Galactomannan Antigen Detection 1084
(1-3)-β-D-Glucan 1085
Aspergillus Polymerase Chain Reaction (PCR) 1085
Newer Tests 1087
Monoclonal Antibodies 1087
Urinary Antigen Detection 1088
Volatile Organic Compounds 1088
MicroRNA 1089
Radiological Diagnosis 1089
Positron Emission Tomography (PET) 1089
Diagnostic Algorithm 1090
Treatment of Invasive Aspergillosis 1090
Voriconazole 1090
Isavuconazole 1094
Other Azoles 1094
Echinocandins 1095
Polyenes 1097
New Antifungals Currently Under Investigation 1097
Combination Therapy 1098
Voriconazole plus Echinocandin Therapy 1098

A. Pérez-Cortés Villalobos · S. Husain (✉)
Multi-Organ Transplant Unit, Division of Infectious Diseases, University Health Network, Toronto, ON, Canada
e-mail: Armelle.Perez-Cortes@uhn.ca; shahid.husain@uhn.ca

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_44

Liposomal Amphotericin B plus Echinocandin Therapy 1099
Duration of Therapy 1099
Susceptibility Testing 1099
Prevention 1100
HSCT Recipients 1101
SOT Recipients 1101
Conclusions 1103
Key Points 1103
References 1104

Introduction

Aspergillus is an ubiquitous mold and found worldwide in soil, food, water, and compost. Contamination of the hospital air supply can occur when construction or renovation is conducted. *Aspergillus fumigatus* is by far the most common species, isolated in 73% of cases [1].

The annual burden of invasive aspergillosis (IA) is estimated to be more than 300,000 cases worldwide [2]. Immunocompromised patients are at highest risk of acquiring the disease.

The usual route of infection for IA is through inhalation of the conidia into the lung or paranasal sinuses. Posttransplant patients have reduced pulmonary host defenses, which inhibits the activity of pulmonary macrophages. If the conidia are not eradicated, they may enlarge and germinate to form hyphae causing disseminated infection by invading blood vessels and parenchymal tissue.

The Transplant-Associated Infection Surveillance Network (TRANSNET) performed a prospective surveillance of invasive fungal infections (IFIs) and identified an overall incidence of 3.4% (range by site, 0.9%–13.2%) among hematopoietic stem cell transplant (HSCT) recipients [3], and a 12-month cumulative incidence of 3.1% (1.2%–6.1%) among solid organ transplant (SOT) recipients [1].

The incidence of IA in HSCT recipients increased during the 1990s, as a multitude of factors such as the receipt of T cell-depleted or CD34-selected stem cell products, corticosteroids, neutropenia, lymphopenia, graft versus host disease (GVHD), cytomegalovirus (CMV) disease, and respiratory virus infections increased this incidence after HSCT [4]. The most important risk factor is prolonged neutropenia, as presented in Table 1.

The incidence of IA in HSCT recipients is bimodal, with the first peak in incidence occurring early after transplantation (<40 days). Several groups have identified a second risk period late after engraftment (40–180 days post-transplant) associated with GVHD and CMV infection [5]. The mean time until diagnosis is 180 days in some case series [1, 4, 5]. The mortality rate in HSCT recipients with IA ranges from 66.6% to 80% and does not differ for those with early onset versus late onset of infection [3, 9]. IA is one of the causes of non-relapse-related mortality in non-myeloablative HSCT recipients, with 9% of overall mortality and 39% of non-relapse mortality in this patient population being due to invasive mold infections [9]. In the PATH alliance analysis, 90-day survival in the allogeneic stem cell

Table 1 Risk factors for invasive aspergillosis in SOT and HSCT recipients [1, 4–7]

SOT patients	HSCT patients
Liver transplant Re-transplantation or reoperation Rejection or change immunosuppression Acute kidney injury -Renal replacement therapy Fulminant hepatic failure MELD >30 CMV infection	**Early IA (<40 days after transplant)** Aplastic anemia, myelodysplastic syndrome, and multiple myeloma [4] Transplantation with T cell-depleted or CD34-selected stem cell products Delayed neutrophil engraftment or prolonged neutropenia CMV disease
Lung transplant Single lung transplant Early airway ischemia Rejection or change immunosuppression Pre-transplant colonization Positive intraoperative *Aspergillus* culture in CF patient Hypogammaglobulinemia CMV infection	**Late IA (days 41–180 after transplant)** Delay engraftment of T lymphocytes Neutropenia Respiratory viruses (influenza and parainfluenza) [7] Graft-versus-host disease Corticosteroids
Heart transplant *Aspergillus* colonization Reoperation Posttransplant hemodialysis Rejection or change immunosuppression Existence of an episode of IA in any patient within the heart transplant program, 2 months before or after transplant Pretransplant diagnosis of COPD High duration of steroids CMV infection	
Kidney transplant Previous pulmonary disease Cardiovascular diseases [8] Delayed graft function Rejection or change immunosuppression CMV infection	

transplant recipient with IA was 59.7% [5]. The most common species of this fungus is *Aspergillus fumigatus*. Figure 1 shows the distribution of *Aspergillus* species in HSCT recipients reported in different cohorts.

In SOT recipients, the overall incidence of IA is slightly lower than in HSCT [6]. Lung transplant recipients report higher incidence compared to other solid organ transplants and are comparable to allogenic hematopoietic stem cell transplant recipients (Fig. 2). Some of the risk factors for developing early IA (<3 months) in SOT include complications in the postoperative period such as the use of vasopressors >24 h after surgery, intensive care readmission, development of renal failure, hemodialysis, and infections due to bacteria and CMV. The risk factors for developing late IA were age >50 years, chronic transplant rejection, and changes in immunosuppression [6]. Risk factors differ, depending on the transplanted organ, and are presented in (Table 1).

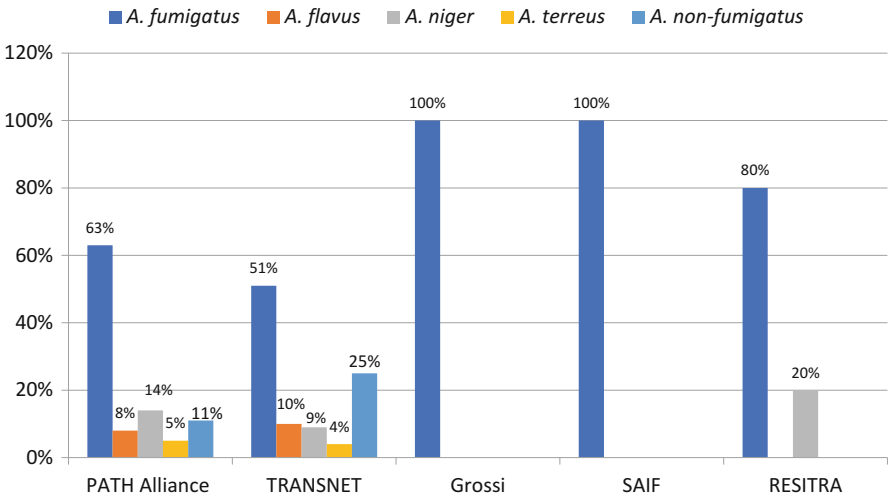


Fig. 1 Distribution of *Aspergillus* infections in lung transplant [1, 3, 5, 10–12]

The median time of presentation in HSCT recipient case series was 184 days after transplant [1]. In the SOT population, IA in liver transplant recipients occurred earlier ranging up to 17 days after transplant [14]. In lung transplant recipients, IA occurred at a median of 7.7 months posttransplantation [15].

Cryptic Emergence of Resistant *Aspergillus* Species and Azole-Resistant Strains

Antifungal resistance in *Aspergillus* spp. is increasingly being recognized and poses additional therapeutic challenges. *A. fumigatus* is eukaryotic with a complex cellular composition, and unlike bacterial pathogens, it does not develop rapid resistance in response to antifungal exposure. Mutations in the *Cyp51A* gene which encodes the Cyp51 enzyme responsible for the last step in ergosterol biosynthesis have been the resistance mechanisms documented in clinical isolates [16]. These resistance mechanisms, which include TR_{46}/Y121F/T289A, TR_{53}, and TR_{34}/L98H, have been implicated in isavuconazole, voriconazole, posaconazole, and itraconazole resistance [17]. G54 and M220 have been associated with resistance to itraconazole and posaconazole [17], while Y121F has been associated with resistance to voriconazole alone [18].

Azole resistance can also be an intrinsic phenotype, as is known to occur in some cryptic *Aspergillus* species related to *A. fumigatus*, specifically *A. lentulus* and *A. pseudofischeri.* On the other hand, wild-type *A. fumigatus* and *A. flavus* are sensitive to azoles [16]. Resistance can be acquired by two major routes, either through long-term azole therapy or via the utilization of azole compounds in the environment, particularly in agriculture [19].

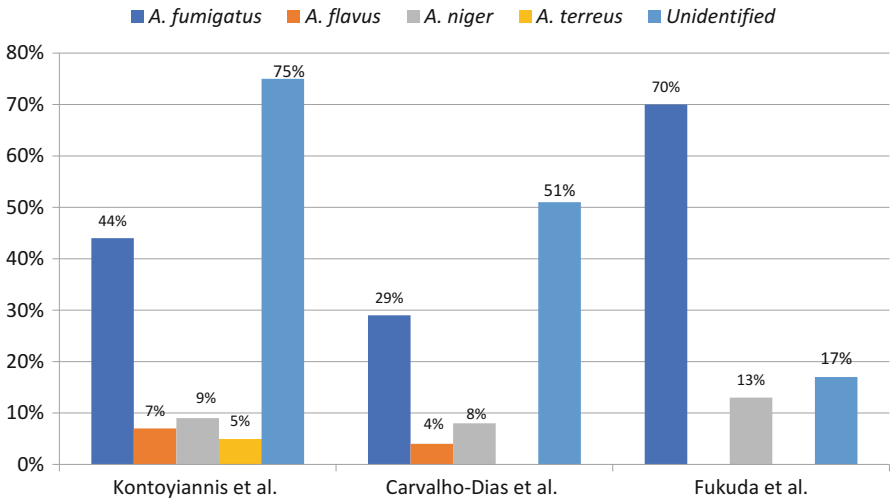


Fig. 2 Distribution of *Aspergillus* species in HSCT [3, 9, 13]

Acquired resistance to azoles in *A. fumigatus* has become a public health concern. A prospective multicenter international survey that included 3,788 *Aspergillus* isolates in 22 centers from 19 countries showed 3.2% of clinical *A. fumigatus* isolates to be azole resistant [20]. However, in a prospective surveillance study in the Netherlands, the prevalence of itraconazole resistance in *A. fumigatus* was 5.3% (range 0.8%–9.5%); 64% were azole naive, and the case fatality rate of patients with azole-resistant invasive aspergillosis was 88% [21]. A recent surveillance study of fungal infection in SOT recipients in the USA showed a low rate of triazole resistance (1/181) in *A. fumigatus* isolates [22]. Additionally, in a cohort of 22 lung transplant recipients with *A. fumigatus*, targeted antifungal prophylaxis did not lead to *Cyp51A* resistance mutations [23]. A retrospective study of breakthrough invasive mold infections that occurred during posaconazole and voriconazole prophylaxis in hematologic malignancy and SOT patients reported 24 breakthrough infections; IA accounted for 21% of cases, and *A. ustus* emerged as the predominant *Aspergillus* pathogen (43% of the cases) [24].

Diagnosis of Aspergillosis

The diagnosis of IA relies on a combination of host, imaging, microbiology, and pathology findings. The standard definitions for IFIs, published by the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) Consensus Group [25], were developed to maintain consistency in clinical and epidemiological studies. Based on these definitions, the diagnosis of IA is often referred to within a scale of certainty: possible, probable, and proven. For the diagnosis of *Proven IA*, there must be histopathologic, cytopathologic, or direct

microscopic examination of a biopsy in which hyphae are seen, accompanied by evidence of associated tissue damage and growth of *Aspergillus* species from culture of a normally sterile and clinically or radiologically abnormal site. A diagnosis of *Probable IA* requires an immunocompromised host with clinical disease and non-definitive microbiological testing (cytology, direct microscopy, and/or culture indicating presence of *Aspergillus* species in a lower respiratory tract specimen or galactomannan antigen index >0.5 in plasma, serum, or bronchoalveolar lavage (BAL) fluid or a serum 1,3 beta D-glucan level of >80 pg/mL). A *Possible IA* diagnosis indicates presence of a compatible clinical disease and an immunocompromised host.

In lung transplant recipients, it is preferred to use the International Society for Heart and Lung Transplantation (ISHLT) guidelines. For the diagnosis of *Proven IA*, there must be a biopsy showing histologic evidence of parenchymal invasion by fungal hyphae, or pseudo-hyphae, or positive culture from sterile tissue alone or sign/symptoms and radiological criteria and laboratory criteria (single positive culture for mold BAL/blood or single positive PCR for mold BAL/blood or positive galactomannan in the BAL or at least two positive sputum cultures/PCRs of fungal organisms). For *Probable IA* the criteria are the same as for proven, except there is negative or absent histology [26]. No category of *Possible IFI* is entertained in the ISHLT definitions, due to a multitude of infectious etiologies that can mimic the radiological patterns of IFI in lung transplant recipients.

Clinical Manifestations

The spectrum of infection caused by *Aspergillus* species is diverse, ranging from allergic responses, asymptomatic colonization, superficial or localized infection, and acute or subacute invasive disease. The clinical presentation and severity depend on the underlying immune defects and risk factors associated with each patient group. In HSCT and SOT recipients, prolonged neutropenia and immunosuppressive therapy cause an increased risk for invasive disease. Table 2 shows the most common presentations of *Aspergillus* infection in different transplant modalities.

Bronchoscopy

Bronchoscopy with bronchoalveolar lavage (BAL) fluid remains the diagnostic procedure of choice for patients with pulmonary aspergillosis. Bronchoscopy should be performed in patients with a suspicion of IA. However, there is no uniform agreement on the best timing for bronchoscopy, and this population of patients commonly present with significant comorbidities that may preclude BAL. The yield of BAL is low in peripheral nodular lesions; in such cases percutaneous or endobronchial lung biopsy should be considered [19].

Table 2 Clinical presentations of infection caused by *Aspergillus* spp.

Type of transplant	Site of infection
HSCT recipients [3, 4]	Common: invasive pulmonary aspergillosis and disseminated infection
Liver transplant recipients [14]	Uniquely predisposed to dissemination of infection beyond the lungs (50–60% cases) Isolation of *Aspergillus* spp. from the respiratory tract of LT recipients is infrequent
Lung transplant recipients [27, 28]	Invasive aspergillosis occurs in 3–15% of lung transplant recipients 58% tracheobronchitis or bronchial anastomotic infections 32% invasive pulmonary aspergillosis 22% disseminated infections with extra-pulmonary involvement *Aspergillus* spp. can be detected in airway sample cultures from 25 to 30% of lung transplant recipients
Heart transplant recipients [10, 28]	Invasive aspergillosis occurs in 6% of heart transplant recipients Common: invasive aspergillosis, disseminated infections Rare: central nervous system infections *Aspergillus* spp. can be detected in 10% of the heart transplant recipients
Kidney transplant recipients [28]	Invasive aspergillosis occurs in 0.7 to 4% of patients Common: invasive aspergillosis, disseminated infections

Microbiology

In the past decade, there has been significant progress in molecular and immunological diagnostic tests for IA. Unfortunately, most of these tests are not widely available, and some are not standardized in the transplant population. Microscopy and culture continue to provide the most certain evidence of IA, and therefore, they should be attempted on appropriate specimens from patients at risk of IA [29]. To detect the presence of fungal elements in biological samples, the most commonly used stain is calcofluor white with 10% potassium hydroxide. While this stain is not specific for *Aspergillus* spp., it nevertheless has the advantage of increased sensitivity and a rapid turnaround time [29]. Other useful stains are Gomori's methenamine silver (GMS) stain and periodic acid-Schiff (PAS). In direct microscopy, *Aspergillus* typically appears as uniform septate hyphae with dichotomous (two-way) acute (45°) angle branching. The hyphae have parallel walls without constrictions and are uniform in width, measuring 3–6 μm in diameter.

Microscopic examination has important limitations that contribute to its low sensitivity. Of note is that the disease is usually required to be at an advanced stage, in order to ensure an abundance of fungal structures for analysis. Furthermore, microscopic examination is also dependent on the number of fields examined and the expertise of the examiner, as the identification of the genus of even the most common pathogenic species of *Aspergillus* can be difficult [30]. Colonies of *Aspergillus* spp. grow rapidly (24–72 h) on a variety of media, growing better in temperatures above 37 °C [30]. Culture of the organism from a sterile site and evidence of tissue

invasion on histopathology are findings that provide the most certain diagnosis of IA. Even though positive culture is the definitive diagnosis of *Aspergill*us infection, we must remember that *Aspergillus* spp. can be a laboratory contaminant or a colonizer of the respiratory tract. Therefore, a positive culture requires careful interpretation, while a negative culture likewise does not rule out a diagnosis of IA.

Immunohistochemistry for *Aspergillus*

Immunohistochemistry (IHC) is a method that selectively identifies specific antigens in tissue cells, based on antigen-antibody recognition. The antibody used in IHC is a polyclonal rabbit anti-*Aspergillus* antibody [31] that conjugates to an enzyme which catalyzes a color-producing reaction. The specificity of the test is determined by the union and binding of an antibody with its counterpart antigen at a light microscopic level. This method has the advantage of being able to identify several fungi, allowing pathological identification of unusual filamentous and yeast-like infections. For the identification of *Aspergillus* spp., polyclonal and monoclonal antibodies have been produced. However, most of these antibodies cross-react with other fungi, thus limiting the use of these tests. Currently none of these tests have received FDA approval.

Serological and Molecular Tests

Due to the limitations of the conventional methods mentioned above, in the last few years, new diagnostic tools have been developed. These new tests attempt to detect antigens and molecular compounds in fungal cell components and thus do not rely on fungal culture.

Galactomannan Antigen Detection

Galactomannan (GM) is a polysaccharide which consists of a mannose backbone and a variable number of galactofuran side chains. GM makes up a major part of the cell wall of *Aspergillus* spp. [32]. Currently, the most common method to determine GM in serum and BAL fluid is a double sandwich enzyme-linked immune assay "Platelia" (Bio-Rad). While the manufacturer recommends an optical index cutoff value of 0.5 in both serum and BAL, due to the large number of false positives in BAL at this cutoff value, the upcoming revision of the EORTC-MSG criteria has instead proposed a cutoff of 1.0 [33, 34].

The *Aspergillus* galactomannan antigen test has important limitations. The GM concentration in vivo is determined not only by the amount of production and secretion from the fungus but also by the degree of angio-invasion that is required to reach the circulation through the alveolar-capillary membrane. Due to the large size of the GM molecule, it cannot freely diffuse from the alveoli through the

endothelial lining of the pulmonary capillaries. As such, angio-invasion is always required [32]. Additionally, GM production is influenced by antifungal therapy, meaning that there is a significant decrease in the sensitivity of this test in patients who are receiving antifungals [35].

Cross-reactivity can occur with this test in cases of histoplasmosis, fusariosis, and talaromycosis (formerly penicilliosis). This is due to the fact that other filamentous fungi may have GM present in their cell wall (although in lower amounts than in *Aspergillus* spp.). False-positive GM has been documented in 13% of cases in liver transplant patients [36] and in 20% of lung transplant patients [37].

The sensitivity of GM can vary depending on the state of immunosuppression of the host. Neutrophils are believed to be involved in the uptake and elimination of GM [32]. This potentially explains the higher sensitivities seen in the context of neutropenia (~70%) such as in the HSCT populations and the decreased sensitivity seen in SOT recipients (~20%) [38].

Testing for BAL GM can improve the diagnosis of IA. The sensitivity of BAL GM has been assessed in a limited number of studies in the transplant population, and it has been seen that by increasing the cutoff value to 1.0, there is an increase in specificity, as outlined in Table 3. In lung transplant recipients, serum GM had a specificity of 95% but a low sensitivity (30%) for the diagnosis of IA [23]. A meta-analysis showed that the serum GM assay may have a greater utility in hematopoietic stem cell transplant recipients than in SOT recipients, in whom the sensitivity and specificity of the test were 22% and 84%, respectively [24]. Therefore, serum GM should not be used to diagnose IA in SOT recipients; BAL GM is preferred for the diagnosis of IPA in SOT recipients. Moreover, screening with GM and β-D-Glucan in liver transplant recipients has been reported to have limited utility [48].

(1-3)-β-D-Glucan

β-D-glucan can be detected by the Tachypleus or Limulus assay. While the presence of β-D-glucan in serum signifies the presence of fungal invasion, unfortunately this test is nonspecific for *Aspergillus* spp. Cross-reactivity with other fungi (including *Candida* and *Pneumocystis*) can lead to false-positive results. A higher sensitivity and specificity have been reported in HSCT recipients. The specificity can be improved in general with multiple positive tests and when combined with a positive culture (Table 3). Currently serum or BAL β-D-glucan is not recommended for early screening and diagnosis of IA in SOT recipients [49].

Aspergillus Polymerase Chain Reaction (PCR)

Multiple investigational DNA detection assays including polymerase chain reaction (PCR) have been developed for the diagnosis of IA in the past few years, with mixed results. Currently the use of PCR is not included in the EORTC/MSG definition of invasive fungal disease due to limited standardization and validation.

Table 3 Diagnostic tests for IA in HSCT and SOT recipients

Diagnostic test	HSCT	SOT
Serum galactomannan	Cutoff value >0.5 Sensitivity 82% Specificity 86% [38]	**Lung transplant** Cutoff value >0.5 Sensitivity 30% Specificity 93% [37] **Liver transplant** Cutoff value >0.5 Specificity 87% [36] **Kidney transplant** Cutoff value >0.5 Specificity 58% [38] **All types of sot recipients** [38] Cutoff value >0.5 Sensibility 22% Specificity 84%
BAL galactomannan	**High-risk HM patients** [39] Cutoff value 1.0 Sensitivity 91.3% Specificity 93%	**Lung transplant** [33] Cutoff value 1.0 Sensitivity 60% Specificity 98% **All types of sot recipients** [34] Cutoff 1.0 Sensibility 100% Specificity 90.8%
β-D-glucan	Sensitivity 50% [31] Specificity 91–99%	**Liver transplant** [40] Cutoff 80 pg/mL Sensitivity 58–75% Specificity 65–83% **Lung transplant** [41] Sensitivity 71–80% Specificity 59–70%
PCR	**HM, HSCT, and mixed populations** **Whole-blood** Sensitivity 84% [42] Specificity 76% **Serum** Sensitivity 64% [42] Specificity 95%	**BAL lung transplant** [43] **pan-*Aspergillus* PCR** Sensitivity 100% Specificity 88% ***A. fumigatus*-specific PCR** Sensitivity 85% Specificity 96%
Radiological findings	235 patients with proven or probable IPA. Presence of halo sign: 35% in HCST recipients and 52% in HM [44] 30 patients with probable and proven IPA with HM and prolonged neutropenia, 63% had halo sign [19]	**Lung transplant** [45] 64 proven or probable IPA: 53% nodular lesions, 47% non-nodular lesions, halo sign 3% **Liver transplant** [46] 25 patients proven IPA: 64% pulmonary nodules, 36% masses, 20% consolidations, 80% halo sign **All types of sot recipients** [47] 46 proven or probable IPA: consolidation or mass 72%, large nodules 59%, ground-glass opacity 50%

The sensitivity and specificity reported in PCR can vary depending on the type of sample, the method for DNA extraction, and the primer used in the PCR for detection of DNA. Other notable issues are that PCR is unable to differentiate between colonization and invasive infection, and it can yield false-positive results due to cross-reactivity with other mold species. Additionally, as most of the existing studies of *Aspergillus* PCR are on patients with hematological malignancies, data is very limited in the SOT population [42]. In a meta-analysis including 25 studies, the sensitivity and specificity of PCR for detection of IA were 84% and 76%, respectively. Diverse patient populations were included, including hematological malignancies, HSCT recipients, and other immunocompromised patients [42]. While experience is limited in the SOT population, the few available studies have been encouraging. A study on the performance of a pan-*Aspergillus* PCR (Viracor-IBT Laboratories) and GM in 150 BAL samples of double lung transplant recipients with a diagnosis of IA showed a sensitivity and specificity of 100% and 88%, respectively. In addition, the sensitivity and specificity of *A. fumigatus*-specific PCR were 85% and 96%, respectively [43]. The performance of the PCR increases when used in combination with another diagnostic test. Positive results for both BAL, PCR, and GM testing improved the specificity to 97%, with minimal detriment to sensitivity (93%) [43]. Likewise, the test showed improved performance with a sensitivity of 64% and specificity of 95%, when at least two PCR results returned positive [42].

The *Aspergillus* PCR is a very promising tool, with a noteworthy advantage in its ability to identify mutations associated with treatment resistance, a potentially useful function in SOT patients that have had previous fungal colonization. There are multiple commercially available PCRs that can identify azole resistance phenotypes such as AsperGenius (PathoNostics BV, Maastricht, the Netherlands) which is a multiplex real-time PCR and melting curve analysis that analyzes *A. fumigatus Cryp51A* with the targets of TR_{34}, L98Hm Y121F, and T289A substitutions [41]. For the analyses of *A. fumigatus* genes other than Cyp51A, there are PCR TaqMan probes that target *Cyp51B* S35 and P294 expression and PCR plus sequencing and real-time RT-PCR that target *Cyp51A*, *mdr1-4*, *atrF*, *cdr1B (abcG1)*, and *mfs56.* Additionally, for the analyses of *A. flavus Cyp51* genes, there is PCR plus sequencing, TaqMan probes that target *Cyp51A*, *Cyp51B*, and *Cyp51*C genes plus 5′ and 3′ regulatory sequences [50]. Furthermore, as the use of PCR in combination with other diagnostic tests was associated with improved diagnostic accuracy, it has the potential to provide an earlier diagnosis.

Newer Tests

Monoclonal Antibodies

The lateral flow device (LFD) is an immuno-chromatographic assay that detects a mannoprotein produced by actively growing *Aspergillus* species, using a monoclonal JF5 antibody. Although initially developed for the detection of the

Aspergillus antigen in human serum, it is now also used for detection in BAL fluid. It is a rapid test (15 min) and can be easily done. In a meta-analysis conducted in 2015 [51], the LFD seemed to display a better performance in BAL. Pooled sensitivity and specificity for proven/ probable IA versus no IA cases were 86% and 93%, respectively [52, 53].

Point-of-care lateral flow devices that test for the detection of *Aspergillus* antigens are currently in development. The most promising is based on the JF5 antibody and has shown good performance in clinical studies done in BAL fluid in cases of probable/proven IPA versus no evidence of IA in SOT recipients. It showed a sensitivity of 94% and specificity of 92% and in hematological malignancies a sensitivity of 67% and specificity of 91%, with an overall sensitivity of 73%, specificity of 90%, positive predictive value of 61%, and negative predictive value of 94% [52]. This test is not commercially available currently.

Urinary Antigen Detection

This assay uses a lateral flow dipstick using the galactofuranose-specific monoclonal antibody (mAb476), which recognizes urine antigens after *Aspergillus fumigatus* pulmonary infection. The per-patient sensitivity and specificity for the diagnosis of proven or probable IA was 80% and 92%, respectively [54]. This assay had a better performance in hematologic malignancy patients, where the sensitivity was 89.5% and specificity was 90.9%. False-positive results were noted in patients with *Histoplasma* infections.

This test could provide considerable clinical utility in high-risk populations, particularly in hematological malignancies, though more data is needed for utilization in clinical practice.

Volatile Organic Compounds

Filamentous fungi can produce a vast array of volatile secondary metabolites. Distinctive volatile metabolites of *A. fumigatus* in vitro include camphene, α- and β-pirene, limonene, and the sesquiterpene compounds α- and β-bergamotene. The volatile metabolite profile of *A. fumigatus* in the breath samples from patients with probable and proven IA can be characterized in vitro using thermal desorption-gas chromatography/mass spectrometry. The detection of α-trans-bergamotene, β-trans-bergamotene, β-vatirene-like sesquiterpene, or trans-geranylacetone using this method identified IA patients with 94% sensitivity and 93% specificity [55]. All these studies were done in heterogeneous cohorts of immunocompromised patients, including patients with hematological malignancies, SOT, and HSCT recipients. While these assays all appear promising, they nevertheless require more study to define performance parameters.

MicroRNA

MicroRNA are a new set of biomarkers currently under examination as potential indicators for the evaluation of fungal exposure, particularly following sub-chronic inhalation exposure to *A. fumigatus*. A 2016 study on mice highlighted the influence of conidia germination on gene expression and the subsequent control it exerted on the ensuing pulmonary immune response. Mice exposed to viable *A. fumigatus* conidia showed increased *Clec*7a, IL-13, and IL-33 expression [56].

Radiological Diagnosis

Radiologic imaging in IA is critical, as it is used to identify the type of lesion, along with the number, the size, and the site of infections. A chest CT scan should be performed in any patient with clinical suspicion of invasive pulmonary aspergillosis (IPA) [19].

The typical findings associated with IA include nodules, consolidative lesions, and wedge-shaped infarcts. In HSCT recipients, a "halo sign" can be observed in the pretransplant period, when the patient is neutropenic. Defined as a pulmonary nodule (>1 cm in diameter) surrounded by a perimeter of ground-glass opacity reflecting hemorrhage, the "halo sign" is transient and is seen only in the first 10 days of angio-invasion before disappearing. The initiation of antifungal treatment in patients with "halo sign" has been associated with improved patient outcomes for early stages, compared to later stages of disease [44].

After engraftment and neutrophil recovery, it is possible to appreciate the appearance of an "air crescent sign" which is representative of nodular opacity, with retraction of infarcted lung tissue, along with crescentic and circular cavitation. A nodule or consolidation, and apparent aspergillomas, may develop at previous sites of pulmonary infiltrates [57]. SOT recipients are more likely than neutropenic patients to show peribronchial consolidation (31% vs. 7%) and ground-glass opacity (38% vs. 7%) and less likely to show halo sign (8% vs. 56%) and air-crescent signs (0% vs. 22%) [58].

Positron Emission Tomography (PET)

For in vivo detection of *Aspergillus* lung infections in humans, radiolabeled *Aspergillus*-specific monoclonal antibodies and iron siderophores hold enormous potential for clinical diagnosis. Positron emission tomography (PET) with [^{18}F] fluorodeoxyglucose ([^{18}F]FDG) has also been studied for the diagnosis of invasive fungal disease. However, a 2018 study demonstrated a lack of specificity with this

method, as the uptake of [^{18}F]FDG during *Aspergillus* lung infection could not be distinguished from that seen during sterile inflammation, lung cancer, or bacterial lung infections [59].

Recent studies have demonstrated the enormous potential of antibody-guided PET/MRI (immunoPET/MRI) using the humanized antibody hJF5. This antibody binds to the antigenic determinant β1,5-galactofuranose, which is present in a diagnostic antigen released by the pathogen during invasive growth in the lung. This humanized antibody is currently undergoing toxicity testing prior to clinical trials, which we will use this *Aspergillus*-specific PET tracer to allow immunoPET/MR imaging of IPA in humans [59].

Diagnostic Algorithm

A proposed algorithm for the diagnosis of IA in HSCT is included in Fig. 3, which includes newer tests, such as *Aspergillus* PCR BAL, and the lateral flow device. The use of the proposed algorithm for diagnosis in SOT recipients is shown in Fig. 4.

Treatment of Invasive Aspergillosis

Early treatment with the right agent appears critical for HSCT and SOT patients with IA. There are three classes of antifungal agents available for the treatment of IA: azoles, echinocandins, and polyenes (Table 4). Currently, voriconazole is the drug of choice for the treatment of IA [19, 25].

Voriconazole

A triazole antifungal that inhibits fungal ergosterol biosynthesis, voriconazole is formulated in both oral and intravenous preparations and is metabolized by CYP2C19>>CYP2C9>CYP3A4 to an inactive metabolite (*N*-oxide voriconazole). Voriconazole is hepatically metabolized, with only 5% of the drug appearing unchanged in the urine. There are many factors that can affect the pharmacokinetics of voriconazole, including age of the patient, patients with single-nucleotide polymorphisms contributing to slow metabolism (high frequency in non-Indian Asian population), liver function, CYP2C19- and CYP3A-interacting medications, diet, antacids, proton pump inhibitors, patient weight, as well as the drug dose and formulation itself [19].

The most common adverse event seen with this medication is visual disturbances, principally photopsia that is self-limited, reversible, and not associated with the drug levels. The presentation of this abnormality typically begins within 30 min of administration of the medication and lasts no longer than 30 min [60]. Hepatotoxicity is common in all the azole drugs, but severe hepatotoxicity is uncommon, and this is often related to the serum voriconazole level.

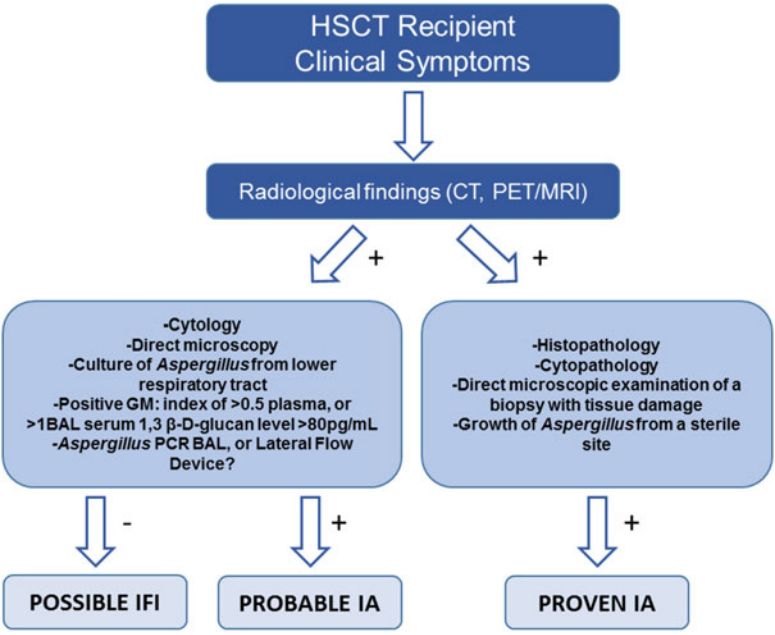


Fig. 3 Diagnostic algorithm of possible invasive fungal infection in HSCT recipients

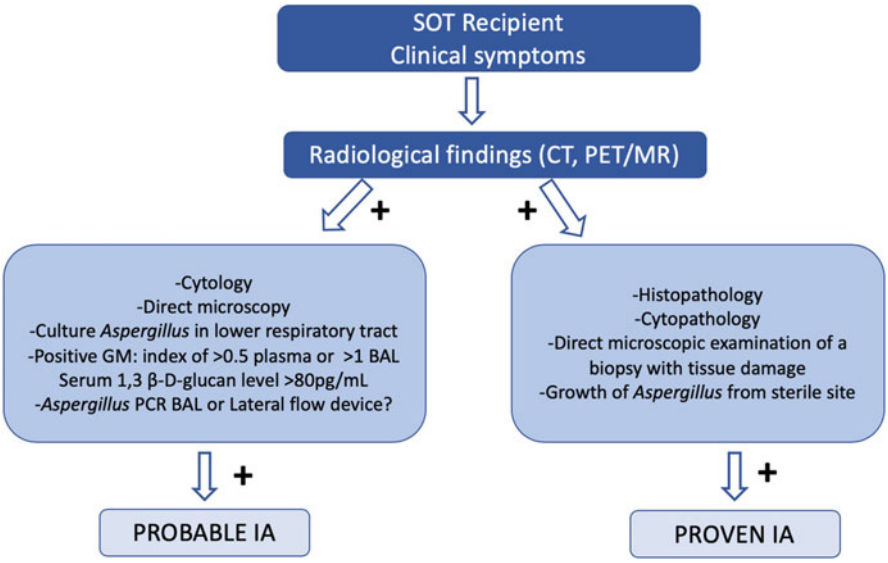


Fig. 4 Diagnostic algorithm for IA in SOT recipients

Table 4 Comparison of the different antifungal therapies for invasive aspergillosis in SOT and HSCT recipients

Drug	Evidence of efficacy	Adverse events	Monitoring
Voriconazole [60–63]	Improved survival compared to amphotericin B (70.8% vs. 57%, respectively) (HR 0.59; 95% CI, 0.40–0.88) [64]	Transient visual disturbances (blurriness, color changes, enhanced vision) seen in 20.6% of patients, <1% require discontinuation. Hepatotoxicity: increased transaminases 13%, 4–8% require discontinuation Rash 6% Hallucination (4.3%) QT prolongation Encephalopathy (levels >5.5mcg/mL) requires discontinuation Enhanced risk of squamous cell carcinoma	Monitor serum level trough concentrations 5–7 days into therapy Target voriconazole troughs >1mcg/mL to <5.5 mcg/mL Once therapeutic, recheck troughs every 1–2 weeks In unstable patients, and when there is uncertainty about concentrations, repeat every 3–5 days. Monitor hepatic function, CNI, and mTOR inhibitor agent levels Monitor cyclosporine levels
Isavuconazole [65, 66]	Non-inferior to voriconazole in invasive mold infections in HSCT and hematologic malignancy. All-cause mortality through day 42 as the primary endpoint (19% vs. 20%, respectively) [67]	Shorten QTc interval Headache 17% Nausea, vomiting, diarrhea (22–26%) LFTs elevations 16% Dyspnea and cough Infusion-related: hypotension, dyspnea, chills (6%)	Evaluate hepatic function prior and during therapy CNI and mTOR inhibitor agent levels Monitor cyclosporine levels Currently, there are no recommendations to monitor drug levels
Posaconazole [19, 68]	Studied in IA and other mycoses refractory or intolerant to conventional antifungal therapy. Overall success 42% for posaconazole and 26% for external control cases collected retrospectively	Generally, well tolerated Nausea, vomiting, diarrhea, abdominal pain Increased liver enzymes Hyperbilirubinemia Adrenal insufficiency	Evaluate hepatic function prior and during therapy CNI and mTOR inhibitor agent levels Monitor cyclosporine levels Currently, there are no recommendations to monitor drug levels
Caspofungin [69, 70]	Previously received amphotericin B, and a favorable response to caspofungin therapy was observed in 45% of	Generally, well tolerated LFT/bilirubin elevations Elevated ALP	Evaluate hepatic function at baseline and every 1–2 weeks during therapy

(continued)

Table 4 (continued)

Drug	Evidence of efficacy	Adverse events	Monitoring
	patients compared to 16% among historical controls, included patients that were HSCT recipients (25%) and SOT recipients (11%)	Histamine-mediated symptoms: rash, facial swelling, and pruritus that are infusion related	Monitor CNI inhibitor agent levels
Anidulafungin [71]	Studied as combination therapy with voriconazole vs. voriconazole monotherapy patients with HM and HSCT recipients. Trend toward decreased mortality at 6 weeks 19.3% vs. 27.5%, respectively (−8.2 percentage points [95% CI, −19.0 to 1.5]; $p = 0.087$)	Generally, well tolerated Histamine-mediated symptoms: rash, facial swelling, and pruritus that are infusion related Phlebitis Fever Diarrhea	Not a substrate, inducer, or inhibitor of CYP450 isoenzymes No significant drug-drug interactions with voriconazole, tacrolimus, ambisome, and cyclosporine No significant increase in LFTs observed
Micafungin [72]	Used as monotherapy for primary treatment, micafungin had a response rate of 50% and 40.9% as salvage therapy	Generally, well tolerated Histamine-mediated symptoms: rash, facial swelling, pruritus, and vasodilation Local phlebitis Fever and chills LFTs elevation	Monitor liver function Monitor sirolimus levels
Amphotericin [19, 64, 73]	**D-AmB**: Voriconazole showed improved survival compared to D-AmB (70.8% vs. 57%, respectively) HR 0.59; 95% CI, 0.40–0.88) [64] **L-AmB**: L-AmB was given to patients with lack of response to prior antifungal therapy; 25% patients were HSCT and 27% SOT; overall, 65% had a favorable clinical response, 44% cured or improved, and 21% stabilized [64]	**D-AmB**: Nephrotoxicity Electrolyte abnormalities Fever, chills and hypotension Anemia Phlebitis **L-AmB**: Infusion reactions Creatinine elevation Anemia Electrolyte abnormalities Transaminase elevations Increased bilirubin	Monitor renal and liver function Monitor serum electrolytes Avoid its use with other nephrotoxic agents

Intravenous voriconazole is administered within a vehicle called cyclodextrin; the accumulation of this substance has been associated with renal toxicity in animal models although this has not been proven in humans [61]. Therefore, oral voriconazole is recommended by the manufacturer in patients with CrCl <50 mL/min. However, in a clinical study, the IV formulation was used with renal dysfunction without any untoward effects [74]. In cases where voriconazole has to be used intravenously, kidney function should be monitored. Administration of voriconazole through an NG tube may require stopping the feed 2 h prior and 2 h after the administration of voriconazole to maximize absorption.

Voriconazole remains the drug of choice in IA [19]. In a randomized controlled trial of 144 patients (29.9% of which were HSCT recipients and 6.2% SOT recipients), voriconazole was compared to amphotericin B in IA. Voriconazole had a successful treatment outcome after 12 weeks of treatment of 52.8% versus 31.6%, respectively, and was associated with improved survival (70.8% in the voriconazole group and 57.9% in the amphotericin B group) [64].

Isavuconazole

Isavuconazonium sulfate is an advanced triazole compound, which is the prodrug of the active isavuconazole. This drug is a potent inhibitor of ergosterol biosynthesis and causes fungal membrane disruption by targeting enzyme 14-α-demethylase. This antifungal is available in oral and intravenous preparations. Isavuconazole is considered a sensitive substrate and a moderate inhibitor of CYP3A4, and accordingly, drug interactions with this antifungal should always be a consideration.

This water-soluble molecule does not need the excipient β-cyclodextrin for intravenous administration and has excellent bioavailability in its oral administration (98%) without any clinically relevant food effects. Isavuconazole is a broad-spectrum antifungal, with FDA approval for treatment of IA and invasive mucormycosis [65].

The efficacy and safety of isavuconazole in the primary treatment of invasive mold disease caused by *Aspergillus* and other filamentous fungi were assessed in a randomized controlled trial that included patients post HSCT or with active malignant diseases. Isavuconazole was found to be non-inferior to voriconazole, as determined using all-cause mortality through day 42 as the primary endpoint (19% vs. 20%, respectively) [66]. It demonstrated fewer drug-related effects than voriconazole (14% vs. 23%, respectively, including less hepatobiliary disorders and eye and skin disorders) [67]. Currently, data concerning the efficacy of isavuconazole in SOT recipients is limited. Isavuconazole can be used in lieu of voriconazole in the treatment of invasive aspergillosis.

Other Azoles

Itraconazole is not a first-line agent for the treatment of IA, as it is an inhibitor and substrate for CYP3A4 and can have serious drug-drug interactions with other chemotherapeutic agents (e.g., cyclophosphamide and vincristine). Additionally,

it has significant pharmacokinetic variation in the absorption and distribution of the drug between patients, due to the needs of an acidic environment for absorption, poor bioavailability, and significant drug interactions. Itraconazole has been shown to have a negative inotropic effect, with reports of congestive heart failure (CHF) associated with its use [75, 76]. The mechanism of itraconazole-induced CHF and its reversibility is unknown. In the presence of CHF, itraconazole should be used with caution. Itraconazole should be reserved for patients with less severe disease as an alternative agent [19].

Posaconazole is an extended-spectrum triazole with in vitro and in vivo activity against *Aspergillus* spp.

Although structurally similar to itraconazole, it has mainly been studied as prophylaxis for IA.

The efficacy of posaconazole as a monotherapy agent for IA has only been assessed in an open-label study of patients with IA and other mycoses, who were refractory or intolerant to conventional antifungal therapy. Posaconazole was compared with data from external control cases, collected retrospectively as a reference group. The overall success was 42% for posaconazole recipients and 26% for control subjects [67]. Posaconazole could be an effective agent for the treatment of IA, given its spectrum of activity and treatment success observed in this uncontrolled trial. However, more studies assessing the efficacy of this drug are needed. The use of posaconazole in primary therapy is further influenced by the fact that steady-state levels of the drug may take 1 week of therapy to be achieved.

Azole antifungal drugs exhibit a wide range and variety of drug-drug interactions, the most common of which are outlined in Table 5.

Echinocandins

Echinocandins are a group of antifungals that inhibit (1,3)-β-D-glucan, an integral component of the fungal cell wall. There are three echinocandins currently available in the market: caspofungin, anidulafungin, and micafungin. These agents have activity against *Aspergillus* spp., and they are considered to have equivalent efficacy. However, caspofungin is the only agent in this class that has been approved by the FDA for the treatment of IA in patients who are refractory to or intolerant of other antifungal therapies [69]. Echinocandins are effective as salvage therapy for IA; primary therapy with an echinocandin is not recommended [49].

The efficacy of caspofungin was assessed in a trial that included patients with IA who were refractory to or intolerant of amphotericin B lipid formulation or triazole therapy. They included patients that were HSCT recipients (25%) and SOT recipients (11%). Almost all of the patients previously received amphotericin B, and a favorable response to caspofungin therapy was observed in 45% of patients compared to 16% among historical controls [70].

Anidulafungin has not been studied as primary or salvage therapy for IA. So far, it has only been reviewed in combination therapy with voriconazole, in patient populations that included hematologic malignancies and HSCT recipients. The

Table 5 Interaction of azoles with other drugs [61, 65, 68, 77]

Azole	Interacting drug	Effect of interaction	Considerations
Voriconazole Itraconazole Posaconazole Isavuconazole	Alprazolam Midazolam	Alprazolam and midazolam serum levels concentrations may be increased	Consider lorazepam, oxazepam, temazepam
Voriconazole Isavuconazole	Atazanavir/ ritonavir Darunavir/ ritonavir Lopinavir/ ritonavir Nevirapine	Voriconazole AUC may be decreased	Follow voriconazole through level
Posaconazole	Amiodarone	Amiodarone serum concentrations may be increased.	Avoid co-administration
Isavuconazole	Lopinavir/ ritonavir	Drug levels of isavuconazole increase 96%	Consider avoiding the combination
Voriconazole Itraconazole	Clopidogrel	May decrease the efficacy of clopidogrel	Avoid co-administration
Voriconazole	Cyclosporine	Cyclosporine AUC increased by 70%	Reduce cyclosporine dose 50% and follow levels
Isavuconazole Posaconazole Itraconazole	Cyclosporine	Cyclosporine serum concentration might be increased	Monitor cyclosporine levels and adjust
Isavuconazole	Mycophenolate mofetil	Increased MMF exposure	Monitor possible MMF toxicities
Voriconazole	Omeprazole	Omeprazole AUC increased by 400% and voriconazole AUC increases 40%	Reduce omeprazole dose by 50% and follow voriconazole trough level
Posaconazole Itraconazole	Omeprazole Pantoprazole	Liquid formulation posaconazole: will decrease posaconazole levels, tablet not affected Itraconazole absorption decreased	Avoid co-administration with liquid azole formulations
Voriconazole Posaconazole	Rifabutin Rifampin	Rifabutin levels might be increased Voriconazole and posaconazole levels might be decreased	Avoid co-administration
Voriconazole	Simvastatin	Simvastatin levels might be increased	Consider pravastatin, atorvastatin or rosuvastatin
Isavuconazole Posaconazole	Atorvastatin	May increase atorvastatin levels	Use caution, monitor LFTs and CK.
Voriconazole Isavuconazole Itraconazole	Sirolimus	Sirolimus serum concentrations may be increased	Monitor sirolimus levels and adjust

(continued)

Table 5 (continued)

Azole	Interacting drug	Effect of interaction	Considerations
Voriconazole Isavuconazole Itraconazole	Tacrolimus	Tacrolimus AUC increased by 300% with voriconazole	Reduce tacrolimus dose to 1/3 of original dose and follow levels.
Voriconazole Posaconazole Itraconazole	Warfarin	Anticoagulation may be increased	Monitor INR

use of combination therapy was not superior to monotherapy with voriconazole. In post hoc analysis, combination of voriconazole and anidulafungin resulted in lower mortality rate among patients with positive galactomannan in the BAL [71]. Anidulafungin is an echinocandin that is not a substrate, inducer, or inhibitor of CYP450 isoenzymes and therefore has no significant drug-drug interactions. In addition, it is eliminated by nonenzymatic degradation and does not require dosage adjustments in patients with renal or hepatic disease [68].

Micafungin has been evaluated in patients with proven or probable IA, including patients with hematologic malignancies and HSCT and SOT recipients. Patients treated with micafungin as monotherapy had a response rate of 50% as primary treatment and 40.9% as salvage therapy [72].

Polyenes

Polyenes bind to ergosterol and disrupt the fungal cell membrane, resulting in leakage of intracellular contents. A notable example of this class is amphotericin B, of which there are currently three formulations: amphotericin B deoxycholate (D-AmB), liposomal amphotericin B, and amphotericin B lipid complex (L-AmB). Amphotericin B deoxycholate is no longer recommended as a therapy due to nephrotoxicity. The lipid formulations are less nephrotoxic and are preferred, even though they have not shown to have better outcomes compared to conventional amphotericin B [19]. Either option is appropriate for initial and salvage therapy of IA.

New Antifungals Currently Under Investigation

Rezafungin (formerly CD101) is an investigational echinocandin under development for the treatment and prevention of IFIs. With a half-life in humans of 130 h, it allows once-weekly treatment and possesses effective in vitro activity and in vivo efficacy against some *Aspergillus* species [78]. Rezafungin has demonstrated potent in vitro activity against *Aspergillus* spp., including azole-resistant *A. fumigatus* isolates, similar to caspofungin and micafungin. However, rezafungin has more potent activity against *A. calidoustus*, as compared to caspofungin [78]. Further studies are warranted to evaluate the in vivo efficacy and its role in the treatment of IA in HSCT and SOT.

Ibrexafungerp (formerly SCY-078) is the first representative of a novel class of structurally distinct glucan synthase inhibitors, "triterpenoids." While currently in phase II oral study for IA, preclinical studies demonstrated potent in vitro and in vivo activity against wild-type and azole-resistant strains of *Aspergillus* and indicated synergistic activity with other antifungal agents in vitro against *Aspergillus fumigatus* [79].

There are other antifungal compounds with novel targets in development which are currently unapproved compounds with novel structures. APX001 is a new compound that has the molecular target of glycosyl phosphatidylin-ositol synthesis (prevents attachment of mannose proteins to the outer cell wall) with potential broad-spectrum potency against *Mucorales*, *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* species [77]. F901318 is a new compound that has the molecular target of dihydroorotate dehydrogenase and has potential activity against *Aspergillus* and *Scedosporium* species, currently undergoing phase II testing [77, 80].

Olorofim (F2G) is another drug that is in phase II development as an oral or intravenous agent for the treatment of mold infections, particularly *Aspergillus* and *Scedosporium*. It is a potent inhibitor of dihydroorotate dehydrogenase, an enzyme involved in the organisms' pyrimidine biosynthesis [80]. The work on host immune cell-targeted approaches is currently in its infancy. A detailed review of new antifungal agents currently in the pipeline was published by Perfect in 2017 [81].

In addition, there are other compounds that could be repurposed such as cyclosporine, tacrolimus, and rapamycin which are mTOR or calcineurin inhibitors already approved for posttransplant immunosuppression and have showed broad antifungal spectrum and could be used alongside existing antifungals [77].

Combination Therapy

Combination therapy in the treatment of IA is not routinely recommended as primary treatment. However, it is commonly used in patients who have not responded to their initial regimen of treatment. Most common combination therapies include voriconazole, as well as an echinocandin.

Voriconazole plus Echinocandin Therapy

The efficacy of voriconazole plus anidulafungin was compared with voriconazole monotherapy for the treatment of IA, in patients with hematological malignancy or HSCT with suspected or documented IA. At 6 weeks of treatment, the combination therapy showed decreased mortality (19.3%) compared to monotherapy (27.5%) (−8.2 percentage points [95% CI, −19.0 to 1.5]; $p = 0.087$) with a similar safety profile in both groups [71].

In a retrospective analysis of HSCT recipients with probable or proven aspergillosis, there was no difference in outcomes in patients who were treated with voriconazole and caspofungin as primary therapy, compared with patients treated with voriconazole as monotherapy [82].

In the SOT population, the combination of voriconazole and caspofungin was assessed as primary therapy for proven or probable cases of IA, compared to a historical control group who received lipid formulation of AmB as primary therapy. The survival rate at 90 days was 67.5% in the combination therapy group, compared to 51% in the control group (HR 0.58, 95% CI, 0.30–1.14, $p = 0.117$). Combination therapy was independently associated with reduced mortality in patients with renal failure and in those with *A. fumigatus* infection [83].

Liposomal Amphotericin B plus Echinocandin Therapy

The studies evaluating this combination of antifungals did not compare voriconazole-based regimens. It has only been evaluated in patients with hematological malignancies, not in HSCT or SOT populations.

Duration of Therapy

The duration of therapy for IA has not yet been established. It is recommended to be continued for a minimum of 12 weeks, depending on the degree and duration of immunosuppression, site of infection, and evidence of disease improvement [19].

Susceptibility Testing

The emerging resistance of antifungal agents is a developing global issue. Recently "AsperGenius" (PathoNostics, Maastricht, the Netherlands) a commercial PCR assay became available, which includes markers specifically for the detection of TR_{46}/Y121F/T289A and TR_{34}/L98H.

Susceptibility testing is of particular relevance in patients suspected to have an azole-resistant isolate, who are unresponsive to antifungal agents, or for epidemiological purposes [29]. Many species of *Aspergillus fumigatus* complex have been associated with high mixed inhibitory concentration (MIC) for amphotericin B (Table 6), and susceptibility testing should be considered. Such isolates should be referred to reference laboratories for MIC testing. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI) criteria can be used for determination of routine MICs to guide clinical management [19].

Table 6 Recommended antifungal regimens in cases of intrinsic resistance [17, 19, 29, 68]

Species	Susceptibilities	Intervention
Aspergillus fumigatus complex *A. lentus* *A. udagawae* *A. viridinutans* *A. thermomutatus* *A. novofumigatus* *A. hiratsukae* *A. pseudofischeri*	Emerging resistance has been reported. Consider local epidemiology of azole resistance	Recommend testing for susceptibility
Aspergillus flavus complex *A. alliaceus*	Partial resistance to amphotericin B. *A. alliaceus* higher MIC for AmB. Less activity of anidulafungin	Recommend testing for susceptibility Consider voriconazole
Aspergillus terreus	Resistant to amphotericin B More susceptible to newer azoles	Recommend using voriconazole, isavuconazole, posaconazole, or itraconazole
Aspergillus calidoustus	Resistant to azoles	Recommend lipid formulations of amphotericin B
Aspergillus tubingensis (*A. niger* complex)	Higher azole MIC common, but no data on clinical impact	Recommend options other than azole monotherapy
Aspergillus niger complex	Isavuconazole, posaconazole, and voriconazole MIC in general one dilution higher compared with *A. fumigatus*; itraconazole MIC in general two steps higher; limited clinical data	Recommend other than itraconazole and isavuconazole [29]
Aspergillus nidulans *Aspergillus tanneri*	May be resistant to amphotericin B	Recommend using voriconazole

Prevention

Given the high frequency of IA, prevention strategies are of great relevance as they increase the clinical success and outcomes of transplant by decreasing risk of IA. Universal prophylaxis and preemptive therapy are the main approaches for prevention. However, these two strategies have differing evidence for efficacy in

HSCT recipients and SOT recipients. Table 7 shows the current considerations of antifungals in IA prophylaxis.

"Universal prophylaxis" involves administration of antifungal therapy – usually for a prolonged period – to all transplant recipients, irrespective of their risk of IA. "Target prophylaxis" involves initiating antifungal therapy based on risk factors. Preemptive therapy is instituted based on the results of culture or biomarkers such as GM and/or β-D-Glucan assays.

HSCT Recipients

The current evidence suggests that the patient populations that benefit from antifungal prophylaxis are patients who have prolonged neutropenia (defined as >7 days of neutropenia) and have a high risk of IA [29] and also patients who are allogenic HSCT recipients with GVHD and have a high risk of IA. Posaconazole was more effective than fluconazole in HSCT recipients with GVHD (OR 0.31, 95% CI 0.13 to 0.75) [88]. Isavuconazole was previously studied in a retrospective study as prophylaxis in HSCT recipients, compared with other azole prophylaxis (voriconazole and posaconazole), isavuconazole showed no difference in cumulative indicence of breakthrough IFIs [84]. Currently, isavuconazole prophylaxis is under investigation in HSCT recipients.

SOT Recipients

While the average incidence of IA is lower in SOT recipients than in HSCT recipients, SOT recipients are treated with prolonged high doses of glucocorticoids or other immunosuppressants, some may be critically ill, and all have an end-stage organ disease. Furthermore, drug-drug interactions and toxicities are more likely to occur in SOT recipients. Accordingly, physicians need to balance the risk and benefit of giving antifungal prophylaxis to this patient population.

In liver transplant recipients, universal prophylaxis against IA is not routinely recommended, and instead targeted prophylaxis is suggested in patients with a higher risk of IA (Table 1) [19]. Targeted prophylaxis in liver transplant was studied in a retrospective cohort, showing that is feasible and safe. It prevented IFIs and reduced the number of patients exposed to antifungals [85]. At this moment, voriconazole and echinocandins have been studied as prophylaxis for IA in liver transplants; however, there is no conclusive evidence that one antifungal is superior in this patient population.

In heart transplant recipients, there is insufficient data to routinely recommend anti-*Aspergillus* prophylaxis, as a majority of the data on prophylactic strategies in heart transplant recipients is reported from single-center studies using targeted prophylaxis in patients with risk factors for IA, using itraconazole, voriconazole, or echinocandins.

Table 7 Recommended anti-*Aspergillus* prophylactic strategies in HSCT and SOT recipients [19, 49, 84–87]

Type of transplant	Prophylaxis strategy	Prophylaxis	Duration
HSCT recipients	Consider prophylaxis in patients with: Prolonged neutropenia Allogenic HSCT with GvHD	Posaconazole Voriconazole	3–4 months [82, 88]
Liver transplant recipients	Consider targeted prophylaxis in patients with any of the following risk factors: Re-transplantation or reoperation Rejection or change immunosuppression Acute kidney injury Renal replacement therapy Fulminant hepatic failure MELD >30 CMV infection	Anidulafungin Caspofungin Micafungin Voriconazole	10–21 days [85]
Heart transplant	Consider targeted prophylaxis *Aspergillus* colonization Reoperation Posttransplant hemodialysis Rejection or change immunosuppression Existence of an episode of IA in any patient within the heart transplant program, 2 months before or after transplant. Pretransplant diagnosis of COPD High duration of steroids CMV infection	Voriconazole Itraconazole Echinocandins Nebulized L-Amb	3 months [87]
Lung transplant recipients	1. Universal prophylaxis 2. Targeted prophylaxis in patients with any of the following risk factors: Single lung transplant Early airway ischemia Rejection or change immunosuppression Pretransplant colonization Induction with alemtuzumab or anti-thymocyte globulin Positive intraoperative *Aspergillus* culture in CF patient Hypogammaglobulinemia CMV infection And that will be followed with BAL cultures and BAL galactomannan	Nebulized L-Amb Voriconazole Posaconazole Isavuconazole	Universal and targeted prophylaxis usually 4–6 months [86, 87]

In lung transplant recipients, the optimal IA prevention strategy has not been defined. Although universal prophylaxis is a common practice in many transplant centers, several studies have used a risk stratification strategy for the use of antifungal prophylaxis in the first year following lung transplant. Currently, target therapy can be employed in centers which are able to follow their patients with BAL culture and BAL GM in patients who have increased risk factors for IA. In a systematic review and meta-analysis, 8.1% of patients receiving universal prophylaxis and 14.3% in the no-prophylaxis arm developed IA. No reduction of IA or *Aspergillus* colonization was seen with universal anti-*Aspergillus* prophylaxis [86]. Currently there is no evidence of the superiority of one antifungal therapy over another.

Conclusions

IA continues to be an important cause of life-threatening infection in HSCT and SOT recipients. Optimal management of this infection involves early diagnosis and early initiation of antifungal therapy. The diagnosis of IA in HSCT and SOT recipients is dependent on a combination of clinical and radiological findings with noninvasive tests such as serum GM and β-D-glucan and also invasive tests such as BAL specimens for fungal staining, culture, BAL GM, and tissue biopsies.

Currently, voriconazole is recommended as the primary therapy for IA. However, there are alternative regimens available, and the decision of which agent to choose depends on organ dysfunction, toxicities, and tolerability. The duration of therapy depends upon the location of the infection, the immunosuppression that the patient is receiving, and the response to therapy. The most effective approach for IA antifungal prophylaxis in SOT and HSCT recipients has not yet been determined and should be selected depending on the patients' risk factors and the logistics of each institution. While the diagnosis and management of IA have advanced significantly over the last few years, there are still many unanswered and unresolved questions.

Key Points

- Invasive aspergillosis has a higher incidence of infection in patients undergoing hematopoietic stem cell transplantation or solid organ transplantation.
- The diagnosis of invasive aspergillosis is based on a combination of host factors and imaging and microbiological findings.
- Improved survival has been noted with early diagnosis and early initiation of antifungal therapy.
- *Proven* infection is established histopathology – showing dichotomously branching septate hyphae, associated tissue damage, and growth of *Aspergillus* species from a culture taken from a normally sterile site.
- Biomarkers such as galactomannan, β-D-glucan, and DNA detection via PCR are useful for establishing the diagnosis of *Probable IA*.
- Voriconazole is recommended for primary therapy to treat all forms of IA.

- Isavuconazole and lipid formulations of amphotericin B (preferably L-AmB) can be considered alternative agents.
- Combination therapy is not recommended for routine use in HSCT and SOT populations; it can be considered in select cases such as disseminated or refractory disease.
- Duration of treatment should be guided by clinical response but is usually administered for a minimum of 12 weeks if it is well tolerated.

References

1. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.
2. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi (Basel). 2017;3(4):57.
3. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. Clin Infect Dis. 2010;50(8):1091–100.
4. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood. 2002;100(13):4358–66.
5. Steinbach WJ, Marr KA, Anaissie EJ, et al. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. J Inf. 2012;65(5):453–64.
6. Gavalda J, Len O, San JR, et al. Risk factors for invasive aspergillosis in solid-organ transplant recipients: a case-control study. Clin Infect Dis. 2005;41(1):52–9.
7. Schauwvlieghe A, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. Lancet Respir Med. 2018;6(10):782–92.
8. Desbois AC, Poiree S, Snanoudj R, et al. Prognosis of invasive aspergillosis in kidney transplant recipients: a case-control study. Transplant Direct. 2016;2(8):e90.
9. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood. 2003;102(3):827–33.
10. Grossi P, De Maria R, Caroli A, Zaina MS, Minoli L. Infections in heart transplant recipients: the experience of the Italian heart transplantation program. Italian Study Group on Infections in Heart Transplantation. J Heart Lung Transplant. 1992;11(5):847–66.
11. Lortholary O, Gangneux JP, Sitbon K, et al. Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005–2007). Clin Microbiol Infect. 2011;17(12):1882–9.
12. San Juan R, Aguado JM, Lumbreras C, et al. Incidence, clinical characteristics and risk factors of late infection in solid organ transplant recipients: data from the RESITRA study group. Am J Transplant. 2007;7(4):964–71.
13. Carvalho-Dias VMH, Sola CBS, Cunha CA, Shimakura SE, Pasquini R, Queiroz-Telles F. Invasive aspergillosis in hematopoietic stem cell transplant recipients: a retrospective analysis. Braz J Infect Dis. 2008;12:385–9.
14. Singh N, Arnow PM, Bonham A, et al. Invasive aspergillosis in liver transplant recipients in the 1990s. Transplantation. 1997;64(5):716–20.
15. Aguilar CA, Hamandi B, Fegbeutel C, et al. Clinical risk factors for invasive aspergillosis in lung transplant recipients: results of an international cohort study. J Heart Lung Transplant. 2018;36(4):S24.

16. Meis JF, Chowdhary A, Rhodes JL, Fisher MC, Verweij PE. Clinical implications of globally emerging azole resistance in *Aspergillus fumigatus*. Philos Trans R Soc Lond Ser B Biol Sci. 2016;371(1709).
17. Verweij PE, Chowdhary A, Melchers WJ, Meis JF. Azole resistance in *Aspergillus fumigatus*: can we retain the clinical use of mold-active antifungal azoles? Clin Infect Dis. 2016;62(3):362–8.
18. Lescar J, Meyer I, Akshita K, et al. *Aspergillus fumigatus* harbouring the sole Y121F mutation shows decreased susceptibility to voriconazole but maintained susceptibility to itraconazole and posaconazole. J Antimicrob Chemother. 2014;69(12):3244–7.
19. Patterson TF, Thompson GR 3rd, Denning DW, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):e1–e60.
20. van der Linden JWM, Arendrup MC, Warris A, et al. Prospective multicenter international surveillance of azole resistance in *Aspergillus fumigatus*. Emerg Infect Dis. 2015;21(6):1041–4.
21. van der Linden JWM, Snelders E, Kampinga GA, et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. Emerg Infect Dis. 2011;17(10):1846–54.
22. Baddley JW, Marr KA, Andes DR, et al. Patterns of susceptibility of *Aspergillus* isolates recovered from patients enrolled in the transplant-associated infection surveillance network. J Clin Microbiol. 2009;47(10):3271–5.
23. Shalhoub S, Luong ML, Howard SJ, et al. Rate of cyp51A mutation in *Aspergillus fumigatus* among lung transplant recipients with targeted prophylaxis. J Antimicrob Chemother. 2015;70(4):1064–7.
24. Lamoth F, Chung SJ, Damonti L, Alexander BD. Changing epidemiology of invasive mold infections in patients receiving azole prophylaxis. Clin Infect Dis. 2017;64(11):1619–21.
25. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813–21.
26. Husain S, Sole A, Alexander BD, et al. The 2015 International Society for Heart and Lung Transplantation Guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients: executive summary. J Heart Lung Transplant. 2016;35(3):261–82.
27. Singh N, Husain S. *Aspergillus* infections after lung transplantation: clinical differences in type of transplant and implications for management. J Heart Lung Transplant. 2003;22(3):258–66.
28. Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. Clin Microbiol Rev. 2005;18(1):44–69.
29. Ullmann AJ, Aguado JM, Arikan-Akdagli S, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect. 2018;24(Suppl 1):e1–e38.
30. Cuenca-Estrella M, Bassetti M, Lass-Florl C, Racil Z, Richardson M, Rogers TR. Detection and investigation of invasive mould disease. J Antimicrob Chemother. 2011;66(Suppl 1): i15–24.
31. Odabasi Z, Mattiuzzi G, Estey E, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin Infect Dis. 2004;39(2):199–205.
32. Mercier T, Guldentops E, Lagrou K, Maertens J. Galactomannan, a Surrogate marker for outcome in invasive aspergillosis: finally coming of age. Front Microbiol. 2018;9:661.
33. Husain S, Paterson DL, Studer SM, et al. *Aspergillus* galactomannan antigen in the bronchoalveolar lavage fluid for the diagnosis of invasive aspergillosis in lung transplant recipients. Transplantation. 2007;83(10):1330–6.
34. Clancy CJ, Jaber RA, Leather HL, et al. Bronchoalveolar lavage galactomannan in diagnosis of invasive pulmonary aspergillosis among solid-organ transplant recipients. J Clin Microbiol. 2007;45(6):1759–65.

35. Leeflang MM, Debets-Ossenkopp YJ, Visser CE, et al. Galactomannan detection for invasive aspergillosis in immunocompromized patients. Cochrane Database Syst Rev. 2008;(4): Cd007394.
36. Kwak EJ, Husain S, Obman A, et al. Efficacy of galactomannan antigen in the Platelia *Aspergillus* enzyme immunoassay for diagnosis of invasive aspergillosis in liver transplant recipients. J Clin Microbiol. 2004;42(1):435–8.
37. Husain S, Kwak EJ, Obman A, et al. Prospective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. Am J Transplant. 2004;4(5):796–802.
38. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis. 2006;42(10):1417–27.
39. Maertens J, Maertens V, Theunissen K, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. Clin Infect Dis. 2009;49(11):1688–93.
40. Akamatsu N, Sugawara Y, Kaneko J, Tamura S, Makuuchi M. Preemptive treatment of fungal infection based on plasma (1 –> 3)beta-D-glucan levels after liver transplantation. Infection. 2007;35(5):346–51.
41. Bhaskaran A, Kabbani D, Singer LG, et al. (1,3) beta-D-Glucan in bronchoalveolar lavage of lung transplant recipients for the diagnosis of invasive pulmonary aspergillosis. Med Mycol. 2017;55(2):173–9.
42. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol. 2014;52(10):3731–42.
43. Luong ML, Clancy CJ, Vadnerkar A, et al. Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. Clin Infect Dis. 2011;52(10):1218–26.
44. Greene RE, Schlamm HT, Oestmann JW, et al. Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. Clin Infect Dis. 2007;44(3):373–9.
45. Singh N, Suarez JF, Avery R, et al. Risk factors and outcomes in lung transplant recipients with nodular invasive pulmonary aspergillosis. J Inf Secur. 2013;67(1):72–8.
46. Qin J, Fang Y, Dong Y, et al. Radiological and clinical findings of 25 patients with invasive pulmonary aspergillosis: retrospective analysis of 2150 liver transplantation cases. Br J Radiol. 2012;85(1016):e429–e35.
47. Lim C, Seo JB, Park SY, et al. Analysis of initial and follow-up CT findings in patients with invasive pulmonary aspergillosis after solid organ transplantation. Clin Radiol. 2012;67(12):1179–86.
48. Singh N, Winston DJ, Limaye AP, et al. Performance characteristics of galactomannan and beta-d-glucan in high-risk liver transplant recipients. Transplantation. 2015;99(12):2543–50.
49. Husain S, Camargo JF. Invasive aspergillosis in solid-organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33(9):e13544.
50. Dudakova A, Spiess B, Tangwattanachuleeporn M, et al. Molecular tools for the detection and deduction of azole antifungal drug resistance phenotypes in *Aspergillus* species. Clin Microbiol Rev. 2017;30(4):1065–91.
51. Pan Z, Fu M, Zhang J, Zhou H, Fu Y, Zhou J. Diagnostic accuracy of a novel lateral-flow device in invasive aspergillosis: a meta-analysis. J Med Microbiol. 2015;64(7):702–7.
52. Heldt S, Hoenigl M. Lateral flow assays for the diagnosis of invasive Aspergillosis: current status. Curr Fungal Infect Rep. 2017;11(2):45–51.
53. Hoenigl M, Heldt S, Prattes J, et al. 2567. Diagnosis of invasive aspergillosis in hematological malignancy patients receiving mold-active antifungals: performance of Interleukin-6 and -8, Asp LFD, and *Aspergillus* PCR in same-day blood and bronchoalveolar lavage fluid samples. Open Forum Infectious Diseases. 2018;5(Suppl 1):S73-S.

54. Marr KA, Datta K, Mehta S, et al. Urine antigen detection as an aid to diagnose invasive aspergillosis. Clin Infect Dis. 2018;67(11):1705–11.
55. Koo S, Thomas HR, Daniels SD, et al. A breath fungal secondary metabolite signature to diagnose invasive aspergillosis. Clin Infect Dis. 2014;59(12):1733–40.
56. Croston TL, Nayak AP, Lemons AR, et al. Influence of *Aspergillus fumigatus* conidia viability on murine pulmonary microRNA and mRNA expression following subchronic inhalation exposure. Clin Exp Allergy. 2016;46(10):1315–27.
57. Georgiadou SP, Sipsas NV, Marom EM, Kontoyiannis DP. The diagnostic value of halo and reversed halo signs for invasive mold infections in compromised hosts. Clin Infect Dis. 2011;52(9):1144–55.
58. Park SY, Kim SH, Choi SH, et al. Clinical and radiological features of invasive pulmonary aspergillosis in transplant recipients and neutropenic patients. Transpl Infect Dis. 2010;12(4):309–15.
59. Thornton CR. Molecular imaging of invasive pulmonary aspergillosis using ImmunoPET/MRI: the future looks bright. Front Microbiol. 2018;9:691.
60. Boyd AE, Modi S, Howard SJ, Moore CB, Keevil BG, Denning DW. Adverse reactions to voriconazole. Clin Infect Dis. 2004;39(8):1241–4.
61. Eiden C, Peyriere H, Cociglio M, et al. Adverse effects of voriconazole: analysis of the French Pharmacovigilance Database. Ann Pharmacother. 2007;41(5):755–63.
62. Pascual A, Calandra T, Bolay S, Buclin T, Bille J, Marchetti O. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis. 2008;46(2):201–11.
63. Stott KE, Hope WW. Therapeutic drug monitoring for invasive mould infections and disease: pharmacokinetic and pharmacodynamic considerations. J Antimicrob Chemother. 2017;72(Suppl 1):i12–i8.
64. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med. 2002;347(6):408–15.
65. Seyedmousavi S, Verweij PE, Mouton JW. Isavuconazole, a broad-spectrum triazole for the treatment of systemic fungal diseases. Expert Rev Anti-Infect Ther. 2015;13(1):9–27.
66. Maertens JA, Raad II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. Lancet. 2016;387(10020):760–9.
67. Walsh TJ, Raad I, Patterson TF, et al. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. Clin Infect Dis. 2007;44(1):2–12.
68. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 8th ed. Philadelphia: PA Elsevier/Saunders; 2015.
69. Mikulska M, Viscoli C. Current role of echinocandins in the management of invasive aspergillosis. Curr Infect Dis Rep. 2011;13(6):517–27.
70. Maertens J, Boogaerts M, Raad I, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Clin Infect Dis. 2004;39(11):1563–71.
71. Marr KA, Schlamm HT, Herbrecht R, et al. Combination antifungal therapy for invasive aspergillosis: a randomized trial. Ann Intern Med. 2015;162(2):81–9.
72. Denning DW, Marr KA, Lau WM, et al. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. J Inf Secur. 2006;53(5):337–49.
73. Chandrasekar PH, Ito JI. Amphotericin B lipid complex in the management of invasive aspergillosis in immunocompromised patients. Clin Infect Dis. 2005;40(Suppl_6):S392–400.
74. Neofytos D, Lombardi LR, Shields RK, et al. Administration of voriconazole in patients with renal dysfunction. Clin Infect Dis. 2012;54(7):913–21.
75. Yusheng Q, Fang M, Gao BX, et al. Itraconazole decreases left ventricular contractility in isolated rabbit heart: mechanism of action. Toxicol Appl Pharmacol. 2013;268(2):113–22.

76. Okuyan H, Altin C. Heart failure induced by itraconazole. Indian J Pharm. 2013;45(5):524–5.
77. Dodds-Ashley E. Management of drug and food interactions with azole antifungal agents in transplant recipients. Pharmacotherapy. 2010;30(8):842–54.
78. Wiederhold NP, Locke JB, Bartizal K, Daruwala P. Rezafungin (CD101) demonstrates potent in vitro activity against Aspergillus, including azole-resistant Aspergillus fumigatus isolates and cryptic species. J Antimicrob Chemother. 2018;73(11):3063–7.
79. Ghannoum M, Long L, Larkin EL, et al. Evaluation of the antifungal activity of the novel oral glucan synthase inhibitor SCY-078, singly and in combination, for the treatment of invasive aspergillosis. Antimicrob Agents Chemother. 2018;62(6)
80. Oliver JD, Sibley GEM, Beckmann N, et al. F901318 represents a novel class of antifungal drug that inhibits dihydroorotate dehydrogenase. Proc Natl Acad Sci U S A. 2016;113(45):12809–14.
81. Perfect JR. The antifungal pipeline: a reality check. Nat Rev Drug Discov. 2017;16:603.
82. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. Clin Infect Dis. 2007;44(4):531–40.
83. Singh N, Limaye AP, Forrest G, et al. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: a prospective, multicenter, observational study. Transplantation. 2006;81(3):320–6.
84. Hamadeh I, Shahid Z, Copelan EA. Use of isavuconazole for prophylaxis against invasive fungal infections in allogeneic stem cell transplant patients: a real world experience. Biol Blood Marrow Transplant. 2018;24(3):S390.
85. Eschenauer GA, Kwak EJ, Humar A, et al. Targeted versus universal antifungal prophylaxis among liver transplant recipients. Am J Transplant. 2015;15(1):180–9.
86. Bhaskaran A, Mumtaz K, Husain S. Anti-Aspergillus prophylaxis in lung transplantation: a systematic review and meta-analysis. Curr Infect Dis Rep. 2013;15(6):514–25.
87. Reichenspurner H, Gamberg P, Nitschke M, et al. Significant reduction in the number of fungal infections after lung-, heart-lung, and heart transplantation using aerosolized amphotericin B prophylaxis. Transplant Proc. 1997;29(1–2):627–8.
88. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med. 2007;356(4):335–47.

Management of Mucorales Infections in Transplant Patients

46

Sharon C.-A. Chen and Monica A. Slavin

Contents

Introduction 1110
Mucorales: The Pathogens 1110
Epidemiology 1111
Causative Pathogens 1111
Risk Factors 1112
Clinical Features 1113
Diagnosis 1114
Imaging 1114
Histopathology 1116
Culture and Microscopy-Based Diagnostic Methods 1117
Serology and Antigen Testing 1118
Molecular-Based Methods 1118
Detection in Blood and Body Fluids 1119
Molecular Techniques to Identify Mucorales from Cultures 1120
Next-Generation Sequencing 1120
Management of Mucormycosis 1120

S. C.-A. Chen (✉)
Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, New South Wales Health Pathology, Westmead Hospital, Sydney, NSW, Australia

Marie Bashir Institute for Emerging Infections and Biosecurity, The University of Sydney, Sydney, NSW, Australia

The National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
e-mail: sharon.chen@health.nsw.gov.au

M. A. Slavin
The National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
e-mail: monica.slavin@petermac.org; monica.salvin@optusnet.com.au

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_45

Susceptibility Data 1121
Antifungal Therapy 1121
Surgical Treatment 1128
Adjunctive Treatments 1129
Conclusions 1130
Key Points 1131
References 1132

Introduction

Patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT), who may also be receiving intensive chemotherapy, are at increased risk of invasive fungal diseases (IFDs). Although the commonest IFDs are invasive aspergillosis and candidiasis, mucormycosis, or infections caused by Mucorales fungi, is the next most common filamentous fungal infection, and its incidence may be rising in these patient groups [1–4]. It is typically rapidly progressive in nature and requires urgent intervention to optimize outcomes [5]. Hence awareness of this condition and of its clinical and species epidemiology and disease patterns is essential. Knowledge of current as well as newer diagnostic and management modalities is also central to informing clinical practice.

This chapter will discuss the epidemiology of mucormycosis including patterns of disease in transplant patients; major pathogenic attributes of the Mucorales, particularly those that may impact developing therapies; and modern diagnostic approaches to these infections. We also focus on antifungal therapy and other management strategies including new therapies for these problematic infections. An appreciation of the pathogens that cause mucormycosis is integral to understanding these infections.

Mucorales: The Pathogens

The term mucormycosis describes infections caused by fungi of the order Mucorales. The term "zygomycosis" which previously was used interchangeably with "mucormycosis" refers also to infections caused by the order *Entomophthorales* and other fungi and is now obsolete for what we now recognize to be Mucorales infections [6, 7]. Across all patient groups including in transplant recipients, the most frequent pathogens are *Rhizopus* spp., *Mucor* spp., and *Lichtheimia* spp., (formerly of the genera *Absidia*), followed by *Rhizomucor* spp., *Cunninghamella* spp., *Apophysomyces* spp., and *Saksenaea* spp. [8, 9]. As there is significant geographic variation, the need to understand local epidemiology is underscored [2, 4, 9]. As such, species identification is essential for epidemiological studies, delineation of disease associations, and investigation of outbreaks of infection. Table 1 summarizes the main genera and species of Mucorales encountered in transplant patients. A number of recent publications detail the taxonomic descriptions of the Mucorales [6, 10].

Table 1 Causative agents of mucormycosis: major genera and species [6, 7, 10]

Genus	Major representative species or species complex
Rhizopus[a]	*R. microsporus*, *R. stolonifer, R. arrhizus* (or *R. oryzae*), *R. delemar* (*R. arrhizus* var. *delemar*)
Mucor	*M. circinelloides* (includes *M. circinelloides f. circinelloides*, *M. circinelloides f. lusitanicus*), *M. irregularis*, *M. indicus*, *M. ellipsoideus*
Lichtheimia	*L. corymbifera*, *L. ornata*, *L. ramosa*, *L. hyalospora*, *L. hylospora*
Rhizomucor	*R. pusillus*
Cunninghamella	*C. bertholletiae*, *C. echinulate*
Apophysomyces	*A. elegans*, *A. trapeziformis*, *A. variabilis*
Saksenaea	*S. vasiformis*, *S. erythrospora*, *S. oblongispora*

[a]The fungal species database Index Fungorum identifies 11 *Rhizopus* species (http://www.indexfungorum.org). However, most clinical *Rhizopus* isolates in culture collections belong to the four species/species complexes listed in Table 1: *R. microsporus*, *R. stolonifer*, *R. arrhizus* (or *R. oryzae*), and *R. delemar* (or *R. arrhizus* var. *delemar*)

Epidemiology

Mucormycosis is most commonly contracted from inhaled sporangiospores which are ubiquitous in air from decaying organic substances and soil in the environment and thus most often infects the airways, lungs, or sinuses [11]. Spores may peak in air after high temperatures or with construction work [12]. Infection may also be cutaneous through inoculation, gastrointestinal (GI) through ingestion, or disseminated affecting any organ including the lungs, heart, brain, liver, esophagus, GI tract, retroperitoneum, kidney, spleen, thyroid, and skin [11, 13].

The US TRANSNET surveillance study performed in 21 transplant centers between 2001 and 2006 revealed a 12-month cumulative incidence of 0.29% in HSCT and 0.07% in SOT, comprising 2% of all IFDs in this population [3]. In this study, the incidence of mucormycosis in HSCT recipients increased over time [3], and this trend was also noted in renal transplant recipients [14]. Both disease prevalence and species epidemiology vary with geography [3].

Causative Pathogens

A recent review identified that in general *Rhizopus* species (213/447 cases, 48%), in particular *Rhizopus arrhizus* (previously *R. oryzae*), are the most common pathogen, followed by *Mucor* spp. (63/447, 14%), consistent with the species distribution noted in earlier large studies including those in transplant patients [3, 15, 16]. Causative Mucorales vary by geographical region. For example, *Apophysomyces* infections are very uncommon in Europe or Africa, while conversely, those caused by *Lichtheimia* have been largely reported from Europe [summarized in 8]. *Saksenaea erythrospora* infections have been documented in North and South America, and the distribution of *Saksenaea vasiformis* infections appears to be

restricted to India, Spain, Australia, and the USA [8]. These epidemiological variations extend to transplant-associated infection.

Risk Factors

In SOT patients, classical risk factors determined by a case-control study were renal failure, diabetes mellitus with or without ketoacidosis, and prior voriconazole and/or caspofungin use, while the calcineurin inhibitor tacrolimus was associated with a lower risk of mucormycosis [16]. Others have also reported an association between malnutrition and mucormycosis [11]. In the US TRANSNET study, 48.1% patients experienced organ rejection in the 30 days before mucormycosis occurred [3]. Almyroudis et al. found that 40% of reported patients in a review of published mucormycosis cases in SOT had increased immunosuppression to prevent rejection within 1 month before onset of mucormycosis [13]. Additional predisposing factors identified in HSCT patients include corticosteroid use, neutropenia, lymphopenia, splenectomy, iron overload, and acute or chronic graft-versus-host disease [3, 11]. In case series not limited to transplant recipients, deferoxamine [15] and minor or major trauma [2, 8, 11, 15] are also associated with mucormycosis. Notably breakthrough cases of mucormycosis have been seen in HSCT and SOT patients receiving either isavuconazole or posaconazole oral solution prophylaxis [17, 18]. Recent studies have found genetic associations increasing risk for IFD focusing on polymorphisms of the interleukin-10 promoter, Toll-like receptor, long pentraxin 3, plasminogen, or mannose-binding lectin genes [12].

Health-care-associated outbreaks of mucormycosis have increasingly been recognized in transplant recipients due to contaminated equipment, intravenous catheters, or air. An outbreak of GI infections caused by *Rhizopus microsporus* affecting hematology patients including four HSCT recipients was linked to allopurinol tablets and packaged food [19]. Other outbreaks, most commonly affecting SOT recipients, have been associated with adhesive bandages, wooden tongue depressors, ostomy bags, water circuitry damage, building construction [20], and locating SOT patients in negative pressure rooms [21]. Contamination of hospital linen related to inadequate washing, drying, or storage has also been identified as a source for pulmonary or cutaneous nosocomial mucormycosis, often affecting transplant recipients [22, 23]. These outbreaks have isolated genetically identical *R. delemar* or *R. microsporus* from linens and affected cases [22, 23]. Mucormycosis in intensive care unit (ICU) patients including transplant recipients was linked to *R. microsporus* and *R. oryzae* contaminated linen carts [24]. A survey of linens in 15 transplant and cancer centers in the USA revealed Mucorales were recovered from $>10\%$ of linens and at 7 sites [25]. Natural disasters such as hurricanes and floods also pose a risk to transplant recipients. Recent reports indicate an increase of cases of mucormycosis in the month after Hurricane Harvey in Texas including two SOT recipients who developed cutaneous infection [26].

With the availability of new immunomodulating therapies and evolution of transplantation practice, transplant recipients may be exposed to new lymphocyte-

depleting monoclonal antibodies, immune checkpoint inhibitors, and small molecule protein kinase inhibitors prior to or after transplantation. It is likely that new risk factors for mucormycosis will emerge in the coming years [16].

Clinical Features

The most common site of mucormycosis in the transplant recipient is pulmonary (53% cases) followed by disseminated infection (13%), sinus (12%), and skin (9%) [3]. This distribution is reflected in other series [12, 13, 17]. Differences in timing and the distribution of sites affected have been seen with different SOT populations. Liver transplant recipients were more likely to have disseminated disease and developed mucormycosis earlier after transplantation than did other SOT recipients at a median of 0.8 versus 5.7 months [16], confirmed by Park et al. where the median onset in liver transplant recipients was 81 days compared with 533 days in other SOT recipients [3]. Mucormycosis occurred later in the lung than in other transplant recipients at a median of 180 days in one study [13]. In renal transplant recipients with mucormycosis, Song et al. found that males predominated (76% cases), diabetes was the most common underlying condition, and rhinocerebral disease was more common than pulmonary disease [14].

Signs and symptoms of mucormycosis in transplant recipients may be nonspecific, and in one review of published cases in renal transplantation, fever was absent in up to half of cases [14]. Symptoms of pulmonary infection include fever, cough, and respiratory distress [14], and in lung transplant recipients, the bronchial anastomosis may be involved [12]. Primary cutaneous infection can occur at the sites of incisions, drains, intravenous catheters, or after skin trauma and may appear necrotic or cellulitic. GI lesions may present as large solitary or multiple ulcerative lesions, frequently with necrotic borders with symptoms of pain, hematemesis, melena or hematochezia, and fever [13]. Rhinocerebral disease (ROCM) is associated with hyperglycemia and acidosis in the SOT recipient and can present with fever, local pain, nasal discharge, facial swelling, and a variety of cranial nerve deficits [13]. In renal transplantation, other signs include oliguria and graft dysfunction with graft mucormycosis occurring in 11.5% and often presenting as acute rejection [14].

In a recent review, compared with other genera, *Rhizopus* spp. were significantly more often isolated in patients with ROCM, while *Cunninghamella* spp. were more common in patients with pulmonary or disseminated disease. *Lichtheimia* spp. or *Saksenaea complex* were more commonly isolated in patients with cutaneous mucormycosis [8].

Risks for poor prognosis have been identified and include infection, host, and treatment factors [12]. Disseminated disease, rhinocerebral infection, malnutrition, renal or hepatic failure, and delayed initiation of treatment have been identified as poor prognostic factors [12, 13 16]. Conversely, the presence of limited surgically resectable infection, discontinuation or reduction of immunosuppression, and early treatment with liposomal amphotericin B have been associated with better outcomes

[12, 13, 16]. Combination antifungal therapy did not improve outcomes [16] (see section "Antifungal Therapy").

Diagnosis

Diagnosis of mucormycosis is often problematic because clinical features are non-specific, frequently mimicking invasive aspergillosis. Radiological features are also non-specific although there are certain signs that are highly suggestive (see section "Imaging"). A multifaceted diagnostic approach comprised of imaging techniques, histological examination of clinical specimens, and microbiological tests (culture- as well as molecular-based) is generally required.

Clinical evaluation of the patient as above to determine likelihood of disease and disease extent is essential to guide diagnostic investigations (Fig. 1). In neutropenic HSCT or SOT patients, the lung is the most common site of infection [3, 9] focusing investigations into the respiratory tract. Diagnostic procedures should be tailored to the site of infection.

Imaging

Respiratory symptoms should lead to early, high-resolution computed tomography (CT) scanning of the chest, which is still the mainstay procedure (Fig. 1). Although insensitive and non-specific, CT features consistent with mucormycosis include lung nodules, with or without the halo sign (a ring of ground-glass opacity surrounding a nodular infiltrate), lung mass, cavitation, and pleural effusion. Of note, pulmonary aspergillosis can also manifest these abnormalities. An early abnormality which may indicate pulmonary mucormycosis is the reversed halo sign – a focal ground-glass opacity associated with a ring or crescent-shaped consolidation [27]. When studied early in neutropenic leukemic patients and followed up by serial scans, sequential morphological changes were observed in one study: (i) reversed halo sign, followed by (ii) consolidation or nodule or mass with halo sign, and (iii) central necrosis and air-crescent sign [28]. In some reports, a diagnosis of mucormycosis was more likely than aspergillosis if a neutropenic patient exhibited >10 distinct nodular infiltrates. Boucier et al. found neutropenic patients were significantly more likely to have a reversed halo sign on their CT scans compared with non-neutropenic patients (79% vs. 31%, $p < 0.05$) [29].

Other imaging abnormalities include the "hypodense" sign on magnetic resonance imaging (MRI) [30] or a central hypodensity in a lung nodule or area of consolidation. The vessel occlusion sign on CT angiography (an interrupted vessel at the border of a focal lesion) is more sensitive and possibly more specific sign of pulmonary mucormycosis in patients with hematological cancer [31]. Imaging of other sites such as the sinus, brain, and abdomen should be performed to aid diagnosis and delineate extent of infection and be considered on a case-by-case

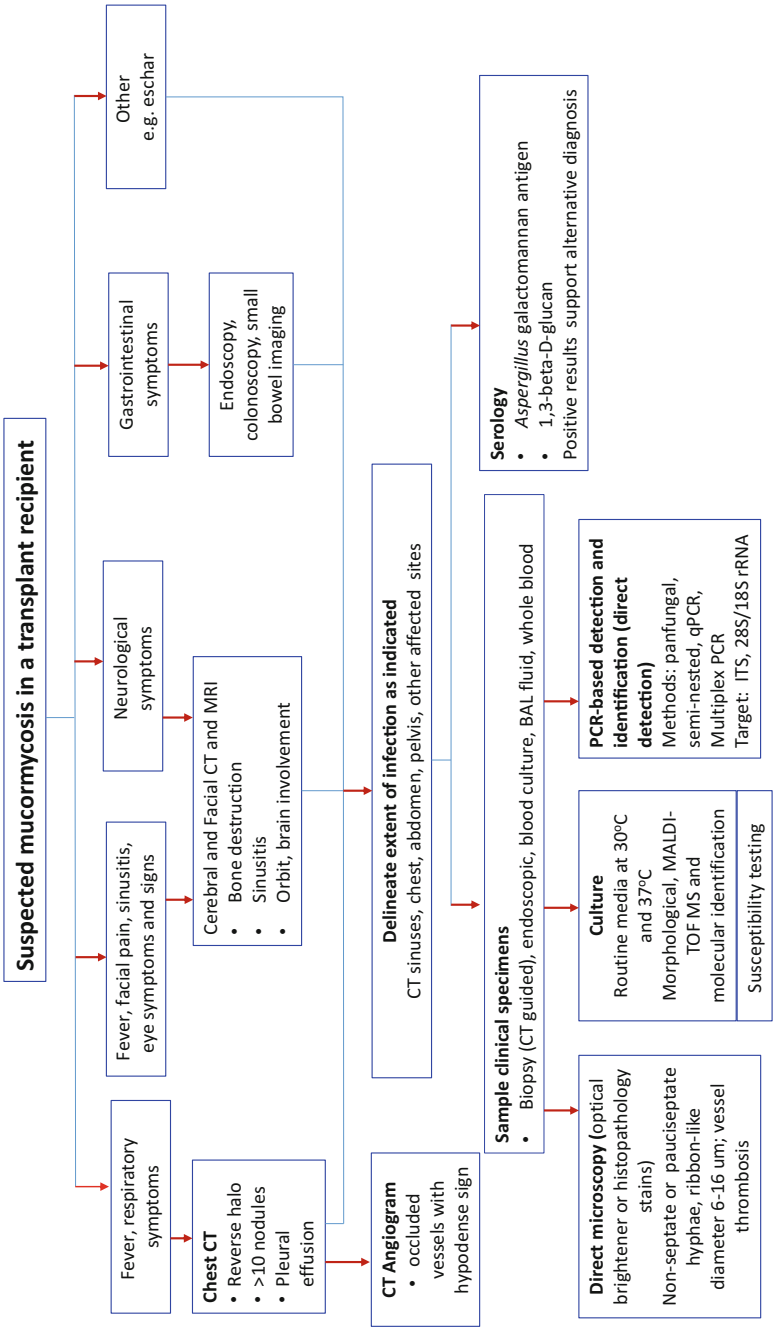


Fig. 1 Diagnostic pathways in a transplant patient suspected to have developed mucormycosis

basis. In some instances, cerebral magnetic resonance imaging (MRI) and imaging of the small bowel may be required. Disease may rapidly progress and follow-up imaging is recommended.

Histopathology

Histopathology and direct microscopy of clinical specimens remains a key diagnostic approach particularly with tissue biopsies (Fig. 2). This is still the case even if the procedure is not capable of identifying genus and species. As Mucorales hyphae are susceptible to physical trauma, care should be taken not to damage the specimen in transport to the laboratory. Of note, the visualization of the hyphae of Mucorales is required to call a "proven" diagnosis of mucormycosis (vs. a probable case or possible infection) [32].

Direct microscopy of specimens with optical brighteners (e.g., calcofluor white) or for histopathology, with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS), and/or Grocott-Gomori's methenamine-silver (GMS) stains, is preferred. Visualizing non-pigmented hyphae associated with tissue invasion confirms an infection.

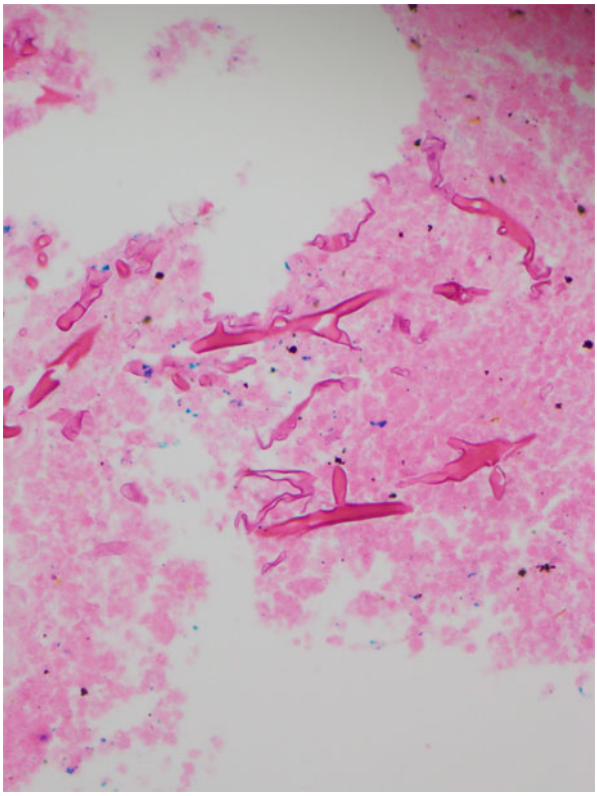


Fig. 2 Periodic acid-Schiff stain of lung tissue showing broad, pauci-septate irregular hyphae of a Mucorales fungus

Characteristic broad (6–25 μM diameter) ribbonlike, wide-angled branching, and pauciseptate or nonseptate hyphae assist in clinching the diagnosis [33] (Fig. 2). However, the specificity of some of these findings is not absolute as hyphae can artefactually appear to be septate or to be seen as having narrow-angle branching due to architectural distortion during processing. The most common cause of incorrect morphological diagnosis remains the misidentification of Mucorales as *Aspergillus* spp. where the non-concordance rate between culture (the gold standard) and histopathological diagnosis can be as low 15% [34]. The application of polymerase chain reaction (PCR) techniques in fresh or formalin-fixed paraffin-embedded tissue (PPFE) [summarized in 35, 36] has been shown to increase specificity, but sensitivity has varied.

Other typical findings in acute lesions of mucormycosis are hemorrhagic infarction, necrosis, and neutrophil infiltration (in non-neutropenic patients). In more longstanding infection, pyogranulomatous inflammation with giant cells and hyphae covered by eosinophilic material (the Splendore-Hoeppli phenomenon) may be seen.

Culture and Microscopy-Based Diagnostic Methods

Notwithstanding modern molecular methods, culture is still a mainstay method to diagnose mucormycosis. Further, it is the only method that enables both species identification and antifungal susceptibility testing. *All specimens from affected body sites should be cultured*. A positive culture from a sterile site, i.e., any tissue including skin lesions, pleural fluid, cerebrospinal fluid, and blood, confirms diagnosis, while a culture from a non-sterile site (e.g., BAL fluid, sinus material), while supportive, must be interpreted in the context of clinical and radiological findings.

Limitations of culture are acknowledged. An invasive procedure (e.g., biopsy, surgical resection) may not be feasible in transplant patients due to severe pancytopenia or other risks that advise against these procedures. Next, culture is insensitive. In one series, respiratory tract samples were culture-negative in 75% of proven pulmonary mucormycosis in cancer patients [37] although the yield of culture appears to have improved over time (50–71%) [9]. In a meta-analysis of diagnostic methods used from 2000 to 2017 [8], a Mucorales pathogen was grown in 79% of cases where culture was attempted; homogenization or grinding of tissue and other specimens leads to lower recovery rates [38].

Laboratories are able to alert clinicians quickly if there is fungal growth as Mucorales grow rapidly (3–5 days) on standard mycological media, visualizing typical cotton candy-like colonies. Identification of the pathogen to genus and species is still reliant on phenotypic methods to visualize fungal sporulation in many laboratories. Certain less common species such as *A. elegans* require special nutrient-poor media to sporulate [38]. Hence knowledge of local epidemiology is useful to inform laboratory practice. Descriptions of morphological structures for species identification using robust fungal identification keys can be found in reference publications [38].

Because identification of mucoralean fungi by morphological methods is difficult especially given the evolving taxonomy, molecular identification methods are often

required for species resolution. However, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) approaches now can aid species or genus identification [39, 40]. Several studies reported good identification when in-house databases were used [39–41]. Commercial databases have performed less well where in one study that tested 111 Mucorales strains, only 49.5% were correctly identified [40]; a correct identification rate of 86% was observed in another study [42]. The establishment of a robust accessible MALDI-TOF MS database is necessary prior to its routine implementation for Mucorales identification.

Serology and Antigen Testing

Unlike the *Aspergillus* galactomannan (GM) test and the beta-1,3-D-glucan (BDG) assays that are used to assist in diagnosis of other fungal infections, there are no commercial serological tests to detect Mucorales in clinical samples. The above tests are typically negative in Mucorales infections and help rule out invasive aspergillosis, although a recent study reported serum BDG positivity in 3/6 patients with *R. arrhizus* infection. Clinical evaluation is of paramount importance given false-positive results and the possibility of mixed infections. *R. arrhizu*s is known to harbor beta-D-glucan synthase, the enzyme that leads to synthesis of BDG [43]. Usually BDG levels in *R. arrhizus* infections are below the limit of detection of the assay.

A recent study has reported an ELISA and lateral flow immunoassay (LFIA) directed against Mucorales mannan molecules that were able to detect *Mucor* spp., *Lichtheimia* spp., and *R. arrhizus* in serum and BAL fluid [44]. A potential rapid point of care test, further evaluation is required to determine its clinical utility.

Molecular-Based Methods

Advances in the field of PCR-based diagnostic methods have been made for the (i) direct detection of Mucorales in clinical specimens and (ii) species identification. Other than tissue specimens, research has focused on identifying Mucorales DNA in blood, BAL fluid, and CSF. Target selection is a key determinant of success. The internal transcribed spacer (ITS) is well-established to provide accurate results and is the preferred gene target [summarized in 45].

Detection in Fresh and Formalin-Fixed Tissue

Numerous studies have shown good clinical utility of PCR-based detection of Mucorales DNA directly from both fresh and FFPE tissue specimens [35, 45]. Most studies have employed in-house techniques, with various PCR formats (e.g., PCR +/− sequencing, quantitative PCR [qPCR]) and assays that remain unstandardized. Assays can be Mucorales-specific or pan-fungal in nature. As mixed fungal infections occur, and because the target fungus is often unknown, many laboratories choose to initially use a broad-range PCR.

Variable sensitivity appears to be the common theme among studies, but specificity is consistently high. Specimen type (and quantity) may affect test performance. Gomez et al. reported excellent performance with PCR and sequencing in an ITS2/D2-directed assay on fresh as well as FFPE tissue and body fluids. The diagnostic yield was higher from samples obtained with open biopsy (76.5%) compared with core needle biopsy (50%) and fine needle aspiration (0%) [46]. The yield is also dependent on whether hyphae are seen in tissue [46, 47]; if these are visualized, the test sensitivity (96.6%) and specificity (98.2%) are high. Indeed, many experts recommend that PCR should mainly be undertaken in tissue containing fungal hyphae. Test sensitivity is reduced with FFPE sections due to low fungal load or DNA fragmentation from formalin (sensitivity 56–65%) [47] although in another study, sensitivity of PCR in both fresh and FFPE tissue was >90% [46].

Detection in Blood and Body Fluids

Detection of Mucorales DNA in blood and other fluids in high-risk patients has been actively pursued for its utility in early diagnosis of mucormycosis. This approach makes clinical sense as infections are angioinvasive; overall, promising results have been observed. Landmark studies for detection of Mucorales DNA in blood fractions include a retrospective evaluation of a combination of three separate 18S rRNA-directed real-time qPCRs targeting *Mucor/Rhizopus*, *Lichtheimia*, and *Rhizomucor* species, for detecting confirmed mucormycosis in immunocompromised patients. In 9/10 patients, Mucorales DNA was detected in serum 3–68 days before standard diagnosis indicating the potential use of qPCR as a screening tool [48]; the utility of this assay for early diagnosis was confirmed in a larger follow-up study (sensitivity 81–92%) where declining DNA load correlated with favorable patient outcomes [49]. Ino et al. reported detection in the blood using broad-range real-time PCR of various Mucorales species from four hematology patients [50]. Genus-specific assays, e.g., a *C. bertholletiae*-targeted PCR, have also been developed for early diagnosis of infection [51].

Mucorales DNA has also been detected in cerebrospinal fluid and BAL fluid. Lengerova et al. validated a high-resolution melt analysis PCR to detect *Rhizopus, Lichtheimia*, and *Mucor* spp. in BAL fluid from hematology patients with pulmonary infiltrates with an assay sensitivity of 100% and specificity of 93% [52]. Both the cell pellet and supernatant fractions of BAL fluid should be tested for optimal yield. Recently, PCR amplification of the novel target *CotH*, a Mucorales-specific gene, yielded positive results when tested on serum, BAL fluid, and urine in patients with mucormycosis [53].

Position of PCR-Based Methodologies

For all clinical specimens, PCR methodologies then have good potential to enable early mucormycosis diagnosis with superior sensitivity and specificity compared with standard microbiological methods. None of these assays are standardized, although this is being studied by the International Society for Human and Animal

Mycology (ISHAM) fungal PCR consortium. The few commercial assays, too, lack sufficient clinical evaluation. These issues need to be addressed prior to more widespread use of PCR assays for mucormycosis diagnosis.

Molecular Techniques to Identify Mucorales from Cultures

Molecular identification techniques are the benchmark for species identification of Mucorales pathogens. Comparison of morphological vs. molecular identification has shown better performance with molecular approaches. As before, target selection is important, and ITS sequencing is the preferred approach for identification of species of Mucorales [54]. The few commercial PCR assays are currently of limited use as these are not designed to resolve species but detect the order Mucorales as a whole or a genus.

A reliable sequence database such as those of the Westerdijk Institute (http://www.westerdijkinsistitute.nl/Collections/BioloMICSSeqeunces.aspx) or ISHAM (http://its.mycologylab.org) should be used for sequence comparison and species identification. Other gene loci that have been evaluated include the 18S and 28S rRNA gene regions, cytochrome b, and *FTR1* genes [45], but there is less evidence of their usefulness.

Next-Generation Sequencing

Next-generation sequencing (NGS) technologies are rapidly changing the landscape of the characterization and tracking of fungal infections. Shelburne et al. reported a metagenomic shotgun approach to identify the uncommon Mucorales, *M. velutinous*, in a patient with acute myeloid leukemia that led to disseminated disease [55]. NGS is still in its early stages with lack of annotated genomes of Mucorales a key limitation. However, whole-genome sequencing approaches have established epidemiological links in outbreaks although the two examples here are not specific for transplant patients [56, 57].

Management of Mucormycosis

Timely treatment of mucormycosis is critical. This typically requires a multidisciplinary approach with a combination of systemic antifungal therapy, surgical resection, and adjunctive measures such as correction of predisposing conditions (e.g., diabetic ketoacidosis), augmenting the host immune response (e.g., with growth factors), and other treatments such as hyperbaric oxygen. *Antifungal agents and surgery are the mainstays of therapy.* Yet prospective clinical studies evaluating the efficacy of antifungal and other treatments for mucormycosis are scarce with only three clinical trials reported [58–60]. Hence guidance for treatment is based largely on case series, expert opinion, animal model studies, and in vitro susceptibility data.

Susceptibility Data

Mucorales are intrinsically resistant to many antifungal drugs, and knowledge of their in vitro susceptibility profiles is clinically relevant [61, 62]. However, there is much discussion on whether the minimum inhibitory concentration (MIC) value itself for a particular isolate-drug pair is helpful. As Mucorales share most (but not all) susceptibility patterns, it is arguable that an MIC is not generally not required. However, there are some differences in susceptibility depending on genus and even within species, and certainly in cases of treatment failure, susceptibility testing can guide alternate therapy. MIC values are essential to establish epidemiological data which can underpin future clinical practice. The use of standard susceptibility testing methods based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or CLSI protocols is preferred [63, 64] as commercial methods, e.g., the E test (bioMerieux, Marcy-l'Étoile, France), produce variable results. Because clinical breakpoints have not been defined for these pathogens, isolates cannot be classified as "susceptible" or "resistant" [65]. Instead MICs are interpreted in the context of epidemiological cutoff values to assess if a drug MIC falls within the wild-type MIC range (i.e., harboring no resistance mechanism).

Overall, amphotericin B is the most active drug against the majority of Mucorales, as shown both in vitro and in vivo animal models of infection although *Cunninghamella* spp. have higher MICs [61]. Among the newer azoles, posaconazole has very good in vitro activity with low MICs (geometric mean [GM] MICs <2 mg/L) against most species except for *Cunninghamella* spp. (GM MICs >4 mg/L) [61]. Some *Rhizopus* strains may also have higher MICs to posaconazole; however the in vivo efficacy of posaconazole is well documented when used as both curative and prophylactic therapy in animals [66, 67]. Isavuconazole also has good activity; although MICs are higher than those of posaconazole (GM MICs 2–three-fold higher) [62, 68], isavuconazole achieves 3–4 times higher blood concentrations [69]. Variation in species susceptibility is demonstrated by isavuconazole showing reduced activity against *M. circinelloide*s [68]. Itraconazole has variable MICs against Mucorales, while voriconazole and the echinocandins exhibit high MICs for all species [61]. Various antifungal combinations against Mucorales have been studied in a few in vitro studies with synergistic effects in some species but with uncertain clinical relevance [70].

Antifungal Therapy

Prevention: Antifungal Prophylaxis

Primary antifungal prophylaxis offered to transplant patients is not directed specifically against mucormycosis but rather a broad range of fungal infections, most commonly candidiasis and aspergillosis. Prophylactic approaches are best established for HSCT recipients, in those with neutropenia and graft-versus-host disease, and are stratified by risk. This discussion is beyond the scope of this chapter but can be found in various expert guidelines [71, 72]. Protocols vary between institutions.

Of note, Mucorales infections have occurred in hematology-oncology patients receiving antifungal prophylaxis including those taking echinocandins and voriconazole [16, 73]. With the current use of posaconazole for prophylaxis, however, breakthrough mucormycosis has become rare with all forms including posaconazole oral suspension [74], posaconazole delayed-release tablets [75], and posaconazole intravenous (IV) infusions [76]. Should mucormycosis at an institutional level be of particular concern, prophylaxis with posaconazole delayed-release tablets is recommended due to their superior bioavailability over the oral suspension.

Data regarding antifungal prophylaxis in SOT recipients are less robust with relatively few clinical trials and no definitive recommendations. Based on clinical experience, various opinion bodies have published guidelines for prophylaxis according to transplant type and level of patient risk [77, 78]; the more pertinent data and their strength of recommendations [79] are summarized in Tables 2 and 3. All of the azoles, echinocandins, and amphotericin B formulations have been studied for antifungal prophylaxis with varying success; these may either be offered universally or targeted at high-risk subgroups. Protocols are often institution-specific, and there is no consensus on duration of prophylaxis.

In liver transplantation, recipients at high risk for invasive yeast and mold infections (Table 2) should receive antifungal prophylaxis [80] as in its absence; about 36% of these patients have developed an IFD. An inhaled amphotericin B formulation is one of the more commonly used drugs in lung transplantation although broad-spectrum azoles now offer a practical alternative [81] (Table 2). Amphotericin B has the advantage of having activity against the Mucorales, but the protective effect of inhaled formulations is limited to the airways. Both amphotericin B deoxycholate and lipid formulations of amphotericin B have been shown to be efficacious. Duration of use is highly variable ranging from 3 months to lifelong prophylaxis (in most transplant centers, 6–12 months). For systemic prophylaxis or where an azole is otherwise used, an appreciation of the complex drug pharmacokinetics, drug interactions with transplant rejection medications, and safety profiles is imperative.

Transplant physicians are often asked about secondary prophylaxis or whether to continue or recommence antifungal therapy in a patient who has had mucormycosis and who has responded to treatment, but who is scheduled for a new episode of immunosuppression. Expert opinion recommends continuation or restarting the last agent effective in that patient with or without surgical resection of the disease focus [82].

First-Line Antifungal Therapy

Investigations to confirm the diagnosis in suspected mucormycosis are essential but should not delay the initiation of therapy, as delay in treatment is an independent predictor of poor outcomes. More specifically, Chamilos et al. reported that delayed frontline amphotericin B-based therapy in hematology patients with mucormycosis resulted in a twofold increased mortality (83% vs. 49%) [5].

Table 2 Antifungal prophylaxis in sold organ transplant patients: recommendations according to transplant type. (Modified from Gavalda et al. and Kennedy et al. [77, 78])

Organ transplant	Population	Antifungal drug	Duration
Kidney	–	None	–
Pancreas	All	Fluconazole (A-III)[a]	1–2 weeks
Pancreas-kidney	*High risk*: Enteric drainage, hemodialysis, acute rejection and poor initial graft function, posttransplant laparotomy, vascular thrombosis, post-perfusion pancreatitis anastomotic problems	Echinocandin (A-III) or liposomal amphotericin B (A-III)	Varies with risk factors
Intestinal	All	Fluconazole (A-III)	3–4 weeks or until anastomosis healed and there is no rejection
Liver	*If one major or two minor criteria* Major: Re-transplantation, fulminant liver failure, MELD score ≥30, renal replacement therapy Minor: MELD score 20–30, split, living donor, choledochojejunostomy, transfusion requirement high, renal failure, early re-intervention, multifocal *Candida* colonization or infection	Micafungin or caspofungin (A-II) Liposomal amphotericin B (A-II) Amphotericin B lipid complex (A-II) Anidulafungin (B-III)	2–4 weeks or until all risk factors have resolved
Lung and lung-heart	Either all patients OR Guided strategy (induction with alemtuzumab or thymoglobulin, acute rejection, single-lung transplant, *Aspergillus* colonization pre or during first year post transplant, acquired hypogammaglobulinemia	Nebulized liposomal amphotericin B 25 mg (A-II): 3 times/week until healed bronchial suture, once/week for 2–6 months, once every 2 weeks >6 months. If guided prophylaxis, load 25 mg 3 times a week for 2 weeks and then once a week Nebulized amphotericin B lipid complex 50 mg (B-II). Load once every	Nebulized liposomal amphotericin B: Indefinitely or minimum 12 months or guided by presence of risk factors Nebulized amphotericin B lipid complex: Minimum 12 months or guided by risk factors Voriconazole: Determined by risk factors, minimum 4 months

(continued)

Table 2 (continued)

Organ transplant	Population	Antifungal drug	Duration
		2 days for 2 weeks and then 50 mg once a week Voriconazole (B-II)	
Heart	High risk: Acute rejection hemodialysis, re-exploration	Itraconazole, (A-III) Voriconazole (B-III) Posaconazole (B-III) Echinocandins (B-III)	Minimum 3 months

[a]Grading: according to ESCMID scoring systems, based on strength of recommendation and quality of evidence [79]

Table 3 Definition of strength of recommendation and quality of evidence [79]

Strength of recommendation	**Definition**
Grade A	Strong support for a recommendation for use
Grade B	Moderate support for a recommendation for use
Grade C	Marginal support for a recommendation for use
Grade D	Support for a recommendation against use
Quality of evidence	**Definition**
Level I	Evidence from at least 1 properly designed randomized, controlled trial (orientated on the primary endpoint of the trial) Note: Poor quality of planning, inconsistency of results, indirectness of evidence, etc. would lower the SoR
Level II	Evidence from at least 1 well-designed clinical trial (incl. Secondary endpoints), without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); from multiple time series; or from dramatic results of uncontrolled experiments Note: Every level II evidence must have at least one added index
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

Antifungal drugs presently used to treat mucormycosis are limited to amphotericin B formulations, posaconazole, and isavuconazole [58]. The principles and preferred antifungal treatment of mucormycosis in transplant patients are similar to those in other populations. Guidelines for antifungal therapy have been published [79, 83], but these were written prior to the clinical studies of isavuconazole. In the hematology and HSCT setting, the recommendations from ECIL (ECIL-6) were updated in 2017 [84].

Commensurate with in vitro data and across all three guidelines mentioned above, the preferred first-line agent for all forms of mucormycosis is a lipid formulation of amphotericin B – all favor liposomal amphotericin B (L-AMB) at a dose of 5–10 mg/kg/day (Table 4). Small case series and registry data have reported use of L-AMB with good outcomes although response rates remain at ≤50% for primary

Table 4 First-line antifungal monotherapy for treatment of mucormycosis in transplant patients and other populations (adapted from the ECIL-6, ESCMID, and the Australian and New Zealand Mycoses Interest Group (ANZMIG) guidelines) [79, 84, 85]

Antifungal therapy	Dose if known	Grade of recommendation	Comment
Liposomal amphotericin B	5–10 mg/kg/day	B II [95] B [94] AII [89]	Liposomal amphotericin B is preferred over other formulations in renal failure and in central nervous system infection Higher initial dose in CNS disease (BIII); 10 mg/kg/day
Amphotericin B, lipid complex	5 mg/kg/day	B II [95]) A II or AIII [89]	Has been used in solid organ transplant patients
Amphotericin B colloidal dispersion	3–4 mg/kg/day	CII [95]	Not available in the USA
Amphotericin B deoxycholate	1–1.5 mg/kg/day	DII [89]	If lipid formulations are unavailable
Isavuconazole, orally or intravenous	3 × 200 mg days 1–2 then 1 × 200 mg/day from day 3	BII [89]	$N = 21$ patients −11 hematology, 4 diabetes mellitus, overall mortality comparable to amphotericin B formulations
Posaconazole Delayed-release tablet or intravenous formulation	2 × 300 mg day 1 then 1 × 300 mg from day 2	BII [89]	Higher trough levels than posaconazole oral suspension Intravenous use if oral dosing is not possible
Posaconazole oral suspension	4 × 200 mg/day or 2 × 400 mg/day	CIII [95] CII [89]	No data to support its use as first-line treatment

mucormycosis [9, 59, 85–87]. There is no requirement to escalate doses over several days; the full daily dose should be given from the first treatment day. If significant renal toxicity ensues, the dose may be reduced though expert opinion does not recommend doses under 5 mg/kg/day [79]. A prospective non-comparative trial assessed the efficacy and safety of first-line high-dose L-AMB at 10 mg/kg/day for the first month of treatment [59]. Although patients receiving this high dose tended to have higher response rates (45%), this requires confirmation before the dose can be recommended. Renal function should be monitored as the use of doses of 10 mg/kg/day resulted in increased (albeit mostly reversible) serum creatinine levels [59, 87]. The use of high-dose L-AMB at 10 mg/kg/day in CNS involvement is supported by clinical data [9].

Other amphotericin B formulations have also been used with good outcomes. For decades, amphotericin B deoxycholate has been the cornerstone of therapy with successful use in multiple publications [9, 15, 88]. However, its efficacy is limited by substantial toxicity, particularly at the doses and long treatment durations needed for mucormycosis [79] and particularly in the context of kidney transplantation. Although discouraged, if lipid formulations or other alternatives are not available,

it should not be withheld as first-line therapy. Amphotericin B lipid complex at a dose 5 mg/kg/day has been used successfully in kidney transplant patients, although there are fewer data on efficacy compared with L-AMB [9, 89]. Use of amphotericin B is limited by the need for IV administration.

The latest marketed triazole, isavuconazole, has received much interest in efficacy studies in mucormycosis following promising in vitro and preclinical data. In a small comparative matched control study against amphotericin B formulations ($n = 33$ patients, historical cohort), the efficacy of isavuconazole as first-line treatment ($n = 21$ patients) was similar [58]. To this end, isavuconazole (either IV or oral) has been licensed for first-line treatment of mucormycosis in the USA. Posaconazole oral suspension has also been used successfully in first-line treatment [9, 86]. The advent of the delayed-release tablet with improved bioavailability exposure and the IV infusion formulation [76] both lend themselves to future comparative drug efficacy studies. At present, if amphotericin B formulations cannot be used and isavuconazole is not available, then posaconazole delayed-release tablets or infusion could be considered for first-line treatment (Table 4) [90]. However, in the ECIL-6 guidelines, posaconazole is positioned as an option in maintenance or salvage therapy [84].

In a recent review of management strategies of mucormycosis [88] in 851 patients, an amphotericin B formulation was given as initial therapy as monotherapy in 88% of cases, with posaconazole oral suspension as initial therapy in 11 cases (800 mg daily in divided doses); death was only observed in 1 patient. Of 174 cases of mucormycosis in renal transplant patients, the overall survival in those receiving either posaconazole or lipid amphotericin B was significantly higher than that in patients receiving amphotericin B deoxycholate (92.3% vs. 73.4% vs. 47.4%); however the timing of initiation of these therapies was not described, and the authors concluded that a lipid amphotericin B formulation is preferred [14].

Drug toxicity with the azoles is well appreciated, in particular hepatotoxicity. In contrast to other mold-active azoles, isavuconazole is less hepatotoxic although it can cause shortening of the QTc interval [91].

Step-Down Therapy

Commonly, after initial treatment with amphotericin B therapy and when the patient is clinically stable, step-down therapy to an oral agent is instituted. The use of posaconazole delayed-release tablets is preferred. Alternatively, isavuconazole may also be used. Posaconazole oral suspension can be used but is discouraged now that formulations with higher exposure are available. In a large review of 96 cases of mucormycosis (2003–2011) where posaconazole was a component of therapy, the complete response rate was 64% and partial response rate 7.3%. Posaconazole was predominantly used in combination with another antifungal or as second-line treatment [88, 92].

Is There a Role for Up-Front Antifungal Combination Therapy?

There are no robust data from large comparative studies to guide recommendations for combination antifungal therapy. In animal models, both polyene-echinocandin

and polyene-azole (primarily, posaconazole) combinations have shown improved survival rates with no antagonism observed [93].

Data from case reports and a few small patient series report promising results with use of combinations of polyenes and azoles or polyenes plus echinocandins [94, 95]. Combined amphotericin B lipid complex and caspofungin offered superior survival over amphotericin B alone in 1 study in 11 ROCM patients (6 had diabetes) [94]. In another study of 36 patients, 52% had combination L-AMB and caspofungin therapy with a more favorable prognosis [95]. However, other studies in hematology patients failed to demonstrate benefits of double or triple antifungal combinations [96]. Data from two IFD registries – the SEIFEM and FUNGISCOPE – indicated that combination L-AMB and posaconazole may have benefits in very aggressive forms of mucormycosis [97]. Hence combination therapy may be rationally administered with possible though unproven benefit. Disadvantages include drug interactions, toxicity, and cost. It would be important to study the potential clinical benefits of polyene combined with either delayed-release posaconazole tablets or IV posaconazole.

Salvage Antifungal Treatment

Treatment failure is often multifactorial, with both drug and host factors contributing to slow or absent clinical response. Source control and augmentation of host immune response where possible are all essential. Drug-related reasons for treatment failures are disease that is truly refractory to the antifungal used or toxicity or intolerance to the first-line antifungal agent(s). For amphotericin B formulations, renal toxicity is especially relevant, while azole-induced hepatotoxicity is not uncommon.

Because only two drug classes – the polyenes and azoles – have proven efficacy in mucormycosis, salvage treatment mostly indicates a switch to use of the other class. In patients failing amphotericin B or where this cannot safely be used, isavuconazole salvage treatment had yielded favorable patient outcomes [58]. Salvage therapy with posaconazole oral suspension achieved cure in a number of case series and two non-randomized clinical studies [9, 90, 92, 98]. These data strongly support the use of both isavuconazole and posaconazole as salvage treatments when amphotericin B is no longer an option. As before, posaconazole delayed-release tablets or infusions are strongly preferred over posaconazole oral suspension. In cases of primary treatment failure with isavuconazole or posaconazole, lipid-based amphotericin B formulations have been used with efficacy [90].

Treatment Duration

The duration of therapy for treatment of mucormycosis is not well-defined. As transplant patients are often seriously ill, typically a minimum of several weeks to months of therapy are given or treatment continued until the risks are reduced due to recovery of neutrophil counts or immunosuppression reduced or discontinued. In addition, there should be resolution of signs of infection and substantive improvement of radiological abnormalities. Radiologic improvement may be difficult to determine after surgical resection because of postoperative scarring or distortion of

anatomical structures. Findings of gross surgical assessment are also important. In all cases, treatment duration follows a personalized approach.

Data from studies employing isavuconazole therapy have reported the median duration of isavuconazole first-line or salvage treatment to be 84 days in a single-arm open-label study combined with a retrospective case-control analysis [58]. Among posaconazole oral suspension studies, treatment duration ranged from 1 week to almost 3 years (mean 6 months) [99]. This wide range of duration is expected due to both the diversity of clinical manifestations and the complexity of the underlying diseases.

Therapeutic Drug Monitoring (TDM)

Triazole antifungals may have unpredictable pharmacokinetics in patients with mucormycosis due to unpredictable bioavailability and/or drug-drug interactions. In some cases, these factors may lead to treatment failure or drug toxicity. Although the therapeutic range of posaconazole for mucormycosis is unknown, routine TDM is strongly recommended in patients receiving treatment with posaconazole. Based on preclinical data and following simulation, serum trough posaconazole concentrations of 1–1.5 mg/L (where MICs are up to 0.25 mg/L) up to 4 mg/L (for isolates with elevated MICs, e.g., 2 mg/L) are recommended to reduce the risk of treatment failure [99]. Notably, serum levels of >2 mg/L may only be achievable in patients with non-licensed doses of posaconazole tablets or the IV formulation. Such supratherapeutic levels have been linked with liver toxicity, hypokalemia, and hypertension [100].

At present, there is no clinical evidence to indicate a need for routine TDM with isavuconazole. However, measurement of drug concentrations could be useful in suspected treatment failure or if there are drug interactions, suspected drug toxicity, uncertain pharmacokinetics (e.g., in obese patients), or after switching from IV to oral therapy**.** In the SECURE isavuconazole clinical trial for aspergillosis, in patients where TDM was performed, >90% of patients achieved predicted target exposure (>1 μg/ml), but there was no association between serum levels and treatment response or liver toxicity [101].

Surgical Treatment

Surgery is essential for local control of mucormycosis, prevention of disease extension to adjacent structures, and removal of necrotic tissue. This is especially pertinent as antifungal agents penetrate poorly into devitalized tissue. Disseminated disease, or involvement of difficult-to-access sites such as the central nervous system, however, poses limitations.

Procedures that have been shown to be helpful include lung resection or lobectomy, debridement of rhino-orbital-cerebral mucormycosis (ROCM), debridement of skin/soft tissue infection, and resection of affected viscera, such as the spleen, GI tract, or even transplanted organs. Often transplant recipients are too ill to undergo surgery, but this option should still be considered as several studies report higher

survival rates with surgical intervention [9, 26]. A review of 106 mucormycosis cases in solid organ transplant recipients from 1970–2002 found reduced mortality (34.3%) among patients receiving both surgery and antifungals compared with those with antifungal therapy alone (62.5%) [13]. In a case-control study of 50 solid organ recipients (infection sites, 48% lung, 26% ROCM, 22% cutaneous/soft tissue), surgical resection was strongly associated with treatment success by multivariate analysis [16]. Song et al. observed survival rates of 70.2% in renal transplant patients receiving combined surgery and antifungals compared with 32.4% and 36.4% in those receiving antifungal drugs alone and surgery alone, respectively (36.4%) [15]. The ECIL-6 guidelines strongly recommend surgical intervention for cutaneous/soft tissue infection and ROCM and moderately recommend surgery of pulmonary lesions [84].

The principles of surgical management include early, extensive, and frequent debridement, careful assessment of resection margins, and minimizing morbidity of surgery. In ROCM, as near to complete debridement of affected tissue increased survival in solid organ transplant recipients [102]. It is important to customize the extent of surgery along the tissue planes of affected sites to reduce cosmetic disfigurement. In lung infection, excision is difficult where disease is multifocal; however resection of lesions in unifocal disease will not only debulk disease but may prevent bleeding and has been associated with increased survival [9, 103]. In hepatic mucormycosis, liver resection per se has been feasible as has removal of liver abscesses with good outcomes. In the event of SOT graft mucormycosis, the transplanted organ should be removed; whether re-transplantation is feasible should be considered on a case-to-case basis.

Surgery is a mainstay for cutaneous and soft tissue infection and should be prompt and aggressive as infection is rapidly progressive. While resection of all grossly abnormal tissue is essential, removal of some adjacent surrounding healthy-looking tissue also appears to be important for best outcomes. However, defining a margin of clearance is not straightforward, and sometimes, sacrifice of limbs or expendable bony structures (e.g., ribs) is necessary. Although frozen sections may be low-yield, the presence of fungal elements often necessitates immediate re-excision and can guide planning of repeat debridement. Ideally the same surgeon performs the debridements in order to achieve consistent reassessment [26].

Adjunctive Treatments

Topical Antifungals

The use of topically administered amphotericin B in conjunction with systemic antifungals in the management of various forms of mucormycosis has been described [summarized in 88]. Amphotericin B has been administered into the eye (for retinitis), into wounds, impregnated into bone cement, and inhaled or locally instilled into the lung via bronchoscopy. Instances of intrathecal administration percutaneously or via an Ommaya reservoir are also reported. However, data supporting these approaches are lacking given the inadequate descriptions of drug

doses and methods of solution preparation as well as a paucity of information regarding the stability and safety of these formulations.

Iron Chelators

Iron is essential for the metabolism of many fungi, and strategies to starve Mucorales of iron such as through the use of iron chelators make logistic sense. However, despite extensive study, the utility of these compounds in the treatment of mucormycosis remains uncertain.

Deferasirox (Exjade, Novartis) is one chelator that cannot be used as a siderophore by Mucorales. The drug has been shown to be efficacious and safe, largely in patients with diabetes [104]. However, a double-blind randomized controlled study showed increased mortality in deferasirox-treated patients as compared to those treated with an amphotericin B formulation [60]. This study enrolled mainly patients with hematologic malignancy and cautioned against the use of deferasirox. Deferasirox is known to be potentially toxic to the bone marrow and kidneys and may cause harm to neutropenic patients with mucormycosis [105]. Future research in this space is warranted, though for the current time adjunctive deferasirox use should be avoided.

Augmentation of Host Immunity and Reversal of Host Risk Factors

In neutropenic or highly immunosuppressed hematologic malignancy and SOT patients with mucormycosis, granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) has been added to antifungal treatment in a small number of patients with good results [106]. A report of successful use of combination of the immunomodulator nivolumab and interferon-γ in a patient with intractable abdominal mucormycosis was recently published [107]. Overall, data suggest that growth factors may be useful in neutropenic patients with mucormycosis. In patients with diabetes mellitus, correction of hyperglycemia and ketoacidosis is essential.

Hyperbaric Oxygen

Clinical experience with the use of hyperbaric oxygen as adjunctive therapy for mucormycosis is limited to case reports or small case series [108] and has involved a wide spectrum of clinical presentations with only small numbers of hematologic malignancy and transplant patients. At present, data indicate that there is insufficient evidence to define the efficacy of this expensive treatment, but it may be considered in refractory disease.

Conclusions

In conclusion, Mucorales infections in transplant patients are uncommon. However, the disease progresses rapidly with dissemination and high mortality. Epidemiology varies with geographic region, and the population of patients at risk is

expanding with the introduction of newer more intense immunosuppressive therapies. Early diagnosis is essential for optimal outcomes. There have been recent advances in molecular-based methods for direct detection of Mucorales DNA in clinical specimens including in blood of high-risk hematologic malignancy patients.

There are no specific preventative or antifungal prophylaxis measures against mucormycosis. Large studies or randomized controlled trials investigating the efficacy of treatment regimens are still lacking. Surgical debridement should be performed whenever feasible. The drug of choice remains liposomal amphotericin B ($\geq$5 mg/kg/day), with dose adjustment in renal failure if necessary. In cases of extensive disease or rapid progression, the addition of isavuconazole or posaconazole may be considered. Isavuconazole or posaconazole may be administered as maintenance therapy. Treatment should be continued until resolution of clinical or imaging findings and reconstitution of the host immune system.

Key Points

- Mucorales infections are emergent in both stem cell as well as solid organ transplant recipients whereby those receiving intense anti-rejection therapies or newer immunomodulating therapies may be at increased risk. Outbreaks of infection can occur.
- *Rhizopus spp*. remain the most common causative agent worldwide.
- Diagnosis should be multifaceted involving imaging, histopathology of affected sites for visualization of characteristic broad pauci-septate hyphae, culture, and molecular-based tests.
- Cases of suspected or diagnosed infection require a rapid or immediate response to imitation of therapy as mortality is high and infection is rapidly progressive.
- Management is multidisciplinary involving early surgical resection or debridement-affected sites and areas of necrosis, antifungal therapy, and reversal of immunosuppression where possible.
- There are no robust data from randomized controlled treatment trials; hence, recommendations for antifungal therapy are based on relatively small comparative studies, open-label studies, and expert opinion.
- The first-line antifungal agent (drug of choice) is liposomal amphotericin B 5–10 mg/kg/d. Alternatively, isavuconazole (either IV or oral; 600 mg/d for days 1–2, then 200 mg/d)) has been licensed for first-line treatment of mucormycosis in the USA. If amphotericin B formulations cannot be used and isavuconazole is not available, then posaconazole delayed-release tablets or infusion (600 mg/d for day 1, then 300 mg/d) could be considered for first-line treatment.
- In cases of particularly aggressive or disseminated infection, combination antifungal therapy should be considered. Polyene-azole (posaconazole typically) combination has been used with varying success, whilst polyene-echinocandin

combinations have been used with good result in small numbers of patients with rhino-orbital-cerebral disease.
- Data strongly support the use of both isavuconazole and posaconazole both as (i) "step-down" therapy following amphotericin B use, and (ii) as salvage treatments when amphotericin B is no longer an option.
- The use of iron chelators to treat mucormycosis is not recommended.

References

1. Cuenca-Estrella M, Bernal-Martinez L, Isla G, Gomez-Lopez A, Alcazar-Fouli L, Biutrago MJ. Incidence of zygomycosis in transplant recipients. Clin Microbiol Infect. 2009;15:37–40.
2. Slavin MA, van Hal SJ, Sorrell TC, Lee A, Marriot DJ, Daveson K, et al. Invasive infections due to filamentous fungi other than *Aspergillus*: epidemiology and determinants of mortality. Clin Microbiol Infect. 2015;21:490.e1–490.e10.
3. Park BJ, Pappas PG, Wannemuehler KA, Alexander BD, Anaissie EJ, Andes DR, et al. Invasive non-*Aspergillus* mould infections in transplant recipients, United States, 2001–2006. Emerg Infect Dis. 2011;17:1855–64.
4. Guinea J, Escribano P, Vena A, Munoz P, Martinez-Jimenez MDC, Padilla B, et al. Increasing incidence of mucormycosis in a large Spanish hospital from 2007–2015: epidemiology and microbiological characterisation of the isolates. PLoS One. 2017;12:e0179136.
5. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with haematologic malignancy who have zygomycosis. Clin Infect Dis. 2008;47:503–9.
6. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoramycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. Clin Infect Dis. 2012;54(Suppl 1):S8–S15.
7. Mendoza L, Vilela R, Voelz K, Ibrahim A, Voigt K, Lee SC. Human fungal pathogens of Mucorales and Entomophthorales. Cold Spring Harb Perspect Med. 2015;5:a019562.
8. Jeong W, Keighley C, Wolfe R, Lee W, Slavin M, Kong DCM, et al. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clin Microbiol Infect. 2019;25:26–34.
9. Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, Lagrou K, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) working group on zygomycosis between 2005 and 2007. Clin Microbiol Infect. 2011;17:1859–67.
10. Gryganskyi AP, Golan J, Dolatabadi S, Mondo S, Robb S, Idnurm A, et al. Phylogenetic and phylogenomic definition of *Rhizopus* species. G3 Genes Genomes Genet. 2018;8:2007–18.
11. Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis. 2012;54(Suppl 1):S23–34.
12. Crabol Y, Lortholary O. Invasive mould infections in solid organ transplant recipients. Scientifica (Cairo). 2014;2014:821969.
13. Almyroudis NG, Sutton DA, Linden P, Rinaldi MG, Fung J, Kusne S, et al. Zygomycosis in solid organ transplant recipients in a tertiary transplant Centre and review of the literature. Am J Transplant. 2006;61:2365–74.
14. Song Y, Qiao J, Giovanni G, Liu G, Yang H, Wu J, Chen J. Mucormycosis in renal transplant recipients: review of 174 reported cases. BMC Infect Dis. 2017;17:293.
15. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis. 2005;41:634–53.

16. Singh N, Aguado JM, Bonatti H, Forrest G, Gupta KL, Safdar N, et al. Zygomycosis in solid organ transplant recipients: a prospective matched case control study to assess risks for disease and outcome. J Infect Dis. 2009;200:1002–11.
17. Fung M, Schwartz BS, Doernberg SB, Langelier C, Lo M, Graff L, et al. Breakthrough invasive fungal infections on isavuconazole prophylaxis and treatment: what is happening in the real-world setting? Clin Infect Dis. 2018;67:1142–3.
18. Auberger J, Lass-Flörl C, Aigner M, Clausen J, Gastl G, Nachbaur DJ. Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-Centre institutional retrospective observational study. J Antimicrob Chemother. 2012;67:2268–73.
19. Cheng VC, Chan JF, Ngan AH, To KK, Leung SY, Tsoi HW, et al. Outbreak of intestinal infection due to *Rhizopus microsporus*. J Clin Microbiol. 2009;47:2834–43.
20. Rammaert B, Lanternier F, Zahar JR, Dannaoui E, Bougnoux ME, Lecuit M, et al. Health-associated mucormycosis. Clin Infect Dis. 2012;54(Suppl 1):S44–54.
21. Novosad SA, Vasquez AM, Nambiar A, Arduino MJ, Christensen E, Moulton-Meissner H, et al. Notes from the field: probable mucormycosis among adult solid organ transplant recipients at an acute care hospital – Pennsylvania, 2014–2015. MMWR Morb Mortal Wkly Rep. 2016;65:481–2.
22. Duffy J, Harris J, Gade L, Sehulster L, Newhouse E, O'Connell H, et al. Mucormycosis outbreak associated with hospital linens. Pediatr Infect Dis J. 2014;33:472–6.
23. Cheng VCC, Chen JHK, Wong SCY, Leung SSM, So SYC, Lung DC, et al. Hospital outbreak of pulmonary and cutaneous zygomycosis due to contaminated linen items from substandard laundry. Clin Infect Dis. 2016;62:714–21.
24. Teal LJ, Schultz KM, Weber DJ, Gergen MF, Miller MB, DiBiase LM, et al. Invasive cutaneous *Rhizopus* infections in an immunocompromised patient population associated with hospital laundry carts. Infect Control Hosp Epidemiol. 2016;37:1251–3.
25. Sundermann AJ, Clancy CJ, Pasculle AW, Liu G, Cumbie RB, Driscoll E, et al. How clean is the linen at my hospital? The Mucorales on unclean linen discovery study of large United States transplant and cancer centers. Clin Infect Dis. 2019;68:850–3.
26. Kueht M, Villarreal JA, Reece E, Galvan N, Mysore K, Restrepo A, et al. Cutaneous mucormycosis in solid organ transplant recipients after Hurricane Harvey: short and long term management. Plast Reconstr Surg Glob Open. 2019;7:e2041.
27. Nam BD, Kim TJ, Lee KS, Kim TS, Han J, Chung MJ. Pulmonary mucormycosis: serial morphological changes on computed tomography correlate with clinical and pathologic findings. Eur Radiol. 2018;28:789–95.
28. Legouge C, Caillot D, Chretien ML, Lafon I, Ferrant E, Audia S, et al. The reversed halo sign: pathognomonic pattern of pulmonary mucormycosis in leukemic patients with neutropenia. Clin Infect Dis. 2014;58:672–8.
29. Boucier J, Heudes P-M, Morio F, Gastinne T, Chevallier P, Rialland-Battisti F, et al. Prevalence of the reversed halo sign in neutropenic patients compared with non-neutropenic patients. Data from a single-centre study involving 27 patients with pulmonary mucormycosis (2003–2016). Mycoses. 2017;60:526–33.
30. Sassi C, Stanzani M, Lewis RE, Facchini G, Bazzochi A, Cavo M, et al. The utility of contrast-enhanced hypodense sign for the diagnosis of pulmonary invasive mould disease in patients with haematological malignancies. Br J Radiol. 2018;91:20170220.
31. Stanzani M, Sassi C, Lewsi RE, Tolomelli G, Bazzocchi A, Cavo M, et al. High resolution computed tomography angiography improves the radiographic diagnosis of invasive mould disease on patients with haematological malignancies. Clin Infect Dis. 2015;60:1603–10.
32. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/invasive fungal infections

cooperative group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46:1813–21.
33. Gaurner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev. 2011;24:247–80.
34. Shah AA, Hazen KC. Diagnostic accuracy of histopathologic and cytopathologic examination of *Aspergillus s*pecies. Am J Clin Pathol. 2013;139:55–61.
35. Dadwal SS, Kontoyiannis DP. Recent advances in the molecular diagnosis of mucromycosis. Expert Rev Mol Diagn. 2018;18:845–54.
36. Gholinejad-Ghadi N, Shokohi T, Seifi Z, Reza-Aghili S, Roilides E, Nikkhaha M, et al. Identification of Mucorales in patients with proven invasive mucormycosis by polymerase chain reaction in tissue samples. Mycoses. 2018;61:909–15.
37. Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV. Zygomycosis in the 1990s in a tertiary care cancer centre. Clin Infect Dis. 2000;30:851–6.
38. Garcia-Hermoso D, Alanio A, Lortholary O, Dromer F. Agents of systemic and subcutaneous mucormycosis and entomophthoromycosis. In: Carroll KC, Pfaller MA, Landry ML, Patel R, McAdam AJ, Richter SS, Warnock DW, editors. Manual of clinical microbiology. 12th ed. Washington, DC: American Society for Microbiology Press; 2019. p. 2163–86; chapter 124.
39. Dolatabadi S, Kolecka A, Versteeg M, de Hoog SG, Boekhout T. Differentiation of clinically relevant Mucorales *Rhizopus microsporus* and *R. arrhizus* by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). J Med Microbiol. 2015;64:694–701.
40. Shao J, Wan Z, Li R, Yu J. Species identification and delineation of pathogenic Mucorales by matrix-assisted laser desorption ionisation time of flight mass spectrometry. J Clin Microbiol. 2018;56:e01886–17.
41. Schrodl W, Heydel T, Schwarze VU, Hoffmann K, Grosse-Herenthey A, Walther G, et al. Direct analysis and identification of pathogenic *Lichtheimia* species by matrix-assisted laser desorption ionisation time of flight analyser-mediated mass spectrometry. J Clin Microbiol. 2012;50:419–27.
42. Rychert J, Slechta ES, Barker AP, Miranda E, Babady NEM, Tang YW, et al. Multicentre evaluation of the Vitek MS v33.0 system for the identification of filamentous fungi. J Clin Microbiol. 2018;56:e01353–17.
43. Angebault C, Lanternier F, Dalle F, Schrimpf C, Roupie AL, Ddupuis A, et al. Prospective evaluation of serum beta-D-glucan testing in patients with probable or proven fungal disease. Open Forum Infect Dis. 2016;3:ofw128.
44. Barnham-Maurisch AR, Hubbard B, Kvam AJ, Gates-Hollingsworth M, Green HR, Soukup E, et al. Conservation of mannan synthesis in fungi of the Zygomycota and Ascomycta reveals a broad diagnostic target. mSphere. 2018;3:e00094–18.
45. Millon L, Scherer E, Rochhi S, Bellanger A-P. Molecular strategies to diagnose mucormycosis. J Fungi. 2019;5:24.
46. Gomez CA, Budvytiene I, Zemek AJ, Banaei N. Performance of targeted fungal sequencing for culture-independent diagnosis of invasive fungal infection. Clin Infect Dis. 2017;65:2035–41.
47. Lau A, Chen S, Sorrell T, Carter D, Malik R, Martin P, et al. Development and clinical application of a panfungal PCR assay to detect and identify fungal DNA in tissue specimens. J Clin Microbiol. 2007;45:380–5.
48. Millon L, Larosa F, Lepiller Q, Legrand F, Rocchi S, Daguindau E, et al. Quantitative polymerase chain reaction detection of circulating DNA in serum for early diagnosis of mucormycosis in immunocompromised patients. Clin Infect Dis. 2013;56:e95–e101.
49. Millon L, Herbrecht R, Grenoiullet F, Morio F, Alanio A, Letscher-Bru V, et al. Early diagnosis and monitoring of mucormycosis by detecting circulating DNA in serum: retrospective analysis of 44 cases collected through the French surveillance network of invasive fungal infections (RESSIF). Clin Microbiol Infect. 2016;22:810.e1–8.

50. Ino K, Nakase K, Nakamura A, Nakamori Y, Sugawara Y, Miyazaki K, et al. Management of pulmonary mucormycosis based on a polymerase chain reaction (PCR) diagnosis in patients with haematologic malignancies: a report of four cases. Intern Med. 2017;56:707–11.
51. Bellanger AP, Berceanua RS, Valot B, Fontan J, Chauchet A, et al. Development of a quantitative PCR detecting *Cunninghamella bertholletiae* to help in diagnosing this rare and aggressive mucormycosis. Bone Marrow Transplant. 2018;53:1180–3.
52. Lengerova M, Racil Z, Hrncirova K, Kocmanova I, Volfova P, Ricna D, et al. Rapid detection and identification of mucormycetes in bronchoalveolar lavage samples from immunocompromised patients with pulmonary infiltrates by use of high-resolution melt analysis. J Clin Microbiol. 2014;52:2824–8.
53. Baldin C, Soliman SSM, Jeon HH, Alkhazraji S, Gebremaraim T, Gu Y, et al. PCR-based approach targeting Mucorales specific gene family for the diagnosis of mucormycosis. J Clin Microbiol. 2018;56:e00746–18.
54. CLSI. Interpretative criteria for bacteria and fungi identification by DNA target sequencing: approved guideline MM-18A. Wayne: Clinical and Laboratory Standards Institute; 2008.
55. Shelburne SA, Ajami NJ, Chibucos MC. Implementation of a pan-genomic approach to investigate holobiont-infecting microbe interaction: a case report of a leukemic patient with invasive mucormycosis. PLoS One. 2015;10:e0139851.
56. Etienne KA, Gillece J, Hilsabeck R, Schupp M, Colman R, Lockhart SR, et al. Whole genome sequence typing to investigate the *Apophysomyces* outbreak following a tornado in Joplin, Missouri, 2011. PLoS One. 2012;7:e49889.
57. Garcia-Hermoso D, Criscuolo A, Lee SC, Legrand M, Chaouat M, Denis B, et al. Outbreak of invasive wound mucormycosis in a burn unit due to multiple strains of *Mucor circinelloides* f. *circinelloides* resolved by whole genome sequencing. MBio. 2018;9:e00573–18.
58. Marty FM, Ostrosky-Zeichner L, Cornely OA, MUllane KM, Perfect JR, Thompson GR 3rd, et al. Isavuconazole treatment for mucormycosis: a single-arm open-label trial and case-control analysis. Lancet Infect Dis. 2016;16:828–37.
59. Lanternier F, Poiree S, Elie C, Garcia-Hermoso D, Bakouboula P, Sitbon K, et al. Prospective pilot study of high-dose (10 mg/kg/day) liposomal amphotericin B (L-AMB) for the initial treatment of mucormycosis. J Antimicrob Chemother. 2015;70:3116–23.
60. Spellberg B, Ibrahim AS, Chin-Hong PV, Kontoyiannis DP, Morris MI, Perfect JR, et al. The Deferasirox-AmBisome therapy for Mucormycosis (DEFEAT Mucor) study: a randomized, double-blinded, placebo-controlled trial. J Antimicrob Chemother. 2012;67:715–22.
61. Alastruey-Izquierdo A, Castelli MV, Cuesta I, Monzon A, Cuenca-Estrella M, Rodriguez-Tudela JL. Activity of posaconazole and other antifungal agents against Mucorales strains identified by sequencing of internal transcribed spacers. Antimicrob Agents Chemother. 2009;53:1686–9.
62. Dannaoui E. Antifungal resistance in Mucorales. Int J Antimicrob Agents. 2017;50:617–21.
63. Arendrup MC, Meletiadis J, Mouton JW, Lagrou K, Hamal P, Guinea J and the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds. EUCAST Definitive Document E.DEF 9.3.1. (www.eucast.org/astooffungi/) (Accessed 3 Feb 2020).
64. CLSI. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, CLSI Standard M38. 3rd ed. Wayne: Clinical and Laboratory Standards Institute; 2017.
65. Espinel-Ingroff A, Chakrabarti A, Chowdhary A, Cordoba S, Dannaoui E, Dufresne P, et al. Multicentre evaluation of MIC distributions for epidemiologic cuttoff value definition to detect amphotericin B, posaconazole, and itraconazole resistance among the most clinically relevant species of Mucorales. Antimicrob Agents Chemother. 2015;59:1745–50.
66. Salas V, Pastor FJ, Calvo E, Sutton DA, Chander J, Mayayo E, et al. Efficacy of posaconazole in a murine model of disseminated infection caused by *Apophysomyces variabilis*. J Antimicrob Chemother. 2012;67:1712–5.

67. Spreghini E, Orlando F, Gianni D, Barchiesis F. *In vitro* and *in vivo* activities of posaconazole against zygomycetes with various degrees of susceptibility. J Antimicrob Chemother. 2010;65:2158–63.
68. Arendrup MC, Jensen RH, Meletiadis J. In vitro activity of isavuconazole and comparators against clinical isolates of the Mucorales order. Antimicrob Agents Chemother. 2015;59: 7735–42.
69. Kaindl T, Andes D, Engelhardt M, Saulay S, Larger P, Groll AH. Variability and exposure - response relationships of isavuconazole plasma concentrations in the phase 3 SECURE trial of patients with invasive mould diseases. J Antimicrob Chemother. 2019;74:761–7.
70. Biswas C, Sorrell T, Djordjevic JT, Zuo X, Jolliffe KA, Chen SC. In vitro activity of miltefosine as a single agent and in combination with voriconazole or posaconazole against uncommon filamentous fungal pathogens. J Antimicrob Chemother. 2013;68:2842–6.
71. Maertens JA, Girmenia C, Brüggemann RJ, Duarte RF, Kibbler CC, Ljungman P, European Conference on Infections in Leukaemia (ECIL), a joint venture of the European Group for Blood and Marrow Transplantation (EBMT), the European Organization for Research and Treatment of Cancer (EORTC), the Immunocompromised Host Society (ICHS) and; European Conference on Infections in Leukaemia (ECIL), a joint venture of the European Group for Blood and Marrow Transplantation (EBMT), the European Organization for Research and Treatment of Cancer (EORTC), the Immunocompromised Host Society (ICHS) and the European LeukemiaNet (ELN), et al. European guidelines for primary antifungal prophylaxis in adult haematology patients: summary of the updated recommendations from the European conference on infections in leukaemia. J Antimicrob Chemother. 2018;73:3221–30.
72. Fleming S, Yanakou C, Haeusler GM, Clark J, Grigg A, Heath C, et al. Consensus guidelines for antifungal prophylaxis in haematological malignancy and haemopoietic stem cell transplantation, 2014. Intern Med J. 2014;44:1–15.
73. Pang K, Godet C, Fekkar A, Scholler J, Nivoix Y, Letscher-Bru V, et al. Breakthrough invasive mould infections in patients treated with caspofungin. J Infect. 2012;64:424–9.
74. Lerolle N, Raffoux E, Socie G, Touratier S, Sauviggnon H, Porcher R, et al. Breakthrough invasive fungal disease in patients receiving posaconazole primary prophylaxis: a 4-year study. Clin Microbiol Infect. 2014;20:O952–9.
75. Duarte RF, Lopez-Jimenez J, Cornely OA, Laverdiere M, Helfgott D, Haider S, et al. Phase 1b study of new posaconazole tablet for prevention of invasive fungal infections in high-risk patients with neutropenia. Antimicrob Agents Chemother. 2014;58:5758–65.
76. Cornely OA, Robertson MN, Haider S, Grigg A, Geddes M, Aoun M, et al. Pharmacokinetics and safety results from the phase 3 randomized, open-label, study of intravenous posaconazole in patients at risk of invasive fungal disease. J Antimicrob Chemother. 2017;72:3501.
77. Gavalda J, Meije Y, Fortun J, Roilides E, Saliba F, Lortholary O, et al. Invasive fungal infections in solid organ transplant recipients. Clin Microbiol Infect. 2014;20(Suppl 7):27–48.
78. Kennedy CC, Razonable RR. Fungal infections after lung transplantation. Clin Chest Med. 2017;38:511–20.
79. Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group; European Confederation of Medical Mycology, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosis 2013. Clin Microbiol Infect. 2014;20(Suppl 3):5–26.
80. Saliba F, Delvart V, Ichai P, Kassis N, Botterall F, Mihaila L, et al. Fungal infections after liver transplantation: outcomes and risk factors revisited in the MELD era. Clin Transpl. 2013;27: E454–61.
81. Neoh CF, Snell GI, Kotsimbos T, Levvey B, Morrissey CO, Slvin MA, et al. Antifungal prophylaxis in lung transplantation – a worldwide survey. Am J Transplant. 2011;11:361–6.
82. Nosari A, Ravini M, Cairoli R, Cozzi P, Marbello L, Marenko P, et al. Surgical resection of persistent pulmonary fungus nodules and secondary prophylaxis are effective in preventing fungal relapse in patients receiving chemotherapy or bone marrow transplantation for leukemia. Bone Marrow Transplant. 2007;39:631–5.

83. Blyth C, Gilroy N, Guy S, Chambers S, Cheong E, Gottlieb T, et al. Consensus guidelines for the treatment of invasive mould infections in haematological malignancy and haemotopoietic stem cell transplantation. Intern Med J. 2014;44 https://doi.org/10.1111/imj:12588.
84. Tissot F, Agrawal S, Pagano L, Petrikkos G, Groll A, Skiada A, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and haematopoietic stem cell transplant patients. Haematologica. 2017;102:433–44.
85. Shoham S, Magill SS, Merz WG, Gonzalez C, Seibel N, Buchanan WL, et al. Primary treatment of zygomycosis with liposomal amphotericin B: analysis of 28 cases. Med Mycol. 2010;48:511–7.
86. Ruping MJ, Heinz WJ, Kindo AJ, Rickerts V, Lass-Florl C, Beisel C, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. J Antimicrob Chemother. 2010;65:296–302.
87. Cornely OA, Maertens J, Bresnik M, Ebrahimi R, Ullmann AJ, Bouza E, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). Clin Infect Dis. 2007;44:1289–97.
88. Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Chen SC-A, et al. The contemporary management and clinical outcomes of mucormycosis: a systematic review. Int J Antimicrob Agents 2019;53:589–597.
89. Forrest GN, Mankes K. Outcomes of invasive zygomycosis infections in renal transplant recipients. Transpl Infect Dis. 2007;9:161–4.
90. Skiada A, Lass-Florl C, Klimko N, Ibahim A, Roilides E, Petrikkos G. Challenges in the diagnosis and treatment of mucormycosis. Med Mycol. 2018;56:S93–S101.
91. Mellinghoff SC, Bassetti M, Dorfel D, Hagel S, Lehners N, Plis A, et al. Isavuconazole shortens the QTc interval. Mycoses. 2018;61:256–60.
92. Vehreschild JJ, Birtel A, Vehreschild MJ, LIss B, Farowski F, Kocharek M, et al. Mucormycosis treated with posaconazole: review of 96 case reports. Crit Rev Microbiol. 2013;39:310–24.
93. Ibrahim AS, Gebremariam T, Schwartz JA, Edwards JE Jr, Spellberg B. Posaconazole mono- or combination therapy for the treatment of murine Zygomycosis. Antimicrob Agents Chemother. 2009;53:772–5.
94. Reed C, Bryant R, Ibrahim AS, et al. Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. Clin Infect Dis. 2008;47:364–71.
95. Jenks JD, Reed SL, Seidel D, Koehler P, Cornely OA, Mehta SR, et al. Rare mold infections caused by Mucorales, *Lomentospora prolificans* and *Fusarium*, San Diego: the role of antifungal combination therapy. Int J Antimicrob Agents. 2018;52:706–12.
96. Kyvernitakis A, Torres HA, Jiang Y, Chamilos G, Lewis RE, Kontoyiannis DP. Initial use of combination treatment does not impact survival of 106 patients with haematologic malignancies and mucormycosis: a propensity score analysis. Clin Microbiol Infect. 2016;22:811e1–8.
97. Pagano L, Cornely O, Busca A, Ciara M, Cesaro S, Gasbarrino C, et al. Combined antifungal approach for het treatment of invasive mucormycosis in patients with hematological disease: a report from the SEIFEM and FUNGISCOPE registries. Haematologica. 2013;98:e127–30.
98. Kim JH, Benefield RJ, Ditolla K. Utilization of posaconazole oral suspension or delayed-released tablet salvage treatment for invasive fungal infection. Mycoses. 2016;59:726–33.
99. Lewis RE, Albert ND, Kontoyiannis DP. Comparative pharmacodynamics of posaconazole in neutropenic murine models of invasive pulmonary aspergillosis and mucormycosis. Antimicrob Agents Chemother. 2014;58:6767–72.
100. Mahmood M, Abu Saleh O, Sohail MR. Hypokalemia and hypertension associated with supratherapeutic posaconazole levels. Antimicrob Agents Chemother. 2017;61:e00019–7.
101. Desai AV, Kovanda LL, Hope WW, Andes D, Mouton JW, Kowalski D, et al. Exposure-response relationships for isavuconazole in patients with invasive aspergillosis and other filamentous fungi. Antimicrob Agents Chemother. 2017;61:e01034–17.
102. Sun HY, Forrest G, Gupta KL, Aguado JM, Lortholary O, Julia MB, et al. Rhino-orbito-cerebral zygomycosis in solid organ transplant recipients. Transplantation. 2010;90:85–92.

103. Chretien ML, Legouge C, Pages PB, Lafon I, Ferrant E, Plocque A, et al. Emergency and elective pulmonary surgical resection in haematological patients with invasive fungal infections: a report of 50 cases in a single centre. Clin Microbiol Infect. 2016;22:782–7.
104. Spellberg B, Andes D, Perez M, Anglim A, Bonilla H, Mathisen GE, et al. Safety and outcomes of open-label deferasirox iron chelation therapy for mucormycosis. Antimicrob Agents Chemother. 2009;53:3122–5.
105. Zhao Z, Li S, Liu G, Yan F, Ma X, Huang Z, et al. Body iron stores and heme-iron intake in relation to risk of type 2 diabetes: a systematic review and meta-analysis. PLoS One. 2012;7: e41641.
106. Pagano L, Offidani M, Fianchi L, NOsari A, Candoni A, Picardi M, et al. Mucormycosis in hematologic patients. Haematologica. 2004;89:207–14.
107. Grimaldi D, Pradier O, Hotchkiss RS, Vincent JL. Nivolumab plus interferon-gamma in the treatment of intractable mucormycosis. Lancet Infect Dis. 2017;17(1):18.
108. John BV, Chamilos G, Kontoyiannis DP. Hyperbaric oxygen as an adjunctive treatment for zygomycosis. Clin Microbiol Infect. 2005;11:515–7.

Non-*Aspergillus* Fungal Infections in Transplant Patients

47

Sanjay G. Revankar

Contents

Introduction 1139
Epidemiology 1140
Diagnosis 1141
Clinical Disease 1143
Therapy 1144
  Superficial and Deep Local Infections 1145
  Pneumonia 1146
  Central Nervous System Infection 1146
  Disseminated Infection 1146
Alternative Therapy 1147
Prophylaxis 1148
Infection Control 1148
Conclusion 1148
Key Points 1149
References 1149

Introduction

Non-*Aspergillus* fungal infections in recipients of organ (SOT) and stem cell (HSCT) transplantation are uncommon yet present unique challenges. The variety of clinical presentations and often refractory nature of the infections compounds the difficulty in managing these patients. In this chapter, we will focus on a group of fungi that are seen rarely in clinical practice, though increasingly reported in recent years due to an increased number of immunocompromised patients at risk. Outside the commonly recognized filamentous fungi such as *Aspergillus* and *Mucorales* are a large and heterogenous group of fungi that are associated with diverse clinical

S. G. Revankar (✉)
Division of Infectious Diseases, Wayne State University School of Medicine, Detroit, MI, USA
e-mail: srevankar@med.wayne.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_46

presentations. For the purposes of this discussion, we will consider the dematiaceous fungi (associated with phaeohyphomycosis), *Fusarium* (*F.*) spp., *Scedosporium* (*S.*) spp., and Microsporidia, which have been recently reclassified as fungi. Phaeohyphomycosis is caused by a large group of darkly pigmented fungi that are difficult to categorize into a simple framework and represent dozens of unique organisms [1]. Although rare, these infections are being increasingly seen in a variety of clinical syndromes in both immunocompromised and normal individuals. Transplant patients in particular are at high risk due their prolonged immunosuppression. *Fusarium* spp. are often associated with plants and traumatic inoculation and may be drug resistant [2]. *Scedosporium* spp. are emerging as important causes of invasive and refractory mycoses in immunocompromised patients [3]. Microsporidia have typically caused gastrointestinal disease, though in highly immunocompromised patients, disseminated infection is not uncommon [4]. As the numbers of immunocompromised patients from transplantation increases, management of these unusual infections will continue to present a challenge for clinicians.

Epidemiology

The most common fungal infections after SOT and HSCT are candidiasis and aspergillosis, characteristically presenting as fungemia and pulmonary infection, respectively. However, non-*Aspergillus* infections account for 7–10% of fungal infections and have varied clinical presentations [5]. Lung transplant patients have the highest rate of invasive mold infection at 12.5%, followed by small bowel transplants (11.6%), lung (8.6%), and liver (4.7%), with renal transplants having the lowest risk (1.3%) though most infections occur in renal transplants due to the higher number of transplants performed [5]. Non-*Aspergillus* mold infections usually present 10–14 months after transplant. Liver transplant recipients may have earlier infections, with an average presentation less than 3 months after transplant [5]. Risk factors for fungal infections in general include prolonged surgery, hepatic and renal dysfunction, and concurrent cytomegalovirus (CMV) and HHV-6 infection. Specific organ transplants have unique risks. For lung recipients risks include airway colonization with mold (typically *Aspergillus*), CMV infection, and anastomotic ischemia. Risks for kidney recipients include pretransplant dialysis, diabetes, tacrolimus, and prolonged and high-dose steroid use. Fungal infection risk in heart recipients is increased with CMV disease, posttransplant dialysis, and the presence of *Aspergillus* in bronchoalveolar lavage [6]. In general, these uncommon fungal pathogens are found in soil, associated with plants, or in environmental water sources, and distributed worldwide.

Dematiaceous fungi are a large and heterogeneous group of primarily molds. Some studies have suggested that dematiaceous fungal infections are more common in warmer climates [7]. Specific fungi appear to be geographically restricted, such as *Rhinocladiella (R.) (*formerly *Ramichloridium) mackenziei*, which has primarily been seen in patients from the Middle East [8]. Exposure is likely from inhalation or minor trauma, which is typically not noticed by the patient. Surveys of outdoor air

for fungal spores routinely observe dematiaceous fungi [9]. As they are ubiquitous in the environment, individuals are constantly exposed to them, though they remain uncommon causes of disease. Most life-threatening infections due to these unusual fungi are seen in immunocompromised patients, with the possible exception of CNS infection caused by dematiaceous fungi. Incidence of phaeohyphomycosis is 0.16–3.6%, depending on the type of transplant [7, 10]. Cases are fairly evenly divided between HSCT and SOT patients. Average diagnosis is made at 20 months posttransplant [11]. In one large series, the most common genus was *Exophiala*, and overall mortality was 19%. Cases were sporadic, and no clear pattern was observed [12].

Fusarium and *Scedosporium* are uncommon in HSCT patients (3.8%) and distinctly rare in SOT, accounting for 0.2–0.7% of cases of fungal infection [13]. Rates have been low and generally stable over time, though mortality is high. *F. solani* is the most common species causing disease [14]. Exposure is generally thought to be from environmental sources such as plants and soil, though food has been implicated as a risk factor, as *Fusarium* can be isolated from a variety of foods, including fruit, nuts, vegetables, and cereals [15]. Other molds have also been isolated but with unclear significance. *Scedosporium* is found in the environment, typically in soil and water sources [16]. Near-drowning is frequently associated with pneumonia and even disseminated infections due to this fungus. Multiple species are in this genus with recently changing taxonomy. Common species are *S. apiospermum* and *S. boydii* [16]. Median time to diagnosis is 4 months after transplant [14].

Microsporidia are commonly found in water sources and typically cause self-limited diarrheal illness, though in transplant patients, dissemination can be seen [4]. Though there are over 1400 species of Microsporidia, only about 1% are known to infect humans, including *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* [4].

Diagnosis

The diagnosis of infections due to non-*Aspergillus* fungi requires a high degree of clinical suspicion, especially in transplant patients. Careful assessment of signs and symptoms should lead to further imaging and/or acquisition of appropriate tissue samples for diagnostic testing. Radiologically, nodular lesions are the most common finding in the lung, though the "halo sign" is less frequent than in neutropenic patients with aspergillosis [6]. Biopsy of relevant lesions is also essential, as blood and sputum cultures are usually unrevealing. It is important to recognize that the histology of *Fusarium* and *Scedosporium* infections is similar to Aspergillus, so that results of culture are often critical to making a specific diagnosis [14]. In contrast, the histologic appearance of phaeohyphomycosis is often characterized by irregular hyphal elements and beaded, yeastlike forms [1]. However, it may still be difficult to differentiate this pathologically from infection due to other molds. In such cases, the Fontana-Masson stain, which is specific for melanin, can usually be used to confirm the presence of dematiaceous hyphae [1]. Currently, the diagnosis of these infections initially relies on pathologic examination of clinical specimens and careful

gross and microscopic examination of cultures, occasionally requiring the expertise of a mycology reference lab for unusual or newly described pathogens. Despite the various testing methodologies available in the modern clinical laboratory, culture remains the mainstay of diagnosis.

Unfortunately, no specific diagnostic tests are available to identify these fungi, particularly to the species level. No specific serologic, antigen, or polymerase chain reaction (PCR) methods are routinely available, which is partly due to the tremendous diversity of these pathogens. However, antigen tests that have been primarily useful for *Candida* and *Aspergillus*, such as serum β-D-glucan and serum or bronchoalveolar lavage (BAL) galactomannan, occasionally may be cross-reactive with this group of fungi, but this is not consistent [17, 18]. Galactomannan may be positive in *Fusarium* infections, and serum β-D-glucan, which is found in most fungal species, has also been positive in cases of phaeohyphomycosis, *Fusarium*, and *Scedosporium* infections [19, 20]. A recent study examined the utility of bronchoalveolar lavage β-D-glucan levels and found that BAL measurements were no better than serum levels in diagnosis [21]. One useful aspect of antigen testing was the finding that serum β-D-glucan may have good negative predictive value [6].

Studies have also begun to examine the potential of identifying species within this diverse group of fungi using PCR of highly conserved regions of ribosomal DNA [22]. Molecular identification has become more useful as one study demonstrated by identifying eight non-sporulating clinical isolates of dematiaceous fungi to the species or genus level by a combination of internal transcribed spacer (ITS) and D1/D2 rRNA typing [23]. Various PCR methodologies are under development for *Fusarium* and *Scedosporium*, but not currently available for routine clinical use [24]. Quantitative PCR for fungal diagnosis from tissue samples using specific primers has been able to detect *Fusarium* and *Scedosporium*, including a *Fusarium* isolate from tissue samples initially considered consistent with *Aspergillus*. PCR was found to be positive in 80% of cases with negative culture [25].

Matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectrometry is a useful method for microbial identification [26]. It is broadly effective across different groups of organisms grown in different conditions. It is rapid, providing results within a few minutes, though samples from an actively growing culture are needed. Sensitivity is good, needing only 10^4 organisms for detection [26]. However, difficulties in identifying molds arise due to differences in spores vs hyphae and the inherent biological complexity of fungal organisms. Clinically important fungi have been included in most databases, though uncommon molds are generally not reliably identified [26, 27]. Further database refinement is needed in order to be clinically useful, though this is time-consuming and laborious. MALDI-ToF for rare mold identification is still a work in progress.

The diagnosis of microsporidial infection is generally through microscopic examination of tissue samples or stool and use of specific stains [4]. Electron microscopy of tissue samples is very sensitive but time-consuming and not readily available [4]. PCR diagnosis of *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* has been described and may be more sensitive than microscopy but is not routinely available [28].

Clinical Disease

Many infectious syndromes are observed with these fungi (Table 1), ranging from superficial infections such as keratitis and subcutaneous nodules to invasive infections such as brain abscess and disseminated disease [1, 6]. In particular, phaeohyphomycosis encompasses many clinical syndromes due to a wide variety of dematiaceous fungi [29]. Clinical presentation may be indolent, particularly in solid organ transplant patients. Superficial infections are the most common form of infection due to these fungi, predominantly subcutaneous nodular lesions [12]. Although they rarely lead to life-threatening disease, significant morbidity can occur depending on the site of infection and response to therapy. There are numerous case reports of subcutaneous infection due to a wide variety of species [30, 31]. Minor trauma is the usual inciting factor, though it may be unrecognized by the patient. Lesions typically occur on exposed areas of the body and often appear as isolated cystic or nodular lesions. Occasionally, dissemination can occur, which can be refractory to therapy with high mortality [31, 32]. Keratitis is an uncommon but is a serious infection due to these fungi. Dematiaceous fungi account for up to 8–17% of cases, particularly in tropical regions, though *Fusarium* is common as well [33]. Deep local infection such as septic arthritis and osteomyelitis is rare and often difficult to treat, frequently requiring both surgical and combination medical therapy [34]. Pneumonia usually occurs in highly immunocompromised patients and may be due to a wide variety of species [35]. It is unclear what specific risk factors may contribute to pulmonary infection with these fungi, which are commonly found in the environment, though studies have found an association between pretransplant colonization and subsequent pneumonia in lung transplant patients [6]. Central

Table 1 Clinical syndromes associated with specific non-*Aspergillus* fungi and suggested therapy

Clinical syndrome	Commonly associated fungal genera (species)	Therapy
Subcutaneous nodules	*Exophiala* *Alternaria* *Fusarium*	Surgery +/- Voriconazole
Keratitis	*Curvularia* *Fusarium*	Topical natamycin +/- Itraconazole Voriconazole
Pneumonia	*Ochroconis* *Exophiala* *Scedosporium*	Voriconazole
Brain abscess	*Cladophialophora (bantiana)* *Rhinocladiella (mackenziei)* *Ochroconis*	Consider combination therapy – see discussion
Disseminated disease	*Lomentospora (prolificans)* *Curvularia* *Fusarium*	Consider combination therapy – see discussion
Microsporidiosis	*Encephalitozoon (cuniculi)* + others *Enterocytozoon (bieneusi)*	Albendazole Fumagillin

nervous system infection is a rare clinical manifestation, though it remains one of the most difficult to cure and mortality rates are high. The pathogenesis may be hematogenous spread from an initial, presumably subclinical pulmonary focus. Brain abscess is the most common presentation, with *Cladophialophora (C.) bantiana* the most common species isolated [36, 37]. Disseminated infection is the most uncommon manifestation of infection seen with dematiaceous fungi [38]. *L. prolificans* is the most common cause, but other species can be seen [32, 38]. In contrast to most invasive mold infections, blood cultures are positive in over half the cases, usually seen with *L. prolificans* [3, 38]. Infection with this species was also associated with septic shock. Infection with *L. prolificans* was associated with nearly 100% mortality in the absence of recovery from neutropenia [3, 39]. Survival in transplant patients with *L. prolificans* infection was associated with surgical resection (where feasible) and recovery of neutropenia, but not antifungal therapy [13].

Fusarium and *Scedosporium* cause disease with various clinical presentations and may also cause disseminated infection, though primarily in HSCT patients [14, 24]. *Fusarium* generally causes subcutaneous lesions and pneumonia, with the latter having high mortality, especially in lung transplant recipients [40]. Fungemia is also seen and, while mortality is high, can be treated successfully, even with subsequent HSCT [41]. However, disseminated *Fusarium* infection during HSCT is generally fatal, despite aggressive therapy [42]. Overall mortality rates are high, reported as 79% for *Fusarium* in a large registry and 58–72% for *Scedosporium* in SOT patients [3, 13].

Microsporidiosis in transplantation is uncommon but can be associated with severe disease. Chronic diarrhea is a typical presentation, especially in renal transplants [43, 44]. Graft dysfunction may be the presenting feature in renal transplants, in the absence of other symptoms [45]. Interestingly, donor-derived infections have also been described, with a predominantly encephalitic presentation [46, 47]. Dissemination is also observed but can be treated effectively if diagnosed promptly [47].

Therapy

There are no standardized therapies for infections caused by these uncommon fungi, though guidelines by the *European Society of Clinical Microbiology and Infectious Diseases* (ESCMID) and the American Society of Transplantation (AST) have been published, mostly based on animal models, case series, and expert opinion [48, 49]. There is no single antifungal agent that is effective against all these fungal species in all clinical settings. The choice of drug is made more difficult by the fact that these are rare infections, with no randomized clinical trials to guide therapeutic decisions. However, triazole agents demonstrate the broadest in vitro activity, including voriconazole, posaconazole, isavuconazole, and itraconazole. However, the dematiaceous fungi *L. prolificans* and *Scopulariopsis brumptii* are generally resistant to all available antifungals [50]. Length of therapy is generally based on clinical response and ranges from several weeks to several months or longer, including potentially lifelong suppressive therapy in immunocompromised patients with

infections such as brain abscess or endocarditis due to refractory pathogens [51]. The diversity of the pathogens and hosts makes it impossible to find a "one-size-fits-all" therapeutic strategy.

Voriconazole, posaconazole, and isavuconazole are better tolerated than itraconazole with a broader spectrum of activity and would be the preferred drugs for the non-*Aspergillus* molds. Though there is greater clinical experience associated with itraconazole, particularly against dematiaceous fungi, voriconazole and posaconazole are generally more active in vitro, particularly against *Fusarium* and *Scedosporium*. Voriconazole is the only agent FDA approved as salvage therapy for *Fusarium and Scedosporium* infections and may have improved clinical response vs other agents [3]. Voriconazole may also have advantages for central nervous system infections due to its ability to achieve good cerebrospinal fluid levels, unlike itraconazole [52]. All azoles likely achieve adequate brain tissue levels [53]. Posaconazole is very well tolerated with broad activity, particularly against *Aspergillus* and other molds, including *Fusarium* and *Scedosporium* [54]. Isavuconazole is the most recent broad-spectrum azole with tolerability comparable to fluconazole and less drug interactions than voriconazole and itraconazole [55]. Although clinical data are very limited, some efficacy with these uncommon fungi has been shown in small studies [56]. Itraconazole has in vitro activity against the vast majority of dematiaceous fungi and extensive clinical experience [1], but recent use has been limited due to adverse effects which have led to a black box label from the US Food and Drug Administration (FDA) cautioning against use in patients with ventricular dysfunction due to itraconazole's negative inotropic effects.

Therapy in patients with organ transplantation should be continued longer than in other settings, and the use of surgery (when appropriate) should be strongly considered. Management of drug interactions is critically important when using triazole antifungals, as all the above have significant effects on many drugs used in transplant patients, notably calcineurin inhibitors [57]. Monitoring of drug levels of both immunosuppressives and triazoles (except perhaps isavuconazole) should be routinely performed.

Superficial and Deep Local Infections

Superficial infections are the most common form of infection due to these fungi in transplant patients. Oral systemic therapy with an azole in conjunction with surgery is usually employed and often successful, though relapses can occur [12, 23, 29]. Surgical excision alone has also been successful in a number of cases [23, 30]. Keratitis is often treated with topical polyenes, such as natamycin and amphotericin B, but oral and topical itraconazole has been found to be useful as well, particularly in refractory cases [1]. Recent clinical experience suggests voriconazole and posaconazole are potentially useful agents as well [58]. Penetrating keratoplasty can be performed in those patients failing initial therapy, though many patients are left with residual visual deficits [1]. Deep local infection such as septic arthritis and osteomyelitis is rare and often difficult to treat, frequently requiring both surgical and combination medical therapy [59].

Pneumonia

This usually occurs in highly immunocompromised transplant patients and may be due to a wide variety of species [35]. Mortality rates are high in these cases. Experience with voriconazole is accumulating, and it would be favored in this setting given its efficacy in pulmonary aspergillosis [13, 14]. Posaconazole has also been reported as successful in a case report of *Phaeoacremonium* pneumonia [60].

Central Nervous System Infection

Though this is a rare clinical manifestation of infection, it remains one of the most difficult to cure, and mortality rates are high, regardless of therapy. *C. bantiana* is the most common species isolated, usually seen in brain abscesses [29]. In general, available evidence and recommendations suggest combination therapy is preferred, though it remains unclear which combination is optimal. One review showed that amphotericin B, flucytosine, and itraconazole may be associated with improved survival, though it was used in a small number of cases [8]. Guidelines suggest voriconazole, flucytosine, and an echinocandin, with or without amphotericin B, based on animal models and case reports [48]. Complete excision of brain abscesses appeared to have better outcomes than aspiration or partial excision [61]. Outcomes are poor, with an overall mortality >70% [61]. Posaconazole has also been used successfully, including cases of *Rhinocladiella* and *Scedosporium* brain abscess [62, 63]. Some patients are placed on indefinite suppression with voriconazole or posaconazole once the infection has been successfully treated [51].

Disseminated Infection

This is the most uncommon manifestation of infection seen with these fungi. There are no antifungal regimens associated with improved survival in disseminated phaeohyphomycosis, including multiple combination therapies [13, 29]. Anecdotal reports of successful treatment may be instructive, however [29]. *Fusarium* and *Scedosporium* may also cause disseminated infection, though primarily in hematopoietic stem cell transplant patients [13, 14]. Combination therapy with voriconazole and amphotericin B or an echinocandin has shown promising results in fusariosis, though with limited data [24, 40, 41].

Therapy for microsporidial infections is limited to albendazole and fumagillin, based on small studies. Albendazole may be effective for most species, except *Enterocytozoon bieneusi* [4], where fumagilin has been used with success [43]. Duration of therapy varies widely, depending on the severity of infection and host immune status, ranging from 2 weeks to 12 months [45]. Albendazole is better tolerated and can be given for prolonged therapy. Fumagillin is associated with thrombocytopenia and neutropenia, and therapy is usually 2–4 weeks [64].

Alternative Therapy

There are few useful alternatives to triazole antifungal agents against these uncommon fungal infections. Amphotericin B (AmB) may be useful for severe disease given its broad fungicidal activity against most pathogens, though isolates of *Fusarium* and *Scedosporium* are frequently resistant [24]. However, use of lipid AmB preparations allows for much higher doses than possible with standard amphotericin B, which may improve their efficacy against these fungi. The pharmacodynamics of these formulations are different from standard amphotericin B and may also affect their overall efficacy for specific infections [65]. However, given the need for parenteral administration, use of these agents is mostly confined to serious infections in unstable patients. Once the infection is under control, longer-term therapy with a broad spectrum oral azole is often reasonable until complete response is achieved.

Terbinafine inhibits ergosterol synthesis but acts on a different target than azoles. It is also considered fungistatic, and its clinical role has been relegated to treatment of dermatophyte infections. However, in vitro activity is quite broad and includes many non-dermatophyte molds [66]. There has been recent interest in potentially expanding its clinical spectrum [66]. However, its extensive binding to serum proteins and distribution into skin and adipose tissue have diminished enthusiasm for its use in treating serious systemic fungal infections [66]. However, terbinafine was used to successfully treat a case of *E. jeanselmei* subcutaneous infection in a heart transplant patient after failing itraconazole [67].

Miltefosine, originally developed as an antineoplastic agent and used in protozoal infections, also has significant antifungal activity against a broad range of pathogens [68]. Clinical experience is anecdotal, though a case of refractory infection due to *Scedosporium prolificans* appeared to respond to the addition of miltefosine [69].

Combination therapy is a potentially useful strategy for refractory infections, though it has not been studied extensively and is not routinely recommended except in cases as mentioned above. Terbinafine in particular appears to provide synergistic activity with azole antifungals, and this may be a useful strategy against refractory subcutaneous infections [66]. The mechanism is presumably potent inhibition of ergosterol synthesis at two different steps of the pathway by these agents. In addition, case reports have suggested that the combination of itraconazole or voriconazole with terbinafine may be synergistic against *S. prolificans* and improve outcomes, though more recent studies cast doubt on this finding [29].

Echinocandins do not have significant activity against filamentous fungi other than *Aspergillus* and would not be recommended as single agents. However, a case of refractory bone and joint infection due to *S. prolificans* was treated effectively with the combination of voriconazole and caspofungin [70].

There are no controlled trials to determine when surgery is most effective for these infections. However, for several of the above clinical presentations, surgery may be curative or significantly improve outcomes. For subcutaneous abscess in

particular, surgery alone has been effective, though in immunocompromised patients, follow-up antifungal therapy is often given. Outcomes for brain abscess may be improved with the use of surgical drainage and/or excision, if possible. Frequently, single lesions are present, which may make surgical resection more feasible. Granulocyte transfusions have been used in patients with refractory fungal infections and neutropenia, though meta-analysis did not show a mortality benefit [71]. Further studies are ongoing, though no randomized controlled studies are available.

Prophylaxis

Voriconazole is commonly prescribed as prophylaxis in high-risk transplant patients (particularly lung), with itraconazole, posaconazole, and aerosolized amphotericin B as alternatives. Duration is usually 3–12 months, depending on the institution [72]. Detection of *Aspergillus* in the first 3 months should be a consideration for preemptive therapy, though non-*Aspergillus* molds have not been associated with post-transplant infection [72]. There are no studies that specifically examine outcomes of prophylaxis for these rare molds. However, the newer azoles with activity against *Aspergillus* would be expected to be effective in prophylaxis for these fungi as well. As these are uncommon infections, even large trials are unlikely to show a significant trend in these infections.

Infection Control

Dematiaceous fungi are routinely found in ambient air, and prevention from this source is not practical, except perhaps in immunocompromised patient units with HEPA filtration precautions for other, more common fungi such as *Aspergillus* [73]. In one hospital study, *Fusarium* was isolated from over half of water system samples, with patient isolates matching some environmental isolates by molecular typing [74]. No person-to-person transmission has been demonstrated.

Conclusion

Non-*Aspergillus* fungi are rarely seen in clinical practice, though they are important agents of disease, particularly in transplant patients. A high index of suspicion is needed when evaluating patients who have undergone organ or stem cell transplantation for possible fungal infection. These fungi often have unique clinical manifestations and appearance in tissue, though often are not detected with routine antigen tests. Culture remains the mainstay for diagnosis. Newer methods of diagnosis, such as PCR or MALDI-ToF, are promising, but not widely utilized. Therapy is individualized, especially for disseminated infections, with combination therapy commonly employed. Though azoles such as

voriconazole and posaconazole have the most recent clinical experience, optimal therapy for most of these infections is unclear, due to a lack of randomized trial data.

Key Points

- Non-*Aspergillus* molds have varied clinical presentations in transplant patients.
- Specific diagnostic testing is not routinely available, but serum galactomannan and β-D-glucan may be positive.
- Therapy is not standardized, though voriconazole and posaconazole are active and have accumulating clinical experience.
- Combination therapy may provide improved outcomes in selected patients.

References

1. Revankar SG, Sutton DA. Melanized fungi in human disease. Clin Microbiol Rev. 2010;23:884–928.
2. Malani AN, Kauffman CA. Changing epidemiology of rare mould infections: implications for therapy. Drugs. 2007;67:1803–12.
3. Seidel D, Meißner A, Lackner M, et al. Prognostic factors in 264 adults with invasive Scedosporium spp. and Lomentospora prolificans infection reported in the literature and FungiScope(®). Crit Rev Microbiol. 2019;10:1–21.
4. Field AS, Milner DA Jr. Intestinal microsporidiosis. Clin Lab Med. 2015;35(2):445–59.
5. Shoham S, Kumar D, Dominguez EA. AST Infectious Diseases Community of Practice. Emerging fungal infections in solid organ transplant recipients: Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019: e13525. https://doi.org/10.1111/ctr.13525.
6. Anesi JA, Baddley JW. Approach to the Solid Organ Transplant Patient with Suspected Fungal Infection. Infect Dis Clin North Am. 2016;30:277–96. https://doi.org/10.1016/j.idc.2015.10.001.
7. McCarty TP, Baddley JW, Walsh TJ, et al. Phaeohyphomycosis in transplant recipients: results from the transplant associated infection surveillance network (TRANSNET). Med Mycol. 2015;53:440–6. https://doi.org/10.1093/mmy/myv018.
8. Revankar SG, Sutton DA, Rinaldi MG. Primary central nervous system phaeohyphomycosis: a review of 101 cases. Clin Infect Dis. 2004;38:206–16.
9. Shelton BG, Kirkland KH, Flanders WD, et al. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol. 2002;68:1743–53.
10. Santos T, Aguiar B, Santos L, et al. Invasive fungal infections after kidney transplantation: a single-center experience. Transplant Proc. 2015;47:971–5. https://doi.org/10.1016/j.transproceed.2015.03.040.
11. Kabir V, Maertens J, Kuypers D. Fungal infections in solid organ transplantation: an update on diagnosis and treatment. Transplant Rev (Orlando). 2019;33:77–86. https://doi.org/10.1016/j.trre.2018.12.001.
12. Schieffelin JS, Garcia-Diaz JB, Loss GE Jr, et al. Phaeohyphomycosis fungal infections in solid organ transplant recipients: clinical presentation, pathology, and treatment. Transpl Infect Dis. 2014;16:270–8. https://doi.org/10.1111/tid.12197.
13. Clark NM, Weigt SS, Fishbein MC, et al. Fungal infections complicating lung transplantation. Semin Respir Crit Care Med. 2018;39:227–54. https://doi.org/10.1055/s-0037-1617443.

14. Lemonovich TL. Mold infections in solid organ transplant recipients. Infect Dis Clin North Am. 2018;32:687–701. https://doi.org/10.1016/j.idc.2018.04.006.
15. Paterson RRM, Lima N. Filamentous fungal human pathogens from food emphasising aspergillus, fusarium and mucor. Microorganisms. 2017;5:E44. https://doi.org/10.3390/microorganisms5030044.
16. Rougeron A, Giraud S, Alastruey-Izquierdo A, et al. Ecology of Scedosporium species: present knowledge and future research. Mycopathologia. 2018;183:185–200.
17. Cuétara MS, Alhambra A, Moragues MD, et al. Detection of (1–>3)-beta-D-glucan as an adjunct to diagnosis in a mixed population with uncommon proven invasive fungal diseases or with an unusual clinical presentation. Clin Vaccine Immunol. 2009;16:423–6.
18. Hachem RY, Kontoyiannis DP, Chemaly RF, et al. Utility of galactomannan enzyme immunoassay and (1,3) beta-D-glucan in diagnosis of invasive fungal infections: low sensitivity for *Aspergillus fumigatus* infection in hematologic malignancy patients. J Clin Microbiol. 2009;47:129–33.
19. Levesque E, Rizk F, Noorah Z, et al. Detection of (1,3)-β-d-Glucan for the Diagnosis of Invasive Fungal Infection in Liver Transplant Recipients. Int J Mol Sci. 2017;18:E862. https://doi.org/10.3390/ijms18040862.
20. Colombier MA, Alanio A, Denis B, et al. Dual invasive infection with Phaeoacremonium parasiticum and Paraconiothyrium cyclothyrioides in a renal transplant recipient: case report and comprehensive review of the literature of Phaeoacremonium Phaeohyphomycosis. J Clin Microbiol. 2015;53:2084–94. https://doi.org/10.1128/JCM.00295–15.
21. Mutschlechner W, Risslegger B, Willinger B, et al. Bronchoalveolar lavage fluid (1,3)β-D-glucan for the diagnosis of invasive fungal infections in solid organ transplantation: a prospective multicenter study. Transplantation. 2015;99:e140–4. https://doi.org/10.1097/TP.0000000000000635.
22. Abliz P, Fukushima K, Takizawa K, et al. Identification of pathogenic dematiaceous fungi and related taxa based on large subunit ribosomal DNA D1/D2 domain sequence analysis. FEMS Immunol Med Microbiol. 2004;40:41–9.
23. Santos DW, Camargo LF, Gonçalves SS, et al. Melanized fungal infections in kidney transplant recipients: contributions to optimize clinical management. Clin Microbiol Infect. 2017;23:333.e9–14. https://doi.org/10.1016/j.cmi.2016.12.024.
24. MW MC, Katragkou A, Iosifidis E, et al. Recent advances in the treatment of Scedosporiosis and Fusariosis. J Fungi (Basel). 2018;4:E73. https://doi.org/10.3390/jof4020073.
25. Salehi E, Hedayati MT, Zoll J, et al. Discrimination of aspergillosis, Mucormycosis, Fusariosis, and Scedosporiosis in formalin-fixed paraffin-embedded tissue specimens by use of multiple real-time quantitative PCR assays. J Clin Microbiol. 2016;54:2798–803.
26. Angeletti S. Matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) in clinical microbiology. J Microbiol Methods. 2017;138:20–9. https://doi.org/10.1016/j.mimet.2016.09.003.
27. Cassagne C, Normand AC, L'Ollivier C, Ranque S, Piarroux R. Performance of MALDI-TOF MS platforms for fungal identification. Mycoses. 2016;59(11):678–90. https://doi.org/10.1111/myc.12506.
28. Heyworth MF. Molecular diagnosis of human microsporidian infections. Trans R Soc Trop Med Hyg. 2017;111:382–3. https://doi.org/10.1093/trstmh/trx070.
29. Revankar SG, Baddley JW, Chen SC, et al. A mycoses study group international prospective study of Phaeohyphomycosis: an analysis of 99 proven/probable cases. Open Forum Infect Dis. 2017;4. https://doi.org/10.1093/ofid/ofx200.
30. Haridasan S, Parameswaran S, Bheemanathi SH, et al. Subcutaneous phaeohyphomycosis in kidney transplant recipients: a series of seven cases. Transpl Infect Dis. 2017; 19. https://doi.org/10.1111/tid.12788.
31. Oberlin KE, Nichols AJ, Rosa R, et al. Phaeohyphomycosis due to Exophiala infections in solid organ transplant recipients: case report and literature review. Transpl Infect Dis. 2017;19. https://doi.org/10.1111/tid.12723.

32. Palmisano A, Morio F, Le Pape P, et al. Multifocal phaeohyphomycosis caused by Exophiala xenobiotica in a kidney transplant recipient. Transpl Infect Dis. 2015;17:297–302. https://doi.org/10.1111/tid.12350.
33. Gopinathan U, Garg P, Fernandes M, et al. The epidemiological features and laboratory results of fungal keratitis: a 10-year review at a referral eye care center in South India. Cornea. 2002;2:555–9.
34. Li JY, Yong TY, Grove DI, et al. Successful control of *Scedosporium prolificans* septic arthritis and probable osteomyelitis without radical surgery in a long-term renal transplant recipient. Transpl Infect Dis. 2008;10:63–5.
35. Campos S, Caramori M, Teixeira R, et al. Bacterial and fungal pneumonias after lung transplantation. Transplant Proc. 2008;40:822–4.
36. Revankar SG, Sutton DA, Rinaldi MG. Primary central nervous system phaeohyphomycosis: a review of 101 cases. Clin Infect Dis. 2004;38:206–16.
37. Reynaud Q, Dupont D, Nove-Josserand R, et al. Rare and unusual presentation of Cladophialophora infection in a pulmonary transplant cystic fibrosis patient. Transpl Infect Dis. 2017;19. https://doi.org/10.1111/tid.12789.
38. Revankar SG, Patterson JE, Sutton DA, et al. Disseminated phaeohyphomycosis: review of an emerging mycosis. Clin Infect Dis. 2002;34:467–76.
39. Rodriguez-Tudela JL, Berenguer J, Guarro J, et al. Epidemiology and outcome of *Scedosporium prolificans* infection, a review of 162 cases. Med Mycol. 2009;47:359–70.
40. Nambiar P, Cober E, Johnson L, et al. Fatal Fusarium infection manifesting as osteomyelitis following previous treatment with amphotericin B in a multi-visceral transplant: case report and review of Fusarium infections in solid organ transplantation. Transpl Infect Dis. 2018;20:e12872. https://doi.org/10.1111/tid.12872.
41. Sheela S, Ito S, Strich JR, et al. Successful salvage chemotherapy and allogeneic transplantation of an acute myeloid leukemia patient with disseminated Fusarium solani infection. Leuk Res Rep. 2017;8:4–6. https://doi.org/10.1016/j.lrr.2017.07.001.
42. Stempel JM, Hammond SP, Sutton DA, et al. Invasive Fusariosis in the Voriconazole Era: Single-Center 13-Year Experience. Open Forum Infect Dis. 2015; 2. https://doi.org/10.1093/ofid/ofv099.
43. Bukreyeva I, Angoulvant A, Bendib I, et al. Enterocytozoon bieneusi Microsporidiosis in Stem Cell Transplant Recipients Treated with Fumagillin. Emerg Infect Dis. 2017;23:1039–41. https://doi.org/10.3201/eid2306.161825.
44. Galván AL, Sánchez AM, Valentín MA, et al. First cases of microsporidiosis in transplant recipients in Spain and review of the literature. J Clin Microbiol. 2011;49:1301–6. https://doi.org/10.1128/JCM.01833-10.
45. Brown M, Longano A, Dendle C, et al. Confirmed microsporidial graft infection in a HIV-negative renal transplant recipient: a case report and review of the literature. Transpl Infect Dis. 2018;20:e12888. https://doi.org/10.1111/tid.12888.
46. Hocevar SN, Paddock CD, Spak CW, et al. Microsporidiosis acquired through solid organ transplantation: a public health investigation. Ann Intern Med. 2014;160:213–20.
47. Smith RM, Muehlenbachs A, Schaenmann J, et al. Three cases of neurologic syndrome caused by donor-derived Microsporidiosis. Emerg Infect Dis. 2017;23:387–95. https://doi.org/10.3201/eid2303.161580.
48. Chowdhary A, Meis JF, Guarro J, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and European Confederation of Medical Mycology (ECMM) joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. Clin Microbiol Infect. 2014;20(Suppl 3):47–75.
49. Shoham S, Kumar D, Dominguez EA, AST Infectious Diseases Community of Practice. Emerging fungal infections in solid organ transplant recipients: Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;12:e13525. https://doi.org/10.1111/ctr.13525.

50. Cuenca-Estrella M, Gomez-Lopez A, Mellado E, et al. Head-to-head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentous fungi. Antimicrob Agents Chemother. 2006;50:917–21.
51. Ray U, Dutta S, Chakravarty C. A *Cladophialophora* brain abscess in a renal transplant recipient. Southeast Asian J Trop Med Public Health. 2016;47:1026–31.
52. Lutsar I, Roffey S, Troke P. Voriconazole concentrations in the cerebrospinal fluid and brain tissue of Guinea pigs and immunocompromised patients. Clin Infect Dis. 2003;37:728–32.
53. Groll AH, Piscitelli SC, Walsh TJ. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. Adv Pharmacol. 1998;44:343–499.
54. Raad II, Hachem RY, Herbrecht R, et al. Posaconazole as salvage treatment for invasive fusariosis in patients with underlying hematologic malignancy and other conditions. Clin Infect Dis. 2006;42:1398–403.
55. Miceli MH, Kauffman CA. Isavuconazole: A New Broad-Spectrum Triazole Antifungal Agent. Clin Infect Dis. 2015;61:1558–65. https://doi.org/10.1093/cid/civ571.
56. Cornely OA, Mullane KM, Ostrosky-Zeichner L, Maher RM, Croos-Dabrera R, Lu Q, Lademacher C, Perfect JR, Oren I, Schmitt-Hoffmann AH, Giladi M, Marty FM, Rahav G. Isavuconazole for treatment of rare invasive fungal diseases. Mycoses. 2018;61(8):518–33.
57. Lempers VJ, Martial LC, Schreuder MF, et al. Drug-interactions of azole antifungals with selected immunosuppressants in transplant patients: strategies for optimal management in clinical practice. Curr Opin Pharmacol. 2015;24:38–44. https://doi.org/10.1016/j.coph.2015.07.002.
58. Hariprasad SM, Mieler WF, Lin TK, et al. Voriconazole in the treatment of fungal eye infections: a review of current literature. Br J Ophthalmol. 2008;92:871–8.
59. Kumashi PR, Safdar A, Chamilos G, et al. Fungal osteoarticular infections in patients treated at a comprehensive cancer Centre: a 10-year retrospective review. Clin Microbiol Infect. 2006;12:621–6.
60. Monaganti S, Santos CA, Markwardt A, et al. Pulmonary phaeohyphomycosis caused by phaeoacremonium in a kidney transplant recipient: successful treatment with posaconazole. Case Rep Med. 2014;2014:902818. https://doi.org/10.1155/2014/902818.
61. Chakrabarti A, Kaur H, Rudramurthy SM, et al. Brain abscess due to Cladophialophora bantiana: a review of 124 cases. Med Mycol. 2016;54:111–9. https://doi.org/10.1093/mmy/myv091.
62. Al Abdely HM, Alkhunaizi AM, Al Tawfiq JA, et al. Successful therapy of cerebral phaeohyphomycosis due to *Ramichloridium mackenziei* with the new triazole posaconazole. Med Mycol. 2005;43:91–5.
63. Mellinghoff IK, Winston DJ, Mukwaya G, et al. Treatment of *Scedosporium apiospermum* brain abscesses with posaconazole. Clin Infect Dis. 2002;34:1648–50.
64. Nagpal A, Pritt BS, Lorenz EC, et al. Disseminated microsporidiosis in a renal transplant recipient: case report and review of the literature. Transpl Infect Dis. 2013;15:526–32. https://doi.org/10.1111/tid.12119.
65. Dodds ES, Drew RH, Perfect JR. Antifungal pharmacodynamics: review of the literature and clinical applications. Pharmacotherapy. 2000;20:1335–55.
66. Revankar SG, Nailor MD, Sobel JD. Use of terbinafine in rare and refractory mycoses. Future Microbiol. 2008;3:9–17.
67. Agger WA, Andes D, Burgess JW. *Exophiala jeanselmei* infection in a heart transplant recipient successfully treated with oral terbinafine. Clin Infect Dis. 2004;38:e112–5.
68. Widmer F, Wright LC, Obando D, et al. Hexadecylphosphocholine (miltefosine) has broad-spectrum fungicidal activity and is efficacious in a mouse model of cryptococcosis. Antimicrob Agents Chemother. 2006;50:414–21.
69. Kesson AM, Bellemore MC, O'Mara TJ, et al. *Scedosporium prolificans* osteomyelitis in an immunocompetent child treated with a novel agent, hexadecylphospocholine (miltefosine), in combination with terbinafine and voriconazole: a case report. Clin Infect Dis. 2009;48:1257–61.

70. Steinbach WJ, Schell WA, Miller JL, et al. *Scedosporium prolificans* osteomyelitis in an immunocompetent child treated with voriconazole and caspofungin, as well as locally applied polyhexamethylene biguanide. J Clin Microbiol. 2003;41:3981–5.
71. West KA, Gea-Banacloche J, Stroncek D, et al. Granulocyte transfusions in the management of invasive fungal infections. Br J Haematol. 2017;177:357–74. https://doi.org/10.1111/bjh.14597.
72. Kennedy CC, Razonable RR. Fungal infections after lung transplantation. Clin Chest Med. 2017;38:511–20. https://doi.org/10.1016/j.ccm.2017.04.011.
73. Patterson JE, Peters J, Calhoon JH, et al. Investigation and control of aspergillosis and other filamentous fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2000;2:22–8.
74. Anaissie EJ, Kuchar RT, Rex JH, et al. Fusariosis associated with pathogenic Fusarium species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. Clin Infect Dis. 2001;33:1871–8.

Management of Cryptococcosis in Transplant Candidates and Recipients

48

John W. Baddley and Graeme N. Forrest

Contents

Introduction 1155
Disease Epidemiology 1156
Epidemiology and Risk Factors in Solid Organ Transplant 1157
Epidemiology and Risk Factors in Stem Cell Transplant 1158
Clinical Impact on Transplant 1158
Diagnosis 1160
Management 1165
Emerging Treatment Modalities 1169
Prevention and Control 1170
Conclusion 1172
Key Points 1173
References 1173

Introduction

Cryptococcus species are encapsulated yeasts responsible for a broad range of infections. There are two major species within the cryptococcal genus, *Cryptococcus neoformans* and *Cryptococcus gattii* [1]. These vary with regard to geographic distribution, populations affected, disease manifestations, and severity [1, 2]. Pulmonary and central nervous system (CNS) diseases are well-known presentations of cryptococcosis, with meningoencephalitis the most frequent and serious manifestation, especially in SOT and HSCT recipients [3]. Infection less frequently involves

J. W. Baddley (✉)
University of Maryland, Institute of Human Virology, Baltimore, MD, USA
e-mail: jbaddley@ihv.umaryland.edu

G. N. Forrest
VA Portland Healthcare System, Portland, OR, USA
e-mail: Graeme.forrest2@va.gov

© This is a U.S. Government work and not under copyright protection in the U.S.; foreign copyright protection may apply 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_47

other sites such as the blood, skin, skeletal system, and prostate [4]. Diagnosis is made with antigen testing from serum or cerebrospinal fluid or culture or histopathology from infected tissues. Treatment of cryptococcosis is based on anatomic site of disease, severity of disease, and the underlying immune status of the patient. Cryptococcal meningitis is treated with induction therapy of an amphotericin B formulation and flucytosine, followed by a prolonged course of fluconazole. For pulmonary disease alone that is less severe, fluconazole for a minimum of 6 months is effective therapy in most patients. Chronic maintenance therapy with fluconazole may be required in transplant patients who remain immunosuppressed and at risk for relapse.

Disease Epidemiology

More than 30 species of the genus *Cryptococcus* have been described, but few are recognized as causing infection in humans [5]. Infection is caused primarily by two species, C*ryptococcus neoformans* and *Cryptococcus gattii*. *C. neoformans* is a fungus that is ubiquitous in the environment. It was isolated initially in nature from peach juice in 1894 by Francisco Sanefelice and was first isolated from soil by Emmons in 1951 [6]. *C. neoformans* has since been isolated from multiple geographic sites worldwide, many of which are contaminated by pigeon or other bird excrement [7]. *C. neoformans* has been isolated less commonly from other sources including fruits and vegetables, decaying wood, dairy products, and excrement from a wide variety of avian species [7].

Cryptococcus gattii, previously regarded as being limited to primarily tropical and subtropical regions, has recently been described throughout the world with the emergence of *C. gattii* infection in Vancouver Island and British Columbia, Canada, and in the Pacific Northwest of the United States since the late 1990s [8–15]. Ecologically, *C. gattii* is found in soil and in association with eucalyptus trees and in other tree species native to biogeoclimatic zones in British Columbia and the Pacific Northwest [1, 16, 17]. With more awareness and use of molecular testing, *C. gattii* is increasingly been identified in areas around the world, with marked genotypic diversity [18].

C. neoformans and *C. gattii* were first distinguished by their antigenic diversity according to serotypes based on structural differences of the polysaccharide capsule: *C. neoformans* strains are classified as serotypes A and D and *C. gattii* strains classified as serotypes B and C [5]. Molecular testing methods have confirmed great diversity within the *C gattii-C neoformans* complex, and new taxonomy has been proposed: *C. neoformans*, divided into *C. neoformans* (serotype A, formerly *C. neoformans* var. *grubii*), and *C. deneoformans* (serotype D, *C. neoformans* var. *neoformans*); *C. gattii* would be recognized as five separate species, namely, *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGIII), *C. tetragattii* (VGIV), and *C. decagattii* (VGIV) [19]. Several other cryptococcal species, *C. albidus* and *C. laurentii*, have been reported to rarely cause disease in humans [20]. Other rare

cryptococcal species that can cause human infection and may be encountered in SOT recipients include *C. albidus* and *C. laurentii* and may only be diagnosed on culture.

In the majority of cases of cryptococcosis, initial infection occurs by inhalation of the organism from an environmental source. Disease typically results from reactivation of dormant infection [21, 22]. Local inoculation can cause skin infection, but skin lesions most often result from disseminated infection from an initial lung source [23, 24]. Presumed human-to-human transmission has been described: one case involved corneal transplantation and another resulted from inoculation of blood from a patient with cryptococcemia [25, 26].

There have been a number of presumed donor-to-donor transmissions of cryptococcosis via solid organs with disease manifestations presenting up to 3 months post-transplant [27–29]. Donor-derived cryptococcosis should be considered when the diagnosis occurs in the recipient within 30 days of transplant, when cryptococcosis is diagnosed in more than one organ recipient from a single donor, or if *Cryptococcus* is documented at the surgical or graft site [3, 27].

Evidence supporting zoonotic transmission of *Cryptococcus* has also been reported [30–32]. In one description, a clinical isolate from a renal transplant recipient with cryptococcal meningitis was indistinguishable on the basis of molecular genotyping from an isolate present in the feces of the patient's pet cockatoo [30].

Epidemiology and Risk Factors in Solid Organ Transplant

Cryptococcosis is the third most common invasive fungal infection in solid organ transplant recipients, less frequent than candidiasis and aspergillosis [33]. Typically, the overall incidence of cryptococcosis in SOT recipients ranges from 0.2% to 5%, and cryptococcosis comprises 8% of all fungal infections [34, 35]. Cryptococcosis is typically a late-occurring infection; with the median time to onset post-transplant ranging from 16 to 21 months [35]. The time to onset is typically earlier (<12 months) for liver and lung transplants, when compared to kidney transplant recipients [36].

Immunosuppression is an important risk factor for cryptococcosis. Among patients without HIV infection, corticosteroid use is associated with an increased risk of cryptococcosis [37–39]. For SOT patients, it remains unclear what dose is related to increased risk [40]. Other immune suppressants such as alemtuzumab and antithymocyte globulin are also associated with increased risk of cryptococcosis [41, 42]. Calcineurin inhibitors (CNI) do not appear to increase the risk of cryptococcosis, but may affect outcomes in patients with cryptococcosis [43, 44]. Receiving a CNI-based regimen was associated with lower odds of disseminated cryptococcal disease when compared to a non-calcineurin-based regimen [44]. This finding may be related to the anti-cryptococcal activity of CNIs, which target the fungal homologs of calcineurin [44, 45].

Additional independent risk factors for cryptococcosis in SOT patients include older age, diabetes, and lung transplant (compared to other SOT types) [36].

Epidemiology and Risk Factors in Stem Cell Transplant

Cryptococcosis is an uncommon infection among HSCT recipients, with fewer than 15 cases reported [34, 35, 46, 47]. The decreased incidence in this population, in contrast to SOT patients, may be related to the widespread use of azole antifungal prophylaxis or differences in immunosuppression [34]. To date, infections by *C. neoformans*, *C. albidus*, *C. laurentii*, and *C. adeliensis* have been reported [34]. The timing of cryptococcosis has ranged from pre-transplant to 6 months post-transplant [34, 47]. Cases have been more common among recipients of autologous stem cell transplants, possibly related to a dominant T-helper 2 phenotype making autologous transplant patients more susceptible [34]. In most cases, disseminated disease (brain, blood, skin) was present.

Clinical Impact on Transplant

The symptoms and signs of cryptococcal infection among transplant patients are typically non-specific; they can be insidious or severe. In patients with pulmonary infection, disease manifestations range from colonization to asymptomatic infection to severe pneumonia with fulminant respiratory failure and acute respiratory distress syndrome (ARDS) [48–50]. However, the diagnosis of colonization in the transplant patient should be interpreted with caution. Because of the propensity of *Cryptococcus* for dissemination to the central nervous system (CNS), a thorough evaluation for extrapulmonary sites of cryptococcal involvement in the transplant patient is recommended [3, 4].

Symptoms of pulmonary cryptococcosis are non-specific and may include fever, chills, cough, malaise, night sweats, dyspnea, weight loss, and, rarely, hemoptysis. Radiographic features are varied and may reveal single or multiple nodular lesions, patchy consolidations, interstitial infiltrates, mass lesions, mediastinal or hilar adenopathy, or, less commonly, pleural effusions [48, 51–53]. Because of these non-specific radiographic findings, it is important that the differential for causative agents is broad. Radiographically, *C. gattii* pulmonary infection in HIV-negative patients may be associated with mass-like lesions that may be mistaken for malignancy [54].

The most common clinical manifestation of cryptococcosis in transplant patients is CNS infection, manifested typically as meningitis, which can be subacute or chronic in nature [55]. A wide range of symptoms and signs can be present with CNS cryptococcosis, but complaints are often mild or non-specific and include headache, nausea and vomiting, malaise, altered mental status, and fevers. Unlike in bacterial meningitis, meningismus is uncommon. Cranial nerve dysfunction may result from increased intracranial pressure, cryptococcal invasion of cranial nerves, or brain parenchymal lesions (cryptococcomas) [5]. Seizures, often a reflection of increased intracranial pressure or focal mass lesions, tend to occur later in the course of disease [56, 57].

Increased intracranial pressure associated with cryptococcal meningitis a prominent finding and causes increased morbidity and mortality [58]. Cryptococci may cause outflow obstruction by blocking passage of CSF across arachnoid villi. In addition, soluble cryptococcal capsular polysaccharide may accumulate in arachnoid villi, leading to alterations in CSF drainage, with subsequent elevation of intracranial pressure potentially leading to hydrocephalus, blindness, deafness, or death [58, 59]. An opening CSF pressure of >250 mm water is found in approximately half of transplant patients. Consequently, routine assessment of suspected cryptococcal meningitis should always include manometry [3, 4]. Brain CT imaging is also important to evaluate for hydrocephalus related to elevated ICP or potential mass lesions (cryptococcomas); however, it has suboptimal sensitivity for evaluating cryptococcomas when compared to MRI. In a recent study evaluating neuroimaging findings in transplant patients with cryptococcosis, outcomes tended to be worse in patients with parenchymal lesions when compared with meningitis or hydrocephalus [55]. Cerebral cryptococcomas are more common in patients with *C. gattii* infection than in patients with *C. neoformans* infection [13, 60].

Other sites where cryptococcal lesions may occur include the skin and soft tissues, prostate gland, liver, kidney, bones, and joints [61, 62]. Cryptococcal skin lesions are seen in up to 10% of transplant patients with cryptococcosis [63, 64]. Disease may manifest as a variety of skin lesions, including pustules, papules, purpura, ulcers, cellulitis, superficial granulomas or plaques, abscesses, and sinus tracts. The majority of lesions are found on the lower extremities and often associated with CNS disease [64]. While cutaneous lesions largely represent hematogenous dissemination in transplant patients, the skin may also be a portal of entry for *Cryptococcus* and a potential source of subsequent disseminated disease in transplant recipients [24].

Cryptococcosis in transplant patients is usually disseminated (extrapulmonary) at time of diagnosis. The majority of patients have meningitis and pulmonary or extrapulmonary infection [43, 44, 65]. In a recent observational study of SOT patients, 61% had disseminated disease: 54% pulmonary and 8.1% skin, soft tissue, or osteoarticular disease [44]. Among SOT organ types, liver transplant recipients were at increased risk for developing disseminated disease. Cryptococcemia is also common among SOT patients, found in up to a third of this patient group [43, 44, 66]. Patients with CNS disease are more likely to be fungemic than those without CNS disease [55].

In the current era, mortality in SOT patients with cryptococcosis ranges from 5% to 30% [34, 36, 67]. In contrast, higher mortality, up to 70%, has been seen in patients infected with *C. gattii* [60]. Prognostic factors for cryptococcal meningitis have been characterized for various patient groups [5, 66, 68]. In general, these include the underlying disease or predisposing condition, the burden of organisms, titers of CSF cryptococcal antigen, mental status at baseline, and the ability to mount an inflammatory response in CSF. Among SOT patients, one study identified altered mental status, absence of headache, and liver failure as predictors of mortality [66]. Among patients living with HIV, lack of inflammation on CSF examination, low CSF glucose, and high cryptococcal antigen are associated with increased

mortality [69]. A factor related to lower mortality is receipt of a calcineurin inhibitor, which may be attributable in part to their synergistic interactions with antifungal agents [44, 70].

Diagnosis

When diagnosing cryptococcosis, it is important to evaluate for site and extent of infection, which may help to inform choice of antifungal agent and duration of therapy [4, 71]. For diagnosing pulmonary cryptococcosis, detection of the organism can be made from tissue biopsy specimens or bronchoalveolar lavage fluid [72]. On routine hematoxylin and eosin staining of lung and other tissues, *C. neoformans* is difficult to identify. Gomori methenamine silver (GMS) or periodic acid-Schiff (PAS) staining allow for identification; the organism can be recognized by its oval shape and narrow-based budding [73]. Mucicarmine staining, which stains the capsule rose to burgundy in color, can help to differentiate *C. neoformans* from *Blastomyces dermatitidis* and *Histoplasma capsulatum* [73]. Prostate or kidney infection may be diagnosed by yeast in the urine, and clinical suspicion is needed to make this diagnosis [61]. Biopsies with histopathology culture of other tissues will confirm the diagnosis of cryptococcosis.

Transplant patients with suspected or proven cryptococcosis should undergo a thorough evaluation for extrapulmonary sites of infection, including a lumbar puncture and cultures of blood, urine, or other tissues, as indicated by clinical signs and symptoms. Blood cultures for *Cryptococcus* may be helpful, as they are positive in up to 45% of patients with meningitis [55]. For evaluation of suspected CNS infection, opening pressure should be determined and CSF samples tested for Gram's and fungal stains, cell count, glucose, protein, fungal culture, and cryptococcal antigen. Culture of the organism will allow for species identification (*C. neoformans* or *C. gattii*); susceptibility testing can be performed if indicated. In research settings, quantitative CSF cultures have been utilized which provide valuable clinical information for determining treatment outcomes [74]. The CSF quantitative culture is a simple technique using a 100 μL input volume of CSF with five 1:10 serial dilutions in water, plating on Sabouraud dextrose agar, and then quantitative culture counting on the plate with the least growth [74]. The change in quantitative culture growth with subsequent serial lumbar punctures gives clinicians valuable feedback on the rate of CSF *Cryptococcus* clearance and when the CSF may be sterilized with treatment.

India ink staining is an inexpensive, rapid method to detect *Cryptococcus* in the CSF [75]. The stain is not taken up by the thick *Cryptococcus* capsule, forming a halo of light by which it can be visualized using a light microscope [75]. However, the sensitivity is not ideal, up to 80% [76]. In patients with early disease and a lower burden of infection (*Cryptococcus* CFU values $<$1,000 per ml of CSF), sensitivity is less than 50% [76].

Cryptococcal antigen testing from CSF and serum has become the preferred method of diagnosis in transplant patients [4]. Latex agglutination (LA) testing

and the lateral flow assay (LFA) are currently available. The LA test for diagnosis of cryptococcal meningitis is sensitive and specific (>90%), but less sensitive for diagnosing *C gattii* infection [13, 60, 77]. Typically, titers using LA are higher with leptomeningeal than intraparenchymal brain lesions, but lower in SOT recipients when compared to patients living with HIV [66]. The LA test requires additional laboratory time and is less useful in resource limited areas [77]. In contrast, the LFA is a point-of-care test that requires no specimen preparation. LFA is more sensitive to polysaccharide across the four cryptococcal serotypes than the LA test. However, this increased sensitivity may lead to false-positive results in patients with low titers (1:2), so clinical correlation is important [78]. Given this and its low cost, LFA is the preferred method [76, 79]. It is important to note that a prozone effect may occur in high cryptococcal burden states [80, 81]. High serum cryptococcal antigen titers are typically present in patients with disseminated and/or CNS disease and lower in patients with isolated pulmonary disease [53]. Cryptococcal antigen testing to evaluate for pulmonary disease is still useful, as there is up to 83% sensitivity of cryptococcal antigen testing in SOT patients with pulmonary involvement [53].

The identification of cryptococcal species (*C. neoformans* vs. *C. gattii*) has become increasingly important, as it may affect choice of antifungal therapy and perhaps outcomes [18, 60, 82]. Currently histopathologic methods and cryptococcal antigen testing are not sufficient to distinguish *C. neoformans* and *C. gattii*. Other options such as canavanine glycine bromothymol blue (CGB) agar, PCR, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or loop-mediated isothermal DNA amplification, high resolution melt analysis, or multilocus sequence typing will determine the correct species but may be available only in specialty laboratories [13, 83–86].

The FilmArray ME panel (Biomerieux, North Carolina), a multiplex PCR test, is approved for the rapid diagnosis of *C. neoformans* and C. *gattii* from CSF. The test can detect up to 20 other viral and bacterial targets and the turnaround time is 1 h [87]. However, there have been concerns about the accuracy of this test in clinical specimens [88]. For example, the FilmArray ME panel demonstrated an overall percent positive agreement (PPA) of only 52% (26/50) for *Cryptococcus neoformans/C. gattii.* The percent positive agreement of the FilmArray assay for *C. neoformans/C. gattii* was 92.3% (12/13) when the results were compared directly to the results of routine fungal smear or culture from initial presentation [88]. The FilmArray ME test for detection of *Cryptococcus* is best when organisms are seen on CSF; however, this is slower and far more expensive than the LFA (Table 1). The concern is that the FilmArray may not be as sensitive when no organisms are present. Even with antifungal therapy, PCR should still detect remnants of cryptococcal organisms [89]. Therefore, a negative PCR should not preclude performing an LFA simultaneously to exclude cryptococcal meningitis [90].

Another multiplex PCR testing panel is the YEAST panel, which targets 21 clinically important fungal organisms [91]. Although not commercially available, a study using reference strains showed that multiplex PCR was 100% specific in identifying *Cryptococcus* [91].

Table 1 Summary of diagnostic tests for *Cryptococcus*

Test	CSF	Serum	*C. gattii*	*C. neoformans*	Turnaround time	Cost	Others	Comments
Approved								
Culture	+	+	+	+	3–14 days	+	CGB test for *C. gattii* identification	Useful for susceptibility testing and species identification
Quantitative culture counting	+	–	+	+	Minutes	+	Good for monitoring treatment responses	Mostly being used in clinical trials but being proposed as clinical marker
Mucicarmine	–	–	+	+	1–2 days	+		Only from tissue
India ink	+	+	–	–	Minutes	+		Low sensitivity, may miss diagnosis in 14% of cases or in low burden infections
Latex agglutination	+	+	+/–	+	1–2 hours	+	Good for following treatment responses	False negatives if high CSF protein or elevated RF in serum. Less sensitive with *C. gattii* infections
Lateral flow assay	+	+	+	+	5 minutes	+	Useful for following treatment response	False positives at low titers. Caution with prozone effect in high burden disease
Multiplex PCR	+	–	+	+	30 minutes	+++ +	Best for diagnosis and not following treatment response	Concern for false negatives, especially if antifungal therapy started

MALDI-MS	+	+	+	+	20 minutes[a]	+		Needs cultures currently which delays diagnosis. Equipment is expensive, but test is cheap
Beta-D-glucan	–	+	+	+	1–2 days[b]	+++		Does not speciate. May be useful for following treatment response. Non-specific
Experimental								
Next-generation sequencing	+	+	+	+	1–2 days	+++ +		Accurate, good for typing and exploring outbreaks
Flow cytometry	+	–	–	–	1–2 days	+++ +		For quantifying burden of disease

Cost: + = <$5; ++ = $6–20; +++ = >$21 and <$100; ++++ = $101–1500

Abbreviations: *CGB* canavanine, glycine, and bromothymol blue, *CSF* cerebrospinal fluid, *RF* rheumatoid factor, *PCR* polymerase chain reaction, *MALDI-MS* matrix-assisted laser desorption/ionization time of flight mass spectrometry

[a]20 minutes is after colony available on culture

[b]Depends whether available locally or sent to specialty laboratory

The other role of PCR is the rapid differentiation of *C. neoformans* from *C. gattii*. Given that CBG agar testing could take some time, the rapidity of real-time PCR could enhance clinical and epidemiological decisions. Currently most testing is available from specialty laboratories [92]. Tavares and colleagues targeted the intergenic spacer 1 (IGS1) region from rDNA locus of *Cryptococcus* with their primers. The minimum limit of detection of both primers was 100 plasmid copies per reaction, and they were highly specific and accurate for each species [92].

MALDI-MS can detect directly from culture organisms within 20 min. Currently, advanced panels can distinguish between *C. gattii* and *C. neoformans*. In a retrospective study of cryptococcal isolates, MALDI-MS was able to identify seven of the eight organisms; however, the one missed was a *Cryptococcus curvatus* isolate, which is currently not in the MALDI-MS database and rarely a human pathogen [86]. Next-generation sequencing (NGS) is able to sequence thousands of organisms in parallel, including having the ability to combine many samples in a single sequencing run and obtain high sequence coverage per sample. NGS-based metagenomic sequencing can detect very low-abundance members of the microbial community that may be missed or are too expensive to identify using other methods. The NGS has a large database that can utilize 150/150 base pair paired-end sequencing to identify small quantities of organisms [93]. The benefit of NGS could be to detect early cryptococcal infection from blood or CSF specimens before infection is evident [93]. Also, when immune reconstitution syndrome occurs, a negative NGS result could help resolve whether to continue antifungal therapy or manage with corticosteroids [93]. It is important to note that these data have not yet been applied to transplant patients. Flow cytometry of the CSF has been used in HIV patients as a way to determine the fungal burden of infection and clinical response to treatment [94].

Serum testing for beta-D-glucan (BDG) has not been considered a diagnostic tool for cryptococcal infections, as early data suggested that it was not expressed in cryptococcal cell walls. However, among HIV-infected Ugandan and South African patients with cryptococcal meningitis, BDG concentrations in the CSF showed 89% sensitivity and 85% specificity from 117 diagnostic CSF specimens [95]. Moreover, CSF concentrations of BDG >500 pg/mL were associated with three times increased risk of 10-week mortality [95]. BDG seems to rapidly normalize with clearance of infection and may be more useful in monitoring treatment response or distinguishing paradoxical IRIS from a relapse of cryptococcal meningitis [95].

Antifungal susceptibility testing of *C. neoformans* has been standardized by the US Clinical and Laboratory Standards Institute (CLSI), but the relationship of MIC to clinical outcomes is not yet validated [96]. The routine use of antifungal susceptibility testing for cryptococcal infection is not recommended. In patients with *C. gattii* infection where genotypes have been associated with increased fluconazole MIC, for those with *C. neoformans* in whom primary therapy has failed or who have relapsed infection, or in patients with cryptococcal infection and recent antifungal exposure (i.e., fluconazole prophylaxis), antifungal susceptibility testing for fluconazole, at a minimum, is recommended [4, 60, 97–99].

Management

Data that inform current treatment guidelines for cryptococcosis in transplant patients are extrapolated from clinical trials among HIV patients and data collected retrospectively from other patient cohorts [3, 4]. The treatment of cryptococcosis is based on site of involvement, severity of illness, and the underlying immunologic status of the patient (Table 2). For patients with CNS disease, the aims of therapy are to eradicate or control the infection, adequately manage elevated intracranial pressure, and prevent long-term neurologic sequelae. For patients with pulmonary disease, the aims of therapy are to eradicate disease and to prevent dissemination to the CNS. The recommendations herein are consistent with guidelines from the Infectious Diseases Society of America (IDSA) [4].

Studies among HIV-negative patients with cryptococcal meningitis were important in defining the efficacy and safety of combination therapy with amphotericin B and flucytosine and the duration of therapy among immunocompromised patients [100, 101]. Combination therapy resulted in higher rates of cure, fewer relapses, and more rapid sterilization of CSF. Treatment recommendations for HIV-negative patients with CNS cryptococcal disease have been adapted from results of more recent studies in patients living with HIV [102, 103]. Specific issues addressed by these studies include use of higher doses of amphotericin B (0.5–1.0 mg/kg/day); use of lipid amphotericin B formulations in patients with renal insufficiency; and treatment with an "induction" regimen of amphotericin B plus 5-flucytosine for

Table 2 Treatment recommendations in transplant patients

CNS, disseminated disease, or moderate to severe pulmonary disease	
Induction	Duration of therapy
Preferred therapy	
Liposomal amphotericin B 3–4 mg/kg/day or amphotericin B lipid complex 5 mg/kg/day plus 5-flucytosine 100 mg/kg/day[a]	Minimum of 2 weeks
Alternative therapy	
Liposomal amphotericin B 3–4 mg/kg/day, or amphotericin B lipid complex 5 mg/kg/day	Minimum of 4–6 weeks
Consolidation	
Fluconazole 400–800 mg/day	8 weeks
Maintenance	
Fluconazole 200–400 mg/day	Minimum of 6–12 months
Pulmonary disease	
Asymptomatic or mild to moderate disease	
Fluconazole 400 mg/day	6–12 months
Severe pulmonary disease, or azole use not an option Same as for CNS disease, above	

[a]Dosages of flucytosine and fluconazole outlined above are in the absence of renal insufficiency. Both require dose reduction for renal insufficiency. Monitoring of flucytosine levels is recommended

2 weeks followed by a "consolidation" regimen with fluconazole for an additional 8–12 weeks [4].

In transplant patients with CNS disease, disseminated disease, or moderately severe to severe pulmonary disease, fungicidal therapy with a lipid formulation of amphotericin B and 5-flucytosine is recommended (Table 2) [4, 104]. The use of a lipid formulation of amphotericin B is preferred over amphotericin B deoxycholate due to decreased nephrotoxicity. Additionally, mortality at 90 days in SOT recipients with CNS cryptococcosis was lower with the use of lipid formulations of amphotericin B when compared to amphotericin B deoxycholate [105]. A lack of 5-flucytosine as induction therapy is an independent risk factor for mycologic failure at week 2 in SOT patients [55, 106, 107]. To avoid adverse effects of flucytosine, including bone marrow suppression and nephrotoxicity, monitoring and maintenance of flucytosine levels (2-h post-dose level of 30–80 μg/mL) are recommended [4, 96, 106].

The recommendation for the management of neurological disease, disseminated cryptococcosis, or moderately severe to severe pulmonary disease is at least 2 weeks of induction therapy with liposomal amphotericin B (3–4 mg/kg/day) or amphotericin B lipid complex (5 mg/kg/day) plus flucytosine (100 mg/kg/day in four equally divided doses every 6 h, adjusted for creatinine clearance). This should be followed by 8 weeks of consolidation therapy with fluconazole (400–800 mg/day). Once induction treatment is completed, 6–12 months of maintenance therapy with fluconazole (200–400 mg/day) is recommended (Table 1). The duration of induction therapy should be extended in patients who are clinically deteriorating, those who fail to improve and have persistently elevated intracranial pressure, those who remain comatose, and those with persistently positive CSF cultures [8]. Fluconazole maintenance therapy may need to be extended in selected patients based on clinical progress or net state of immunosuppression.

Cryptococcal infection of the brain causes a significant inflammatory response resulting in elevations in intracranial pressure that may lead to hydrocephalus, loss of hearing or vision, or death [59]. Raised intracranial pressure (ICP) is a significant factor linked to patient morbidity [108]. Approximately 50–70% of patients with cryptococcal meningitis have elevated ICP [109]. Among HIV patients, therapeutic lumbar punctures were associated with 69% relative survival protection [110]. Lumbar puncture is needed to identify CNS disease and elevated ICP, with CSF samples sent for appropriate diagnostic studies. Repeat lumbar puncture after 2 weeks of induction therapy is helpful to confirm organism clearance with antifungal therapy. Sterile cultures at this time are associated with favorable outcomes in HIV patients [4, 109, 110]. In cases with CSF pressure $\geq$25 cm of CSF or symptoms of increased ICP during induction therapy, serial lumbar punctures should be performed as necessary, with a goal of reducing the CSF pressure by 50% if elevated, or to a normal pressure (<20 cm of CSF). If ICP remains high (>25 mmHg) and symptoms persist, placement of temporary lumbo-peritoneal or external ventricular drains to normalize and monitor CSF pressure should be considered [4, 110, 111]. Permanent ventriculoperitoneal shunting may be indicated if other conservative measures to reduce ICP have failed [4, 111].

Fluconazole 400 mg/day for 6–12 months is recommended in asymptomatic transplant patients and patients with mild to moderate pulmonary disease (Table 2) [3, 4]. Patients with moderate to severe pulmonary disease should be treated similarly to patients with CNS or disseminated infection [4]. Extrapulmonary disease should always be excluded [4, 43]. Positive *C. neoformans* cultures from sterile and non-sterile sites (i.e., sputum) warrant treatment even if the patient is asymptomatic. This is true in lung transplant recipients where *Cryptococcus* may be colonizing the donor allograft; without treatment, infection may become invasive due to post-transplant immunosuppressive therapy [112]. After 6 months of fluconazole therapy, relapse of cryptococcosis is uncommon; therefore the typical duration of therapy is 6–12 months [113]. However, the decision to stop treatment must be based on clinical signs and symptoms, resolution of radiographic abnormalities, and the net state of immunosuppression. Extended treatment with fluconazole is safe in transplant recipients with careful monitoring of drug-drug interactions between fluconazole and calcineurin inhibitors and attention to changes in the QTc interval [114].

Extended-spectrum azoles such as itraconazole, voriconazole, posaconazole, and isavuconazonium offer no advantages over fluconazole for the treatment of *C. neoformans*. These agents are generally more costly and less safe and demonstrate more potential drug interactions with immunosuppressive agents. Itraconazole has been shown to be inferior to fluconazole in the treatment of HIV-associated cryptococcosis [115]. Case reports and case series evaluating voriconazole, posaconazole, and isavuconazonium for treatment of cryptococcosis have been published [116, 117]. Though in most cases these drugs are effective, their role in treatment is unclear, other than an alternative when fluconazole cannot be used. These newer azoles may be useful in the treatment of *C. gattii* genotypes with reduced susceptibility to fluconazole. In vitro data demonstrate their potency against *C. gattii* and suggest that extended-spectrum azoles may offer an oral alternative in transitioning from induction to maintenance therapy [60, 97]. Tacrolimus and sirolimus levels must be closely monitored with co-administration of azoles; dose reduction of these immunosuppressants may be appropriate at the time of azole initiation and discontinuation [118, 119].

The utility of dexamethasone as adjunctive therapy for the management of acute cryptococcal meningitis is not established. In a large randomized controlled trial of cryptococcal meningitis in HIV-infected patients, addition of dexamethasone to standard therapy resulted in slower clearance of the CSF, an increase in serious infections, and no mortality impact compared to placebo [120]. However, clinical data are lacking in transplant recipients and some data are suggestive of clinical benefit after infection clearance [121]. Sertraline, an antidepressant with in vitro activity against *Cryptococcus*, demonstrated increased fungal clearance in one clinical study, though the benefit to SOT patients is unclear [122–124].

Infection management in transplant recipients requires attention to the net state of immunosuppression. Whenever possible, immunosuppression should be reduced during therapy, though this may not be feasible in patients who previously received T cell-depleting agents such as alemtuzumab and thymoglobulin [125]. Ideally, a gradual tapering of immunosuppression can occur while on antifungal therapy,

Table 3 Criteria for diagnosis of IRIS in patients with cryptococcosis

1. New or worsening appearance of any of the following manifestations: a) CNS: clinical or radiographic manifestations consistent with inflammatory process, such as contrast-enhancing lesions on neuroimaging studies; CSF pleocytosis, defined as >5 white blood cells; or increased intracranial pressure, i.e., opening pressure ≥20 mm of water (with or without hydrocephalus) b) Lymph nodes, skin or soft tissue lesions c) Pulmonary lesions detected by chest radiography or CT d) Other focal tissue involvement with histopathology showing granulomatous lesions
2. Symptoms occurring during receipt of appropriate antifungal therapy and could not be explained by a newly acquired infection
3. Negative results of cultures for *C. neoformans* during the diagnostic workup

resulting in eradication of infection with preservation of allograft function. Rapid reductions in immunosuppression may precipitate organ rejection or emergence of immune reconstitution inflammatory syndrome (IRIS). Optimal immunosuppression reduction protocols are not yet established [126].

Restoration of host immunity, particularly if abrupt, may result in adverse outcomes including symptomatic immune reconstitution [127]. The rapid reduction of immunosuppressive therapy in conjunction with initiation of antifungal therapy in transplant recipients may lead to the development of IRIS, with clinical manifestations mimicking worsening cryptococcal disease (Table 3) [62, 113]. IRIS may manifest in many ways, including lymphadenitis, cellulitis, aseptic meningitis, cerebral mass lesions, hydrocephalus, and new pulmonary nodules [62, 113]. Clinically, CNS IRIS appears to have less inflammation and has been found on lumbar puncture to be associated with protein levels ≤50 mg/dL and less than 25 white cells/μL [128]. In evaluating patients living with HIV with IRIS after starting antiretroviral therapy, there was lower interferon gamma (IFN-γ), interleukin (IL-10), and CXCL10 production in patients with higher mortality [129]. These chemokines might enhance T and myeloid cell trafficking into the CNS, resulting in the excessive TH1-type immune responses seen during IRIS [130].

Immunosuppressive agents administered to transplant recipients such as calcineurin inhibitors and corticosteroids exert their effect by preferentially inhibiting Th1 (IL-2 and IFN-α) compared to Th2 (IL-10) responses [131, 132]. Tacrolimus inhibits Th1 to a greater extent than cyclosporine A [133, 134]. The biologic basis of IRIS in SOT recipients is believed to be reversal of a Th2 to Th1 pro-inflammatory response upon withdrawal or reduction of immunosuppression. A potential role of Tregs and Th17 regulatory pathways in the pathogenesis of IRIS in SOT recipients has also been proposed [135]. Potent T cell lymphocyte-depleting agents such as alemtuzumab have also been recognized as a risk factor for IRIS [136].

In SOT recipients with cryptococcosis, IRIS is relatively uncommon, with an incidence of 5–12% [137]. Risk factors independently associated with IRIS are CNS disease and discontinuation of the calcineurin inhibitor [137]. Typically the onset of IRIS is between 4 and 6 weeks after initiation of antifungal therapy [113]. The impact of IRIS on allograft function is just as important with high rates

of graft rejection and allograft failure [137, 138]. Clinically, IRIS in an SOT recipient may present with worsening of clinical symptoms, especially headache. The LP may have elevated opening pressure and white cells, but no growth on cultures and a low level cryptococcal antigenemia. It is a diagnosis of exclusion of relapsed or progressive disease [5]. Currently there are no laboratory markers or clinical criteria that can diagnose IRIS reliably, but exclusion of recurrent or worsening cryptococcosis is important before treatment [126].

There is no proven therapy for IRIS associated with cryptococcosis. Modifications in antifungal therapy are typically not warranted unless viable yeasts are documented in culture. Corticosteroids have been employed anecdotally with success in *Cryptococcus*-associated IRIS in SOT recipients [62, 137]. Corticosteroids in doses equivalent to 0.5–1 mg/kg of prednisone may be considered for major complications related to inflammation in the CNS or severe manifestations of pulmonary or other sites [62]. The efficacy of thalidomide and nonsteroidal anti-inflammatory agents remains unproven [139].

Emerging Treatment Modalities

There is ongoing work to develop new strategies using currently available and approved antifungal agents and to identify more specific anti-cryptococcal drugs based on their ability to neutralize the organism. Current treatment regimens (Table 2) have proven efficacy but are limited by toxicity to both the transplanted organ and patient as well as by cost. Although 5-FC has been available for over 60 years, it has become cost prohibitive in many regions. In addition, lipid formulations of amphotericin B are expensive, as is laboratory testing to monitor safety.

There are several ongoing studies in HIV-infected patients with cryptococcal meningitis that use alternative treatment strategies with amphotericin B, fluconazole, and 5-FC. The AMBITION-CM study (Trial Registration: ISRCTN, ISRCTN72509687) is an open-label, phase 3 randomized controlled non-inferiority trial that is currently being conducted in sub-Saharan Africa [140]. The trial compares cryptococcal meningitis induction therapy with (1) three-dose arm consisting of a single dose (10 mg/kg) of L-AmB given plus 7 days of fluconazole (1200 mg/day) with flucytosine (100 mg/kg/day) to (2) two-dose arm consisting of 7 days of amphotericin B deoxycholate (1 mg/kg/day) with 7 days of flucytosine (100 mg/kg/day). Both groups received 7 days of fluconazole (1200 mg/day) after induction. The primary endpoint is all-cause mortality at 10 weeks, with the study due to close at the end of 2019.

A similar phase 2 study administered short-course, high-dose liposomal amphotericin B in the same setting (Clinical Trial Registration: ISRCTN 10248064). HIV-infected adults with cryptococcal meningitis were randomized to (i) L-AmB 10 mg/kg on day 1 (single dose); (ii) L-AmB 10 mg/kg on day 1 and 5 mg/kg on day 3 (two doses); (iii) L-AmB 10 mg/kg on day 1 and 5 mg/kg on days 3 and 7 (three doses); or (iv) L-AmB 3 mg/kg/day for 14 days (control). All patients received oral fluconazole 1200 mg/day for 14 days. This study looked at early fungal

activity in the CSF of these regimens rather than mortality and showed in a small cohort that single-dose L-amphotericin B with 1200 mg fluconazole was non-inferior. This study is progressing to a phase 3 study [141]. It is unlikely that either of these investigations will be replicated in the transplant populations, but safety and efficacy data could inform alternative treatment strategies in patients not tolerating standard regimens.

Several compounds are in early-stage drug development (Table 4) or have shown efficacy in in vitro or in vivo models. The first is an oral formulation of the standard intravenous therapy. There is an oral encochleated amphotericin B, which is a proprietary cochleate lipid-crystal nanoparticle drug delivery technology. This offers promise of an effective oral antifungal agent for the treatment of cryptococcal meningoencephalitis (Clinicaltrials.gov identifier: NCT03196921).

Punicalagin extracted from *Lafoensia pacari* A. St.-Hil. (Lythraceae) is an ellagitannin from the plant in Brazil, where it has been used for medicinal purposes [142]. In vitro studies show that punicalagin decreased cryptococcal colonies in vitro and was nontoxic. This compound showed promise, but although it slowed growth, it was more fungistatic than fungicidal, suggesting a role as an adjunctive compound rather than primary therapy [142]. VT-1129 is a tetrazole that targets the fungal Cyp51 inhibitor with potent in vitro activity against susceptible and fluconazole-resistant *Cryptococcus* species [143]. VT-1129 acts by inhibiting the fungal cytochrome P450 enzyme Cyp51 (lanosterol 14-α-demethylase), thus inhibiting the biosynthesis of ergosterol in a highly selective manner compared to that of clinically available azole antifungals [143]. This compound in an in vivo study showed that the oral formulation was very potent with persistent clearance of the CSF up to 3 weeks after dosing had stopped; it was compared to fluconazole [143]. The drug has a long half-life (>6 days) and a loading dose achieved high levels in the CSF [144]. Further clinical studies are pending, but this compound offers highly orally bioavailable targeted killing of *Cryptococcus*. It has very few drug interactions which may make it an excellent compound to use in transplant recipients. Lastly, APX001A and its prodrug, APX001, are currently in clinical development for the treatment of cryptococcal meningitis. These compounds target the conserved Gwt1 enzyme required for the localization of glycosylphosphatidylinositol (GPI)-anchored cell wall mannoproteins in fungi [145]. These compounds appear to have synergy, and in vitro studies suggest that the combination with fluconazole can eradicate cryptococcal infections [145]. The development of these new novel and potent compounds with low toxicity and oral bioavailability for the treatment of cryptococcal meningitis is exciting, but clinical study results are at least several years away.

Prevention and Control

Prevention of cryptococcal infection is difficult because *C. neoformans* is ubiquitous in the environment and causes sporadic disease that is relatively uncommon. Primary prophylaxis has been studied in AIDS patients [146, 147]. Although prophylaxis may reduce the frequency of primary cryptococcal disease among those who have CD4

Table 4 New antifungal drugs or synergistic additions to standard antifungal therapies in development

Name	Action	Route	Clinical trial number	Comments
Encochleated amphotericin B	Ergosterol layer	Oral	NCT03196921	Nanoparticle delivery system
Punicalagin	Ellagitannin	Unknown	None	May be adjunctive therapy
VT-1129	Fungal Cyp51 inhibitor – ergosterol	Oral		Potent antifungal activity
APX001A	Target cell wall mannoproteins	Oral		Synergistic with fluconazole
Sertraline	Unknown – possible mTOR	Oral	NCT01802385	Combined with standard therapy. Achieved high CNS levels
Tamoxifen	Unknown – immunomodulation	Oral	NCT03112031	Combined with standard therapy. Fungicidal in vitro with fluconazole and has excellent CNS penetration
Failed or disappeared therapies				
Efungumab (Mycograb)	Heat shock protein 90 inhibitor	IV	NCT00847678	Synergy with amphotericin B in vitro. Study stopped due to poor enrollment
Genaconazole	Ergosterol	Oral/IV	NCT00000677	Not developed
Recombinant interferon-gamma 1b	Increase biologic response converting TH2 to TH1 response	Subcutaneous	NCT00012467	In vivo models only. Closed due to poor enrollment
Lobradimil (RMP-7)	Bradykinin agonist	IV	NCT00002316	Stopped in phase 1. Also did not work with brain cancer

Studies accessed at clinicaltrials.gov as of 10 May 2019
Abbreviations: *NCT* national clinical trial, *IV* intravenous, *mTOR* mammalian target of rapamune, *Cyp* cytochrome P

counts <50 cells/μL, there is little survival benefit [147]. For transplant patients, we do not currently recommend routine antifungal prophylaxis specific for cryptococcosis.

Among patients living with HIV in areas where cryptococcal disease incidence is high, preemptive strategies with use of serum cryptococcal antigen testing to identify asymptomatic infection have been effective at reducing the morbidity and mortality

associated with cryptococcal meningitis [147]. This strategy has not been reported from clinical trials among transplant recipients. Although a positive serum cryptococcal antigen in an SOT recipient correlates with CNS infection, even with use of a highly sensitive assay, a negative screening serum cryptococcal antigen may not exclude CNS disease [57, 148]. If there is a high suspicion for CNS infection, a CSF cryptococcal antigen test is warranted.

In transplant recipients with previous resolved cryptococcosis needing enhanced immunosuppression and at risk for relapse of cryptococcosis, consideration for resuming secondary prophylaxis should be made on an individual case basis. For SOT recipients who experience graft failure with or after cryptococcosis, the ideal timing of re-transplantation is unknown. In kidney transplant recipients, where there is the possibility of a hemodialysis bridge, transplant is reasonable to consider if they have received at least several months of antifungal therapy, have no signs or symptoms attributable to active cryptococcal disease, and have negative cultures from the original site of infection. In SOT populations where no bridging option is available, we prefer that the candidate has completed induction antifungal therapy at a minimum, has no signs or symptoms attributable to active cryptococcal disease, and has negative cultures from the original site of infection.

Because of the rarity of cryptococcosis in the general population, we do not recommend routine screening for cryptococcosis in pretransplant candidates [27]. However, pretransplant candidates with unexplained meningoencephalitis or pulmonary disease and risk factors for cryptococcosis should undergo serum and CSF cryptococcal antigen testing, or further testing such as lumbar puncture or bronchoscopy as indicated by clinical signs and symptoms. For transplant candidates with active cryptococcal disease at the time of evaluation for listing, limited data are available to guide timing of transplant or appropriate candidacy [149]. The recommendations are the same as those for patients with graft failure. In the event that donor cryptococcal disease is discovered or confirmed in the recipient after transplant, fluconazole preemptive therapy is recommended for a minimum of several months and evaluation and monitoring for invasive infection should be performed [27].

Conclusion

Cryptococcosis, mainly caused by *C. neoformans* and *C. gattii*, is the third most common invasive fungal infection in SOT recipients, but is rare among patients with HSCT. The most common manifestations are meningitis and pneumonia. As clinical onset may be insidious and non-specific, headaches, fevers, and mental status changes should warrant diagnostic testing for meningitis. The lateral flow cryptococcal antigen assay of CSF and serum has become an important diagnostic method. Currently, lipid amphotericin B plus 5-flucytosine is used as initial treatment of meningitis, disseminated infection, and moderate to severe pulmonary infection, followed by fluconazole as consolidation therapy. Fluconazole is effective for mild to moderate pulmonary infection. Investigational therapies and newer treatment strategies have shown promise in the treatment of meningitis.

Key Points

- Cryptococcosis is the third most common invasive fungal infection in SOT recipients. It is rare among patients who receive HSCT.
- The most common clinical manifestations of cryptococcosis in transplant patients are CNS infection, typically presenting as meningitis, and pulmonary infection.
- Patients with suspected or proven cryptococcosis should have a thorough evaluation for extrapulmonary disease, including lumbar puncture, blood, and other relevant tissue cultures.
- A lumbar puncture should be performed for diagnosis of CNS disease and evaluation and management of intracranial pressure.
- Cryptococcal antigen testing from blood and CSF is a sensitive test for the diagnosis of cryptococcal meningitis and should be performed using the lateral flow assay over the latex agglutination assay.
- Cryptococcus organisms should be identified to the species level (*C. neoformans* vs. *C. gattii*), as this information may impact treatment choice and duration of therapy.
- Susceptibility testing is recommended for patients who fail primary therapy, have relapse of disease, or develop cryptococcosis with prior antifungal exposure (i.e., fluconazole prophylaxis) or in patients with *C. gattii* genotypes that have been associated with elevated fluconazole MICs.
- A lipid formulation of amphotericin B plus 5-flucytosine is preferred as induction therapy for CNS disease, disseminated disease, or moderate to severe pulmonary disease.
- Fluconazole is preferred for consolidation and maintenance therapy for CNS disease and is the preferred therapy for asymptomatic or mild to moderate pulmonary disease.
- Serial lumbar punctures should be performed for the management of elevated ICP. Temporary or permanent CSF drainage should be considered for in patients where serial lumbar punctures fail to normalize ICP.
- Immune reconstitution syndrome can occur within weeks of start of antifungal therapy and reduced immunosuppression. Exclusion of clinical failure with repeat cultures is warranted before initiating corticosteroid treatment.
- Available data do not support the receipt of antifungal prophylaxis against cryptococcosis in transplant patients.

References

1. Chen SC, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. Clin Microbiol Rev. 2014; 27(4):980–1024.
2. Chen SC, Slavin MA, Heath CH, Playford EG, Byth K, Marriott D, et al. Clinical manifestations of *Cryptococcus gattii* infection: determinants of neurological sequelae and death. Clin Infect Dis. 2012;55(6):789–98.

3. Baddley JW, Forrest GN, AST Infectious Diseases Community of Practice. Cryptococcosis in solid organ transplantation-guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13543.
4. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis. 2010;50(3):291–322.
5. Maziarz EK, Perfect JR. Cryptococcosis. Infect Dis Clin North Am. 2016;30(1):179–206.
6. Emmons CW. Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). Am J Hyg. 1955;62(3):227–32.
7. Levitz SM. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. Rev Infect Dis. 1991;13(6):1163–9.
8. Chambers C, MacDougall L, Li M, Galanis E. Tourism and specific risk areas for *Cryptococcus gattii*, Vancouver Island, Canada. Emerg Infect Dis. 2008;14(11):1781–3.
9. Bartlett KH, Cheng PY, Duncan C, Galanis E, Hoang L, Kidd S, et al. A decade of experience: *Cryptococcus gattii* in British Columbia. Mycopathologia. 2012;173(5–6):311–9.
10. Galanis E, Macdougall L, Kidd S, Morshed M, British Columbia *Cryptococcus gattii* Working Group. Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999–2007. Emerg Infect Dis. 2010;16(2):251–7.
11. MacDougall L, Fyfe M, Romney M, Starr M, Galanis E. Risk factors for *Cryptococcus gattii* infection, British Columbia, Canada. Emerg Infect Dis. 2011;17(2):193–9.
12. Phillips P, Galanis E, MacDougall L, Chong MY, Balshaw R, Cook VJ, et al. Longitudinal clinical findings and outcome among patients with *Cryptococcus gattii* infection in British Columbia. Clin Infect Dis. 2015;60(9):1368–76.
13. Harris JR, Lockhart SR, Debess E, Marsden-Haug N, Goldoft M, Wohrle R, et al. *Cryptococcus gattii* in the United States: clinical aspects of infection with an emerging pathogen. Clin Infect Dis. 2011;53(12):1188–95.
14. Harris JR, Lockhart SR, Sondermeyer G, Vugia DJ, Crist MB, D'Angelo MT, et al. *Cryptococcus gattii* infections in multiple states outside the US Pacific Northwest. Emerg Infect Dis. 2013;19(10):1620–6.
15. DeBess E, Lockhart SR, Iqbal N, Cieslak PR. Isolation of *Cryptococcus gattii* from Oregon soil and tree bark, 2010–2011. BMC Microbiol. 2014;14:323.
16. Kidd SE, Chow Y, Mak S, Bach PJ, Chen H, Hingston AO, et al. Characterization of environmental sources of the human and animal pathogen *Cryptococcus gattii* in British Columbia, Canada, and the Pacific Northwest of the United States. Appl Environ Microbiol. 2007;73(5):1433–43.
17. Kidd SE, Bach PJ, Hingston AO, Mak S, Chow Y, MacDougall L, et al. *Cryptococcus gattii* dispersal mechanisms, British Columbia, Canada. Emerg Infect Dis. 2007;13(1):51–7.
18. Lockhart SR, Iqbal N, Harris JR, Grossman NT, DeBess E, Wohrle R, et al. *Cryptococcus gattii* in the United States: genotypic diversity of human and veterinary isolates. PLoS One. 2013;8(9):e74737.
19. Kwon-Chung KJ, Bennett JE, Wickes BL, Meyer W, Cuomo CA, Wollenburg KR, et al. The case for adopting the "species complex" nomenclature for the etiologic agents of cryptococcosis. mSphere. 2017;2(1).
20. Khawcharoenporn T, Apisarnthanarak A, Mundy LM. Non-neoformans cryptococcal infections: a systematic review. Infection. 2007;35(2):51–8.
21. Saha DC, Goldman DL, Shao X, Casadevall A, Husain S, Limaye AP, et al. Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. Clin Vaccine Immunol. 2007;14(12):1550–4.
22. Brunet K, Alanio A, Lortholary O, Rammaert B. Reactivation of dormant/latent fungal infection. J Infect. 2018;77(6):463–8.
23. Baer S, Baddley JW, Gnann JW, Pappas PG. Cryptococcal disease presenting as necrotizing cellulitis in transplant recipients. Transpl Infect Dis. 2009;11(4):353–8.
24. Neuville S, Dromer F, Morin O, Dupont B, Ronin O, Lortholary O. Primary cutaneous cryptococcosis: a distinct clinical entity. Clin Infect Dis. 2003;36(3):337–47.

25. Beyt BE Jr, Waltman SR. Cryptococcal endophthalmitis after corneal transplantation. N Engl J Med. 1978;298(15):825–6.
26. Glaser JB, Garden A. Inoculation of cryptococcosis without transmission of the acquired immunodeficiency syndrome. N Engl J Med. 1985;313(4):266.
27. Singh N, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, Infectious Diseases Community of Practice(dagger). Am J Transplant. 2012;12(9):2414–28.
28. Sun HY, Alexander BD, Lortholary O, Dromer F, Forrest GN, Lyon GM, et al. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. Clin Infect Dis. 2010;51(9):1062–9.
29. Camargo JF. Donor-derived infections in solid organ transplant recipients: challenging the 30-day paradigm. Transpl Infect Dis. 2017;19(2):e12836.
30. Nosanchuk JD, Shoham S, Fries BC, Shapiro DS, Levitz SM, Casadevall A. Evidence of zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised patient. Ann Intern Med. 2000;132(3):205–8.
31. Lagrou K, Van Eldere J, Keuleers S, Hagen F, Merckx R, Verhaegen J, et al. Zoonotic transmission of *Cryptococcus neoformans* from a magpie to an immunocompetent patient. J Intern Med. 2005;257(4):385–8.
32. Shrestha RK, Stoller JK, Honari G, Procop GW, Gordon SM. Pneumonia due to *Cryptococcus neoformans* in a patient receiving infliximab: possible zoonotic transmission from a pet cockatiel. Respir Care. 2004;49(6):606–8.
33. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.
34. Sun HY, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cell, and tissue transplant recipients: evidence-based evolving trends. Clin Infect Dis. 2009;48(11):1566–76.
35. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. Clin Infect Dis. 2010;50(8):1091–100.
36. George IA, Santos CAQ, Olsen MA, Powderly WG. Epidemiology of cryptococcosis and cryptococcal meningitis in a large retrospective cohort of patients after solid organ transplantation. Open Forum Infect Dis. 2017;4(1):ofx004.
37. Dromer F, Mathoulin S, Dupont B, Laporte A. Epidemiology of cryptococcosis in France: a 9-year survey (1985–1993). French Cryptococcosis Study Group. Clin Infect Dis. 1996; 23(1):82–90.
38. Pappas PG, Perfect JR, Cloud GA, Larsen RA, Pankey GA, Lancaster DJ, et al. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. Clin Infect Dis. 2001;33(5):690–9.
39. Baddley JW, Perfect JR, Oster RA, Larsen RA, Pankey GA, Henderson H, et al. Pulmonary cryptococcosis in patients without HIV infection: factors associated with disseminated disease. EurJ Clin Microbiol Infect Dis. 2008;27(10):937–43.
40. Vilchez RA, Fung J, Kusne S. Cryptococcosis in organ transplant recipients: an overview. Am J Transplant. 2002;2(7):575–80.
41. Silveira FP, Husain S, Kwak EJ, Linden PK, Marcos A, Shapiro R, et al. Cryptococcosis in liver and kidney transplant recipients receiving anti-thymocyte globulin or alemtuzumab. Transpl Infect Dis. 2007;9(1):22–7.
42. Peleg AY, Husain S, Kwak EJ, Silveira FP, Ndirangu M, Tran J, et al. Opportunistic infections in 547 organ transplant recipients receiving alemtuzumab, a humanized monoclonal CD-52 antibody. Clin Infect Dis. 2007;44(2):204–12.
43. Husain S, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. Emerg Infect Dis. 2001; 7(3):375–81.

44. Singh N, Alexander BD, Lortholary O, Dromer F, Gupta KL, John GT, et al. *Cryptococcus neoformans* in organ transplant recipients: impact of calcineurin-inhibitor agents on mortality. J Infect Dis. 2007;195(5):756–64.
45. Gorlach J, Fox DS, Cutler NS, Cox GM, Perfect JR, Heitman J. Identification and characterization of a highly conserved calcineurin binding protein, CBP1/calcipressin, in *Cryptococcus neoformans*. EMBO J. 2000;19(14):3618–29.
46. Park SS, Lee H, Park WS, Hwang SH, Choi SI, Choi MH, et al. A case of disseminated infection with skin manifestation due to non-neoformans and non-gattii *Cryptococcus* in a patient with refractory acute myeloid leukemia. Infect Chemother. 2017;49(2):142–5.
47. Oliver N, Luong T, Tchakarov A, Abdelrahim M, Mulanovich VE, Kontoyiannis DP, et al. Disseminated cryptococcal infection in allogeneic stem cell transplant patients: a rare cause of acute kidney injury. Bone Marrow Transplant. 2016;51(10):1301–4.
48. Brizendine KD, Baddley JW, Pappas PG. Pulmonary cryptococcosis. Semin Respir Crit Care Med. 2011;32(6):727–34.
49. Kerkering TM, Duma RJ, Shadomy S. The evolution of pulmonary cryptococcosis: clinical implications from a study of 41 patients with and without compromising host factors. Ann Intern Med. 1981;94(5):611–6.
50. Perfect JR. The triple threat of cryptococcosis: it's the body site, the strain, and/or the host. mBio. 2012;3(4):e00165–12.
51. Paterson DL, Singh N, Gayowski T, Marino IR. Pulmonary nodules in liver transplant recipients. Medicine (Baltimore). 1998;77(1):50–8.
52. Mueller NJ, Fishman JA. Asymptomatic pulmonary cryptococcosis in solid organ transplantation: report of four cases and review of the literature. Transpl Infect Dis. 2003;5(3):140–3.
53. Singh N, Alexander BD, Lortholary O, Dromer F, Gupta KL, John GT, et al. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. Clin Infect Dis. 2008;46(2):e12–8.
54. McMullan BJ, Sorrell TC, Chen SC. *Cryptococcus gattii* infections: contemporary aspects of epidemiology, clinical manifestations and management of infection. Future Microbiol. 2013;8(12):1613–31.
55. Singh N, Lortholary O, Dromer F, Alexander BD, Gupta KL, John GT, et al. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. Transplantation. 2008;86(5):647–51.
56. Jabbour N, Reyes J, Kusne S, Martin M, Fung J. Cryptococcal meningitis after liver transplantation. Transplantation. 1996;61(1):146–9.
57. Gassiep I, Douglas J, Emeto TI, Crawley K, Playford EG. Cryptococcal infections over a 15 year period at a tertiary facility & impact of guideline management. Mycoses. 2018;61:633.
58. Graybill JR, Sobel J, Saag M, van Der Horst C, Powderly W, Cloud G, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups. Clin Infect Dis. 2000;30(1):47–54.
59. Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, et al. Practice guidelines for the management of cryptococcal disease. Infectious Diseases Society of America. Clin Infect Dis. 2000;30(4):710–8.
60. Forrest GN, Bhalla P, DeBess EE, Winthrop KL, Lockhart SR, Mohammadi J, et al. *Cryptococcus gattii* infection in solid organ transplant recipients: description of Oregon outbreak cases. Transpl Infect Dis. 2015;17(3):467–76.
61. Siddiqui TJ, Zamani T, Parada JP. Primary cryptococcal prostatitis and correlation with serum prostate specific antigen in a renal transplant recipient. J Infect. 2005;51(3):e153–7.
62. Lanternier F, Chandesris MO, Poiree S, Bougnoux ME, Mechai F, Mamzer-Bruneel MF, et al. Cellulitis revealing a cryptococcosis-related immune reconstitution inflammatory syndrome in a renal allograft recipient. Am J Transplant. 2007;7(12):2826–8.
63. Anderson DJ, Schmidt C, Goodman J, Pomeroy C. Cryptococcal disease presenting as cellulitis. Clin Infect Dis. 1992;14(3):666–72.

64. Sun HY, Alexander BD, Lortholary O, Dromer F, Forrest GN, Lyon GM, et al. Cutaneous cryptococcosis in solid organ transplant recipients. Med Mycol. 2010;48(6):785–91.
65. Gassiep I, McDougall D, Douglas J, Francis R, Playford EG. Cryptococcal infections in solid organ transplant recipients over a 15-year period at a state transplant center. Transpl Infect Dis. 2017;19(1):e12639.
66. Wu G, Vilchez RA, Eidelman B, Fung J, Kormos R, Kusne S. Cryptococcal meningitis: an analysis among 5,521 consecutive organ transplant recipients. Transpl Infect Dis. 2002;4(4): 183–8.
67. Lee YC, Wang JT, Sun HY, Chen YC. Comparisons of clinical features and mortality of cryptococcal meningitis between patients with and without human immunodeficiency virus infection. J Microbiol Immunol Infect. 2011;44(5):338–45.
68. Diamond RD, Bennett JE. Prognostic factors in cryptococcal meningitis. A study in 111 cases. Ann Intern Med. 1974;80(2):176–81.
69. Perfect JR, Bicanic T. Cryptococcosis diagnosis and treatment: what do we know now. Fungal Genet Biol. 2015;78:49–54.
70. Kontoyiannis DP, Lewis RE, Alexander BD, Lortholary O, Dromer F, Gupta KL, et al. Calcineurin inhibitor agents interact synergistically with antifungal agents in vitro against *Cryptococcus neoformans* isolates: correlation with outcome in solid organ transplant recipients with cryptococcosis. Antimicrob Agents Chemother. 2008;52(2):735–8.
71. Dromer F, Mathoulin-Pelissier S, Launay O, Lortholary O. Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study. PLoS Med. 2007;4(2):e21.
72. Lortholary O, Nunez H, Brauner MW, Dromer F. Pulmonary cryptococcosis. Semin Respir Crit Care Med. 2004;25:145–57.
73. Gazzoni AF, Severo CB, Salles EF, Severo LC. Histopathology, serology and cultures in the diagnosis of cryptococcosis. Rev Inst Med Trop Sao Paulo. 2009;51(5):255–9.
74. Dyal J, Akampurira A, Rhein J, Morawski BM, Kiggundu R, Nabeta HW, et al. Reproducibility of CSF quantitative culture methods for estimating rate of clearance in cryptococcal meningitis. Med Mycol. 2016;54(4):361–9.
75. Kambugu A, Meya DB, Rhein J, O'Brien M, Janoff EN, Ronald AR, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin Infect Dis. 2008;46(11):1694–701.
76. Kabanda T, Siedner MJ, Klausner JD, Muzoora C, Boulware DR. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal antigen lateral flow assay on cerebrospinal fluid. Clin Infect Dis. 2014;58(1):113–6.
77. Vidal JE, Boulware DR. Lateral flow assay for cryptococcal antigen: an important advance to improve the continuum of HIV care and reduce cryptococcal meningitis-related mortality. Rev Inst Med Trop Sao Paulo. 2015;57(Suppl 19):38–45.
78. Dubbels M, Granger D, Theel ES. Low Cryptococcus antigen titers as determined by lateral flow assay should be interpreted cautiously in patients without prior diagnosis of cryptococcal infection. J Clin Microbiol. 2017;55(8):2472–9.
79. Vijayan T, Chiller T, Klausner JD. Sensitivity and specificity of a new cryptococcal antigen lateral flow assay in serum and cerebrospinal fluid. MLO Med Lab Obs. 2013;45(3):16, 18, 20.
80. Boulware DR, Rolfes MA, Rajasingham R, von Hohenberg M, Qin Z, Taseera K, et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerg Infect Dis. 2014;20(1):45–53.
81. Lourens A, Jarvis JN, Meintjes G, Samuel CM. Rapid diagnosis of cryptococcal meningitis by use of lateral flow assay on cerebrospinal fluid samples: influence of the high-dose "hook" effect. J Clin Microbiol. 2014;52(12):4172–5.
82. La Hoz RM, Pappas PG. Cryptococcal infections: changing epidemiology and implications for therapy. Drugs. 2013;73(6):495–504.
83. Lucas S, da Luz MM, Flores O, Meyer W, Spencer-Martins I, Inacio J. Differentiation of *Cryptococcus neoformans* varieties and *Cryptococcus gattii* using CAP59-based loop-mediated isothermal DNA amplification. Clin Microbiol Infect. 2010;16(6):711–4.

84. Gago S, Zaragoza O, Cuesta I, Rodriguez-Tudela JL, Cuenca-Estrella M, Buitrago MJ. High-resolution melting analysis for identification of the *Cryptococcus neoformans*-*Cryptococcus gattii* complex. J Clin Microbiol. 2011;49(10):3663–6.
85. McTaggart LR, Lei E, Richardson SE, Hoang L, Fothergill A, Zhang SX. Rapid identification of *Cryptococcus neoformans* and *Cryptococcus gattii* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2011;49(8):3050–3.
86. Tarumoto N, Sakai J, Kodana M, Kawamura T, Ohno H, Maesaki S. Identification of disseminated cryptococcosis using MALDI-TOF MS and clinical evaluation. Med Mycol J. 2016;57(3):E41–6.
87. Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, et al. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. J Clin Microbiol. 2016;54(9):2251–61.
88. Liesman RM, Strasburg AP, Heitman AK, Theel ES, Patel R, Binnicker MJ. Evaluation of a commercial multiplex molecular panel for diagnosis of infectious meningitis and encephalitis. J Clin Microbiol. 2018;56(4):e01927–17.
89. Nestor D, Thulin Hedberg S, Lignell M, Skovbjerg S, Molling P, Sundqvist M. Evaluation of the FilmArray Meningitis/Encephalitis panel with focus on bacteria and *Cryptococcus* spp. J Microbiol Methods. 2019;157:113–6.
90. Lee GH, Arthur I, Leung M. False-negative serum cryptococcal lateral flow assay result due to the prozone phenomenon. J Clin Microbiol. 2018;56(4):01878–17.
91. Arastehfar A, Fang W, Pan W, Lackner M, Liao W, Badiee P, et al. YEAST PANEL multiplex PCR for identification of clinically important yeast species: stepwise diagnostic strategy, useful for developing countries. Diagn Microbiol Infect Dis. 2019;93(2):112–9.
92. Tavares ER, Azevedo CS, Panagio LA, Pelisson M, Pinge-Filho P, Venancio EJ, et al. Accurate and sensitive real-time PCR assays using intergenic spacer 1 region to differentiate *Cryptococcus gattii* sensu lato and *Cryptococcus neoformans* sensu lato. Med Mycol. 2015;54:89.
93. Ramachandran PS, Cresswell FV, Meya DB, Crawford ED, DeRisi JL, Boulware DR, et al. Detection of Cryptococcus DNA by metagenomic next-generation sequencing in symptomatic cryptococcal antigenemia. Clin Infect Dis. 2019;68:1978–79.
94. Scriven JE, Graham LM, Schutz C, Scriba TJ, Wilkinson RJ, Boulware DR, et al. Flow cytometry to assess cerebrospinal fluid fungal burden in cryptococcal meningitis. J Clin Microbiol. 2016;54(3):802–4.
95. Rhein J, Boulware DR, Bahr NC. 1,3-beta-D-glucan in cryptococcal meningitis. Lancet Infect Dis. 2015;15(10):1136–7.
96. Clinical and Laboratory Standards Institute. Reference method for broth dilution susceptibility testing of yeasts. 4th ed. Wayne: Clinical and Laboratory Standards Institute; 2017.
97. Thompson GR III, Wiederhold NP, Fothergill AW, Vallor AC, Wickes BL, Patterson TF. Antifungal susceptibility among different serotypes of *Cryptococcus gattii* and *Cryptococcus neoformans*. Antimicrob Agents Chemother. 2008;53:309.
98. Gast CE, Basso LR Jr, Bruzual I, Wong B. Azole resistance in *Cryptococcus gattii* from the Pacific Northwest: investigation of the role of ERG11. Antimicrob Agents Chemother. 2013;57(11):5478–85.
99. Lahiri Mukhopadhyay S, Bahubali VH, Manjunath N, Swaminathan A, Maji S, Palaniappan M, et al. Central nervous system infection due to *Cryptococcus gattii* sensu lato in India: analysis of clinical features, molecular profile and antifungal susceptibility. Mycoses. 2017;60(11):749–57.
100. Dismukes WE, Cloud G, Gallis HA, Kerkering TM, Medoff G, Craven PC, et al. Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. N Engl J Med. 1987;317(6):334–41.
101. Bennett JE, Dismukes WE, Duma RJ, Medoff G, Sande MA, Gallis H, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptoccal meningitis. N Engl J Med. 1979;301(3):126–31.
102. Saag MS, Powderly WG, Cloud GA, Robinson P, Grieco MH, Sharkey PK, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal

meningitis. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group. N Engl J Med. 1992;326(2):83–9.
103. van der Horst CM, Saag MS, Cloud GA, Hamill RJ, Graybill JR, Sobel JD, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. N Engl J Med. 1997;337(1):15–21.
104. O'Connor L, Livermore J, Sharp AD, Goodwin J, Gregson L, Howard SJ, et al. Pharmacodynamics of liposomal amphotericin B and flucytosine for cryptococcal meningoencephalitis: safe and effective regimens for immunocompromised patients. J Infect Dis. 2013;208(2):351–61.
105. Sun HY, Alexander BD, Lortholary O, Dromer F, Forrest GN, Lyon GM, et al. Lipid formulations of amphotericin B significantly improve outcome in solid organ transplant recipients with central nervous system cryptococcosis. Clin Infect Dis. 2009;49(11):1721–8.
106. Dromer F, Bernede-Bauduin C, Guillemot D, Lortholary O. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. PLoS One. 2008;3(8):e2870.
107. Day JN, Chau TT, Wolbers M, Mai PP, Dung NT, Mai NH, et al. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med. 2013;368(14):1291–302.
108. Pappas PG. Managing cryptococcal meningitis is about handling the pressure. Clin Infect Dis. 2005;40(3):480–2.
109. Chang CC, Perfect JR. Repeated therapeutic lumbar punctures in cryptococcal meningitis – necessity and/or opportunity? Curr Opin Infect Dis. 2016;29(6):539–45.
110. Rolfes MA, Hullsiek KH, Rhein J, Nabeta HW, Taseera K, Schutz C, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. Clin Infect Dis. 2014;59(11):1607–14.
111. Shoham S, Cover C, Donegan N, Fulnecky E, Kumar P. *Cryptococcus neoformans* meningitis at 2 hospitals in Washington, D.C.: adherence of health care providers to published practice guidelines for the management of cryptococcal disease. Clin Infect Dis. 2005;40(3):477–9.
112. Kanj SS, Welty-Wolf K, Madden J, Tapson V, Baz MA, Davis RD, et al. Fungal infections in lung and heart-lung transplant recipients. Report of 9 cases and review of the literature. Medicine (Baltimore). 1996;75(3):142–56.
113. Singh N, Lortholary O, Alexander BD, Gupta KL, John GT, Pursell K, et al. An immune reconstitution syndrome-like illness associated with *Cryptococcus neoformans* infection in organ transplant recipients. Clin Infect Dis. 2005;40(12):1756–61.
114. Singh N, Wagener MM, Cacciarelli TV, Levitsky J. Antifungal management practices in liver transplant recipients. Am J Transplant. 2008;8(2):426–31.
115. Saag MS, Cloud GA, Graybill JR, Sobel JD, Tuazon CU, Johnson PC, et al. A comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis. National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1999;28(2):291–6.
116. Alexander BD, Perfect JR, Daly JS, Restrepo A, Tobon AM, Patino H, et al. Posaconazole as salvage therapy in patients with invasive fungal infections after solid organ transplant. Transplantation. 2008;86(6):791–6.
117. Thompson GR 3rd, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F, Ostrosky-Zeichner L, Azie N, et al. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. Clin Infect Dis. 2016;63(3):356–62.
118. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730–44.
119. Dodds-Ashley E. Management of drug and food interactions with azole antifungal agents in transplant recipients. Pharmacotherapy. 2010;30(8):842–54.
120. Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, et al. Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. N Engl J Med. 2016;374(6):542–54.
121. Panackal AA, Marr KA, Williamson PR. Dexamethasone in cryptococcal meningitis. N Engl J Med. 2016;375(2):188.

122. Rhein J, Morawski BM, Hullsiek KH, Nabeta HW, Kiggundu R, Tugume L, et al. Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. Lancet Infect Dis. 2016;16(7):809–18.
123. Trevino-Rangel Rde J, Villanueva-Lozano H, Hernandez-Rodriguez P, Martinez-Resendez MF, Garcia-Juarez J, Rodriguez-Rocha H, et al. Activity of sertraline against *Cryptococcus neoformans*: in vitro and in vivo assays. Med Mycol. 2016;54(3):280–6.
124. Smith KD, Achan B, Huppler Hullsiek K, McDonald T, Okagaki LH, Akampurira A, et al. Increased antifungal drug resistance in ugandan clinical isolates of *Cryptococcus neoformans*. Antimicrob Agents Chemother. 2015;59:7197–204.
125. Singh N, Dromer F, Perfect JR, Lortholary O. Cryptococcosis in solid organ transplant recipients: current state of the science. Clin Infect Dis. 2008;47(10):1321–7.
126. Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. Lancet Infect Dis. 2007;7(6):395–401.
127. Pirofski LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. Adv Exp Med Biol. 2008;635:135–46.
128. Boulware DR, Bonham SC, Meya DB, Wiesner DL, Park GS, Kambugu A, et al. Paucity of initial cerebrospinal fluid inflammation in cryptococcal meningitis is associated with subsequent immune reconstitution inflammatory syndrome. J Infect Dis. 2010;202(6):962–70.
129. Chang CC, Lim A, Omarjee S, Levitz SM, Gosnell BI, Spelman T, et al. Cryptococcosis-IRIS is associated with lower cryptococcus-specific IFN-gamma responses before antiretroviral therapy but not higher T-cell responses during therapy. J Infect Dis. 2013;208(6):898–906.
130. Williamson PR, Jarvis JN, Panackal AA, Fisher MC, Molloy SF, Loyse A, et al. Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy. Nat Rev Neurol. 2017;13(1):13–24.
131. D'Elios MM, Josien R, Manghetti M, Amedei A, de Carli M, Cuturi MC, et al. Predominant Th1 cell infiltration in acute rejection episodes of human kidney grafts. Kidney Int. 1997; 51(6):1876–84.
132. Wiesner DL, Boulware DR. Cryptococcus-related immune reconstitution inflammatory syndrome (IRIS): pathogenesis and its clinical implications. Curr Fungal Infect Rep. 2011;5(4): 252–61.
133. Ferraris JR, Tambutti ML, Cardoni RL, Prigoshin N. Conversion from cyclosporine A to tacrolimus in pediatric kidney transplant recipients with chronic rejection: changes in the immune responses. Transplantation. 2004;77(4):532–7.
134. Dilhuydy MS, Jouary T, Demeaux H, Ravaud A. Cutaneous cryptococcosis with alemtuzumab in a patient treated for chronic lymphocytic leukaemia. Br J Haematol. 2007;137(6):490.
135. Hodge S, Hodge G, Flower R, Han P. Methyl-prednisolone up-regulates monocyte interleukin-10 production in stimulated whole blood. Scand J Immunol. 1999;49(5):548–53.
136. Ingram PR, Howman R, Leahy MF, Dyer JR. Cryptococcal immune reconstitution inflammatory syndrome following alemtuzumab therapy. Clin Infect Dis. 2007;44(12):e115–7.
137. Sun HY, Alexander BD, Huprikar S, Forrest GN, Bruno D, Lyon GM, et al. Predictors of immune reconstitution syndrome in organ transplant recipients with cryptococcosis: implications for the management of immunosuppression. Clin Infect Dis. 2015;60(1):36–44.
138. Singh N, Lortholary O, Alexander BD, Gupta KL, John GT, Pursell K, et al. Allograft loss in renal transplant recipients with *Cryptococcus neoformans* associated immune reconstitution syndrome. Transplantation. 2005;80(8):1131–3.
139. Somerville LK, Henderson AP, Chen SC, Kok J. Successful treatment of *Cryptococcus neoformans* immune reconstitution inflammatory syndrome in an immunocompetent host using thalidomide. Med Mycol Case Rep. 2015;7:12–4.
140. Lawrence DS, Youssouf N, Molloy SF, Alanio A, Alufandika M, Boulware DR, et al. AMBIsome Therapy Induction OptimisatioN (AMBITION): high dose AmBisome for cryptococcal meningitis induction therapy in sub-Saharan Africa: study protocol for a phase 3 randomised controlled non-inferiority trial. Trials. 2018;19(1):649.
141. Jarvis JN, Leeme TB, Molefi M, Chofle AA, Bidwell G, Tsholo K, et al. Short-course high-dose liposomal amphotericin B for human immunodeficiency virus-associated cryptococcal meningitis: a phase 2 randomized controlled trial. Clin Infect Dis. 2019;68(3):393–401.

142. Silva TC, Zara A, Sa F, Bara MTF, Avila RI, Costa CR, et al. Antifungal potential of punicalagin against *Cryptococcus neoformans* species complex. Rev Inst Med Trop Sao Paulo. 2018;60:e60.
143. Wiederhold NP, Najvar LK, Garvey EP, Brand SR, Xu X, Ottinger EA, et al. The fungal Cyp51 inhibitor VT-1129 is efficacious in an experimental model of cryptococcal meningitis. Antimicrob Agents Chemother. 2018;62(9):e01071–18.
144. Wiederhold NP, Xu X, Wang A, Najvar LK, Garvey EP, Ottinger EA, et al. In vivo efficacy of VT-1129 against experimental cryptococcal meningitis with the use of a loading dose-maintenance dose administration strategy. Antimicrob Agents Chemother. 2018;62(11): e01315–18.
145. Shaw KJ, Schell WA, Covel J, Duboc G, Giamberardino C, Kapoor M, et al. In vitro and in vivo evaluation of APX001A/APX001 and other Gwt1 inhibitors against Cryptococcus. Antimicrob Agents Chemother. 2018;62(8):e00523–18.
146. McKinsey DS, Wheat LJ, Cloud GA, Pierce M, Black JR, Bamberger DM, et al. Itraconazole prophylaxis for fungal infections in patients with advanced human immunodeficiency virus infection: randomized, placebo-controlled, double-blind study. National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1999;28(5):1049–56.
147. Awotiwon AA, Johnson S, Rutherford GW, Meintjes G, Eshun-Wilson I. Primary antifungal prophylaxis for cryptococcal disease in HIV-positive people. Cochrane Database Syst Rev. 2018;8:CD004773.
148. Gassiep I, Aye C, Armstrong M, Emeto TI, Heather CS, Norton RE. Correlation between serum cryptococcal antigen titre and meningitis in immunocompetent patients. J Med Microbiol. 2018;67(10):1515–8.
149. Singh N, Sifri CD, Silveira FP, Miller R, Gregg KS, Huprikar S, et al. Cryptococcosis in patients with cirrhosis of the liver and posttransplant outcomes. Transplantation. 2015; 99(10):2132–41.

Pneumocystis jiroveci in Transplant: Recognizing Risk, Understanding Prevention, and Implementing Treatment

49

Shmuel Shoham and M. Veronica Dioverti Prono

Contents

Introduction 1184
Microbiology 1184
Host-Pathogen Interactions 1185
Epidemiology and Prevention (Tables 1 and 2) 1186
Clinical, Radiographic, and Diagnostic Considerations (Fig. 1) 1192
  Clinical Findings 1192
  Radiographic Manifestations 1193
  Direct Microscopy 1193
  Molecular Methods 1194
  Beta-Glucan Testing 1195
  Other Tests 1195
Treatment (Table 3) 1196
  Specific Agents 1197
  Resistance Issues 1198
  Steroids 1198
  Response to Therapy 1199
Conclusion 1199
Key Points 1200
References 1201

S. Shoham (✉)
Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: sshoham1@jhmi.edu

M. V. Dioverti Prono
Infectious Diseases, Johns Hopkins University, Baltimore, MD, USA
e-mail: mdiover1@jhmi.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_48

Introduction

Pneumocystis jiroveci pneumonia (PJP) is at once a classic infection of immunocompromised patients and an emerging fungal infection in transplant recipients. It has been recognized as a problem of medically immunocompromised patients for over half a century, landing into the national consciousness during early days of the human immunodeficiency virus (HIV) epidemic era over 30 years ago, and continues to provide new challenges. The recent epidemiology of PJP has shifted such that about half or more of new cases are in non-HIV-infected patients including solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients. Moreover, the clinical manifestations and outcomes differ between those seen in HIV and transplant recipients. Infection tends to be of more rapid onset, patients are more likely to require ICU care, and death rates are higher.

The goal of this chapter is to help clinicians caring for SOT and HSCT patients understand the changing epidemiology of PJP, be better able to identify whether their patient is at risk for developing it or actually has active disease, and know how to prevent and treat it.

Microbiology

The causative organism of PJP is the fungus *Pneumocystis jiroveci.* Historically, *Pneumocystis species* were classified as protozoa based on their life cycle, morphologic features (large cyst with progeny rupturing to release small trophic forms), and absence of ergosterol in the cellular membrane. However, rRNA analysis and whole genome sequencing have placed these species squarely in the fungal kingdom as ascomycetous fungi [1, 2]. Nevertheless, the protozoal terms cyst and trophozoite continue to be used. For decades, the cause of PJP was referred to as *Pneumocystis carinii*. Advances in understanding the biology of the actual fungus causing PJP have led to taxonomic changes, and the etiological agent of PJP is now recognized to be *P. jiroveci*. In this chapter we will refer to the infection as PJP, but the reader should be aware that it is also sometimes referred to as *Pneumocystis carinii* pneumonia (PCP).

Pneumocystis species have a unique tropism for the lungs and reside within the alveoli. Dissemination beyond the lung, sometimes seen when aerosolized pentamidine prophylaxis is used for prophylaxis, is extremely rare even in profoundly immunocompromised hosts. The human lower respiratory tract is the natural environment for *P. jiroveci*. While there are multiple species within the genus *Pneumocystis*, only *P. jiroveci* is known to cause disease in humans. Other animals can be infected with "their own" species of *Pneumocystis* [3]. *Pneumocystis carinii* for example causes infections in rats. The fungus grows well on epithelial cells within alveoli. Cultivation of the organism in continuous ex vivo axenic culture has not been achieved, although growth of *Pneumocystis* in co-culture with host cells has been described [4, 5].

There are two main fungal forms: trophozoite and cyst. The trophozoites accounts for >90% of pulmonary pneumocystis, with the cyst form making up the rest.

The trophozoites have a single nucleus, multiply by binary fission, are pleomorphic in shape, and are typically about 2 μm in diameter. They have a cell membrane, pseudohyphae, which may help them to attach to host cells, and a fragile cell wall [6]. In active infection, the trophozoites fill up the alveolar space [4].

The cysts (spores) are formed by conjugation of two opposite mating trophozoites. The cysts have a thick outer wall that contains beta-glucans and are much bigger than trophozoites (8–10 μm in diameter). The cysts contain two, four, or eight nuclei. Rupture of the mature cyst, which contains eight intracystic bodies, results in their release to become new trophozoites. The hardy cyst is the transmissible form, deposited into air by an infected individual [7]. Such cysts may then be inhaled by another person and then deposited to the alveoli and release eight spores that subsequently develop into trophic forms and begin the life cycle again [8]. The replication of *Pneumocystis* is generally slow, approximately 7–10 days, a characteristic which is helpful in prophylaxis strategies that use intermittent therapy.

Pneumocystis cell membranes do not have ergosterol. Hence, they cannot be treated with typical antifungal agents like azoles which impair production of ergosterol and amphotericin B, which bind to it. Moreover, it is only the cyst form that contains beta-glucans in its fungal cell wall. Hence the echinocandins are effective against the cyst form but much less so against trophozoites. The role of echinocandins for prophylaxis is currently being evaluated; rezafungin is a novel long-acting echinocandin that currently undergoing clinical testing.

An important characteristic of this fungus is its dependence on synthesizing its own folic acid. Two enzymes are important for this process: dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR). DHPS is inhibited by sulfonamides such as sulfamethoxazole (SMX). DHFR is inhibited by trimethoprim (TMP) but is actually relatively poor at that function. So, while TMP/SMX is the key anti-pneumocystis agent, it is the SMX component that does the lion's share of the work [8].

Variations in genes coding for these enzymes have been instrumental in studying the epidemiology of *P. jiroveci* [8]. Mutations in DHPS are more common than in DHFR and are felt to be driven by drug pressure. The clinical significance of mutation is unclear though as some studies suggest worse outcome in infections with *P. jiroveci* carrying such mutations, but others have not.

Host-Pathogen Interactions

The natural habitat of *P. jiroveci* is at the epithelial layer of human airways, there are no known environmental reservoirs, and the fungus has a worldwide distribution. As detailed above, transmission is via person-to-person spread. It is not necessary to have symptomatic disease to transmit the organism. Aerosols generated by coughing, sneezing, or wheezing serve to propagate the infectious (cyst) form of the fungus [8]. Spread is usually from asymptomatic carriers but can also be from

those in the prodromal or active phases of infection whose fungal burden is higher. Contact with a person who goes on to develop PJP is a risk factor for susceptible hosts to develop clinical disease in the future [9]. The implications of this will be discussed later in the chapter as they pertain to outbreaks and prevention.

Once acquired, pneumocystis infection remains subclinical in non-immunocompromised people, setting up a transient carrier state that is usually cleared within 6 months [10]. Infection in young children is extremely common and is generally cleared spontaneously. Infected adults likely reacquire the infection, perhaps multiple times. The prevalence of asymptomatic infection may reach 20% in non-immunocompromised adults and even higher in those with chronic lung diseases [10]. Carriage in renal transplant recipients has been reported in the 10–20% range [11, 12]. The prevalence and intensity of colonization and the risk for clinically evident infections increase with net state of immunosuppression. The time from acquisition of organism to clinical infection likely varies, but animal studies suggest a period of about 2–3 months from exposure to heavy infection [13]. In sum, infections are predominantly due to recently acquired infection (typically reinfection) and possibly from reactivation of latent infection [14].

Under normal circumstances *P. jiroveci* survives and propagates within the lungs while "flying under the radar" and not causing obvious damage. In chronically immunocompromised patients, organism burden can increase, a brisk inflammatory response can develop, and gas exchange becomes impaired. Patients then present with clinical and radiographic manifestations of disease. At the alveolar level, this is manifested by direct attachment of *Pneumocystis* to respiratory epithelial cells, accumulation of mixture of organisms, cell debris and a foamy exudate in the alveolar lumen, and impaired lung repair all of which lead to symptomatic disease [6].

The critical contribution of the inflammatory reaction to *P. jiroveci* has important ramifications. Fungal components (including beta glucans in the cyst forms) activate an inflammatory reaction that can be profound. The severity of disease correlates more closely with the degree of pulmonary inflammation than with lung fungal burden [6]. Hence in transplant recipients, where the fungal burden is often lower than in those with HIV but the inflammatory response typically stronger, the clinical manifestations can be more severe, even in some cases while the bronchoalveolar lavage fluid stains negative for organisms. Moreover, because inflammation is such an important driver of disease severity, adjunctive treatment with corticosteroids can be a crucial modality.

Epidemiology and Prevention (Tables 1 and 2)

The epidemiology of PJP has shifted multiple times and is currently evolving once again. In the 1940s and 1950s, PJP was identified as an important cause of outbreaks of respiratory failure in premature infants and very young children [15]. Over the next three decades, it was increasingly recognized as a cause of pneumonia in the then growing population of immunocompromised patients. Most cases were in

Table 1 Factors predisposing to *Pneumocystis*

Transplant related	
Solid organ	First 6–12 months after transplant Treatment for rejection
HSCT	First 6–12 months after transplant Systemic treatment for acute or chronic GVHD
Medication related	
Large molecule	Antithymocyte globulin, alemtuzumab, rituximab
Small molecules	Steroids: ≥20 mg prednisone/day for ≥2 weeks Calcineurin inhibitors (cyclosporine, tacrolimus) mTOR inhibitors (sirolimus, everolimus) Antimetabolites (mycophenolate, azathioprine) Kinase inhibitors (ibrutinib and idelalisib)
Other	
Cell count	Absolute neutrophils ≤500 Absolute lymphocytes ≤500 CD4+ T cells ≤200
Cytomegalovirus (CMV) infection	
Exposure to person with PJP infection while having the above risk factors	

patients with hematological malignancies and in those treated with corticosteroids or cytotoxic chemotherapy. Children seemed to be particularly impacted [14]. In the 1980s and 1990s, PJP became intrinsically associated with uncontrolled HIV, and it became nearly synonymous with that diagnosis. In recent years with improvements in HIV care in a growing population of immunocompromised patients (including solid organ and bone marrow transplant recipients), the epidemiology has once again shifted. While uncontrolled HIV infections remain the most common condition associated with PJP, recent data indicate that the proportion of cases and number of deaths in non-HIV PJP (particularly in those with cancer) are surpassing those of HIV-associated infection [16–18]. In France, for example, 59% of PJP cases occurred in non-AIDS patients with 31% in SOT and 8% in HSCT, and non-HIV patients are more likely to require ICU care and have higher death rates [18]. In the USA, over half of PJP deaths are now in non-HIV patients [17].

The main risk factors for PJP are qualitative and quantitative defects in cellular immunity and prolonged use of high doses of steroids (Table 1). Without prophylaxis up to 25% of patients can develop PJP with as little as 16 mg daily of prednisone after 8 weeks or less [19]. Other implicated factors relevant to bone marrow and solid organ transplant recipients, and especially for those with relapsed lymphoid malignancy or posttransplant lymphoproliferative disease (PTLD), include duration and intensity of chemotherapy and duration of neutropenia [20–22].

Antimicrobial prophylaxis is highly successful at preventing PJP in transplant recipients. Prophylaxis is recommended in situations where the risk for PJP is estimated to be more than 3.5% [23]. For that reason, a thorough understanding of the epidemiology of pneumocystis and risk factors for infections is essential when developing prophylaxis protocols for a transplant program. Unlike the situation in

Table 2 Approach to prevention

Drug	Comments	Side effects
TMP/SMX 80/400 (SS) daily, 160/800 thrice weekly	**Agent of choice** (documented superior efficacy) Additional prophylaxis against multiple other organisms (e.g., *Toxoplasma*[a], *Isospora*, *Cyclospora*, susceptible strains of *Streptococcus* spp., *Staphylococcus* spp., *Haemophilus*, *Salmonella*, *Listeria*, *Enterobacteriaceae*[a])	Hypersensitivity (including anaphylaxis and rash), acute nephritis, urinary crystals, hyperkalemia, hypoglycemia, hepatitis, pancreatitis, nausea, vomiting, aseptic meningitis, fever Occasionally, hemolytic anemia in those with G6PD deficiency
Dapsone 100 mg/day	Less activity against *Toxoplasma* Should be avoided in those with severe TMP/SMX reactions due to cross-reactivity	Hemolytic anemia (in those with G6PD deficiency), methemoglobinemia, hepatitis Potential hypersensitivity in those with severe reactions to TMP/SMX "Sulfone syndrome": Fever, rash, lymphadenopathy, hepatitis, and methemoglobinemia
Atovaquone 1,500 mg daily	Less activity against *Toxoplasma* More expensive, less palatable than other options	Rash, nausea, diarrhea, headache, elevated liver enzymes Requires fatty intake for proper absorption
Pentamidine (aerosolized) once monthly	Less effective than TMP/SMX or dapsone Requires trained personnel for administration	Cough, bronchospasm, excessive salivation, sore throat Possible TB transmission, need for TB screening
Pentamidine 4 mg/kg by IV once monthly	Not FDA approved for prevention	Hypotension, pancreatitis, anaphylaxis

[a]Daily dosing preferred

HIV, there are no definitive laboratory markers such as CD4+ count that automatically trigger initiation of PJP prophylaxis. Some have proposed a CD4+ count cutoff of <300/μL; however, patients can develop PJP even with much higher CD4+ counts [22, 24]. Outside of HIV, low CD4+ counts are not sensitive or specific enough to predict susceptibility in transplant patients and should not be use as the sole marker to initiate prophylaxis [14, 25].

For solid organ transplant recipients not receiving effective prophylaxis, the reported PJP rates range from 0.6 to 14% for the kidney, 3–11% for liver, 2–41% for heart, and 6.5–43% for lung transplants [21]. With current prophylaxis protocols, the rate is approximately 1–2% [26]. Moreover, with widespread adaptation of prophylaxis during higher-risk periods, the timing of infection has been pushed further out, and most cases now occur in the first to second year of transplant, which is typically after prophylaxis has been stopped.

Factors leading to elevated risk of infection are often multiple and overlapping [27]. The highest-risk periods are when immunosuppression is at its most intense,

during the first few months posttransplant, and with treatment for acute graft rejection. In those with lung or small bowel transplant, the risk for PJP seems to extend beyond 12 months posttransplant period. The combination of calcineurin inhibitors such as tacrolimus or cyclosporine, mycophenolate, and higher doses of corticosteroids (i.e., ≥20 mg of prednisone per day for ≥2 or more weeks) and T-cell-depleting agents such as thymoglobulin or alemtuzumab substantially increases the odds of developing PJP.

We recommend that programs develop prophylaxis protocols and orders sets such that a period of PJP prophylaxis (e.g., 6 months) is automatically triggered by organ transplantation surgery, receipt of thymoglobulin, alemtuzumab, or high-dose steroids. In those with lung transplantation, the risk for PJP seems to extend beyond 12 months posttransplant; therefore lifelong prophylaxis has been recommended [21]. Lifelong prophylaxis should also be considered for small bowel transplant recipients and prior PJP [28]. Prophylaxis against other organisms such as *Toxoplasma* in heart transplant recipients may also help determine the duration.

Additional risk factors for infection can be more challenging to implement as part of prophylaxis protocol but are nonetheless important. These include older age, CMV infection, lymphopenia (especially absolute lymphocyte count <500), prolonged neutropenia, low CD4+ count, multiple episodes of rejection, and receipt of rituximab [21, 26, 28–33]. Newer data suggest considering PJP prophylaxis in patients with active CMV viremia/disease [34–36].

HSCT recipients are also at substantial risk. Without prophylaxis, the risk for PJP is ~5–16% [21]. As is the case with SOT, TMP/SMX prophylaxis is highly protective. The overall rate for autologous stem cell transplants with prophylaxis is ~0.3% and ~ 0.6–1.0% for allogeneic transplant [37, 38]. As with solid organ transplant, widespread utilization of PJP prophylaxis for at least 6 months post-engraftment or discontinuation of all immunosuppression has led to delayed onset disease, typically beyond 6–12 months after transplant, and mostly in those patients receiving high doses of steroids for treatment of graft-versus-host disease (GVHD) [21]. Timing of infection, however, ranges from within a month to over a year after transplant. In one series, patients developed PJP prior to engraftment (when they were not getting prophylaxis), after prophylaxis was stopped, or when on atovaquone [39].

The highest risk is typically in the first year after transplant and during times of intense immunosuppression. In that regard, acute or chronic GVHD are an important risk driven by use of steroids and other immunosuppressive drugs to control the GVHD, lymphopenia, and low-circulating CD4+ T cells [38]. Published guidelines recommend PJP prophylaxis during at least the first 6 months post-engraftment for allogeneic HSCT and during periods of enhanced immunosuppression [21, 40]. Failure to prescribe or to adhere to a prophylaxis regime during vulnerable periods is an important risk for developing infections [38].

The underlying diseases also impact risk for PJP. Acute lymphoblastic leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and multiple myeloma are all associated with PJP. Even in the absence of transplant, patients with **hematologic malignancies** are at risk, albeit lower than transplant recipients. High-intensity chemotherapy regimens (e.g., R-CHOP14, ABVD, high-dose

methotrexate), receipt of nucleoside analogues (e.g., fludarabine, cladribine), or T-cell-depleting agents (e.g., alemtuzumab, rituximab, bendamustine) place patients at moderate risk. Some guidelines are recommending prophylaxis for up to 6 months after completing treatment [23, 41]. Moreover, since many BMT recipients have residual disease and/or are receiving treatment to prevent recurrences of such diseases, those conditions may carry additional risks.

In that context, while prophylaxis for patients receiving an autologous stem cell is controversial, it should be provided to those with underlying lymphoma or leukemia, intense conditioning regimens, or prior/current treatment with purine analogs (fludarabine, cladribine); it is generally given for 3–6 months posttransplant [40].

Pneumocystis (and other fungal infections) has also been observed in patients receiving the Bruton tyrosine kinase (BTK) inhibitor ibrutinib. Since these patients typically have chronic lymphocytic leukemia (CLL) or lymphoma, it is difficult to definitively determine the specific contribution of this agent to causing such infections. Nevertheless, studies in mice suggest that BTK inhibition increases susceptibility to PJP [42]. Similarly, there is a small but increased risk of PJP during treatment with idelalisib, which is a phosphatidylinositol-3-kinase delta (PI3Kδ) inhibitor used in combination with rituximab for CLL, as well as with Janus Kinase (JAK) inhibitors used in the treatment of myelofibrosis [43, 44].

Transplant recipients who acquire a solid tumor may encounter risk owing to cancer chemotherapy. In the absence of transplant, the incidence of PJP is generally low in patients with solid tumors. Important exceptions are patients receiving temozolomide or high doses of corticosteroids for brain malignancies. The package insert for temozolomide includes a black box warning recommending PJP prophylaxis with its use [23]. Similarly, for patients receiving high-dose steroids as part of cancer therapy, prophylaxis should also be instituted for 6 weeks after tapering doses of steroids containing >16 mg of prednisone or >4 mg of dexamethasone daily for more than 4 weeks [23].

Pneumocystis has long been associated with outbreaks. Molecular epidemiological analyses have indicated that patient-to-patient transmission is the key driver of these outbreaks [45, 46]. Most of the outbreaks have been reported from kidney transplant recipients, but well-documented clusters of cases in other transplant patients and in those with hematological malignancies have also been reported [45]. A key feature of these outbreaks is that infected patients received inadequate or no prophylaxis prior to developing PJP. Control of the outbreaks is typically gained by institution of chemoprophylaxis [47]. The role of isolation precautions is as yet unclear. Those with higher organism burden in their airways might be particularly efficient spreaders. CDC and others advise avoiding placement of someone with PJP in in the same room as an immunocompromised patient [45].

The agent of choice for PJP prophylaxis is *trimethoprim-sulfamethoxazole* (TMP/SMX) due to its superior efficacy when compared to other agents; a large study looking at PJP infections in HSCT recipients found that seven patients developed PJP while on prophylaxis, five of them were on aerosolized pentamidine (AP), and the other two on dapsone; no episodes of PJP were noted on those taking TMP/SMX [48]. Similarly, studies in liver, kidney, and heart transplant recipients

found no cases of PJP in those receiving TMP/SMX [49, 50]. Therefore, TMP/SMX should be used unless there is a clearly documented reaction. If reactions such as rash occurs, desensitization protocols have been successfully applied in up to 80% of patients [40, 51, 52]. This approach should be avoided in those with severe immediate reactions.

In post-HSCT patients, TMP/SMX may delay engraftment due to its myelosuppressive effects, and, therefore, initiation of prophylaxis should be delayed until engraftment occurs. If engraftment is delayed, other agents can be used. Other toxicities include hypersensitivity including rash which could mimic GVHD and even anaphylaxis, hyperkalemia, hypoglycemia, acute interstitial nephritis, hepatitis, nausea, vomiting, pancreatitis, and aseptic meningitis [21, 40]. A study showed no significant difference in adverse events between groups taking TMP/SMX versus placebo; severe adverse events were noted in 3.1% and included severe rash, leukopenia, and thrombocytopenia [53, 54]. Mutations in targets for TMP/SMX (genes encoding dihydropteroate synthase and dihydrofolate reductase) have been detected, as detailed elsewhere in this chapter. But prophylactic failure has not been consistently associated with such mutations [21].

Other less effective options are shown in Table 2 and include dapsone, atovaquone, and aerosolized pentamidine. *Dapsone* is considered a second-line agent when compared to TMP/SMX; there have been reports of breakthrough PJP [41, 48, 55]. A significant proportion of patients with hypersensitivity to TMP/SMX will also react to dapsone (up to 50%), so it should be avoided in those with life-threatening reactions to TMP/SMX [14]. More recently, a study found no breakthrough PJP or cross-reactivity with TMP/SMX; however, it included only a small number of patients [56]. Other toxicities include methemoglobinemia, hemolytic anemia in those with glucose-6-phophate dehydrogenase deficiency, aplastic anemia, agranulocytosis, rash, nausea, and hepatitis. Higher incidence of infections otherwise covered by TMP/SMX was noted in those on dapsone prophylaxis [57].

Atovaquone is tolerated by some patients but not by others. Its taste is unpleasant, and the drug can be expensive; hence patients' adherence can be suboptimal. Absorption may also be compromised in those with poor fat intake, thus limiting its efficacy in those with gastrointestinal disorders such as GVHD [41]. The main side effects include nausea, diarrhea, headaches, and possible liver test elevations. At doses of 1,500 mg daily, it has comparable efficacy to dapsone and aerosolized pentamidine [58, 59]. Mutations in target for atovaquone (cytochrome *b*) have been detected, but prophylactic failure has not been consistently associated with such mutations [21].

Aerosolized pentamidine is less effective and may lead to cough and bronchospasm, which can be overcome by premedicating with beta-adrenergic agonists [21, 40]. Access to a facility that can deliver this type of treatment is not universally available. All patients receiving aerosolized pentamidine need to be screened for tuberculosis, as there have been reports of transmission due to post-administration cough [60]. Cases of disseminated PJP have been reported with aerosolized pentamidine [61]. Small studies which included a total of 96 HSCT patients on aerosolized pentamidine did not report any cases of PJP or discontinuation due to adverse

events [62–64]. However, larger studies have shown otherwise [48, 65]. Pentamidine may also be given by IV as prophylaxis. Typical dose in adults is 4 mg/kg once monthly. Potential side effects include anaphylaxis, hypotension, and pancreatic injury.

Clinical, Radiographic, and Diagnostic Considerations (Fig. 1)

Clinical Findings

The constellation of cough, fever, shortness of breath, and hypoxemia in the context of typical radiographic findings (see below) is the classical presentation of PJP. The cough tends to be dry in nature, fever may be low grade or altogether missing in transplant recipients, and hypoxemia may not be initially present. Fever, tachypnea, tachycardia, and dry rales are the classic constellation of physical exam findings. Progression of disease, sometimes to respiratory failure, can be rapid, evolving over just a few days. Sometimes, however, the progression can be slower playing out over 1–2 weeks or longer. Chest pain and abnormalities on chest auscultation are seen in a

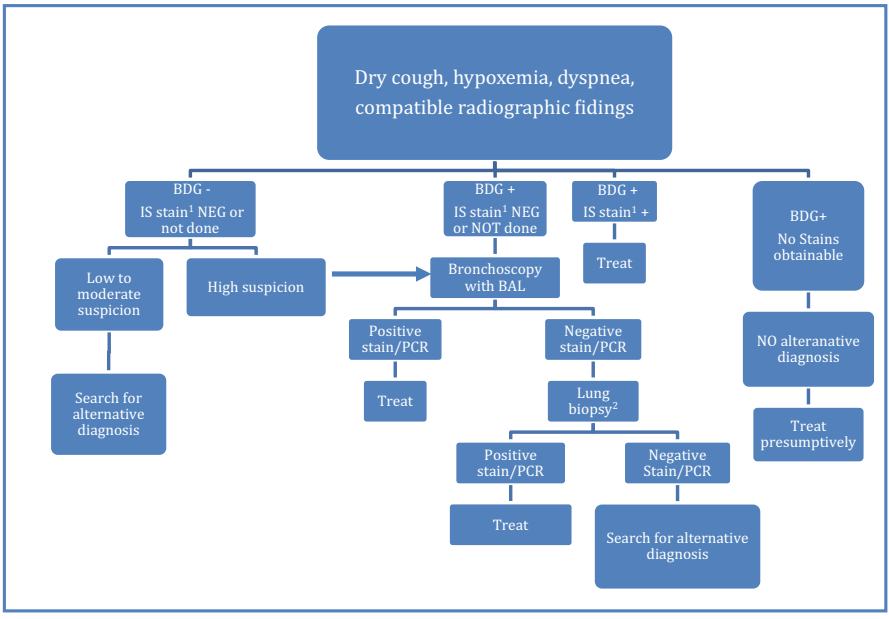


Fig. 1 Suggested diagnostic algorithm. BDG, 1,3-β-D-glucan; BAL, bronchoalveolar lavage; GGO, ground-glass opacities; IS, induced sputum; PCR, polymerase chain reaction; and SOB, shortness of breath. [1]Testing of induced sputum for PJP is associated with multiple false-negative tests and may delay diagnosis. Many go right to bronchoscopy to try to establish the diagnosis via bronchoalveolar lavage. [2]Lung biopsy: transbronchial, video-assisted thoracoscopy (VATS), open lung biopsy

minority of patients (~20–30% of the time). Pneumothorax is a well described, yet rare presentation of PJP and lymphadenopathy is uncommon.

ICU admission is required in nearly 50% of patients, most of which require mechanical ventilation and continuous renal replacement therapy (CRRT). Inhospital mortality is nearly 40% and twice that for patients in ICU. While not diagnostic of PJP, LDH levels of ~500 or higher are associated with worse outcomes (e.g., requirement for ICU level of care and morality) [66].

Radiographic Manifestations

Typical findings in PJP are bilateral interstitial or alveolar/interstitial opacities on chest radiographs and diffuse ground-glass opacities on computed tomography (CT) scans. Septal lines, centrilobular nodules, and, less commonly, focal consolidation, subpleural nodules, cysts, blebs, and pneumothoraxes may also be seen [18]. Pleural effusions are uncommon, and, if present, one should consider an alternative (or additional) diagnosis. The most common radiographic manifestations of PJP are multifocal ground-glass pulmonary opacities, best seen on high-resolution CT scans. These opacities are due to accumulation of intra-alveolar fibrin, debris, and organisms [67]. The appearance of the ground-glass opacities is typically with small nodules (<5 mm), reticulation, and mosaic pattern. Midsize nodules (5–10 mm), which are often due to granulomatous inflammation, may be seen in HIV but are less common in transplant patients [68]. With more advanced disease, lung consolidations can occur, reflecting pulmonary damage from the host immune response. These are more common and occur more rapidly in non-HIV PJP. Pulmonary cysts of varying shape, size, and wall thickness may occur in transplant patients but are more common in HIV [67].

Direct Microscopy

The cornerstone of PJP diagnosis is direct microscopic examination. Appropriate clinical samples include induced sputum, bronchoalveolar lavage (BAL) fluid, or lung tissue. Examination of hypertonic saline-induced sputum is the least invasive approach and may establish the diagnosis in about 50% of HIV PJP patients [69]. However, the yield may be far lower in transplant patients owing to lower organism burden. Yields are better with examination of bronchoalveolar lavage (BAL) obtained via bronchoscopy, and this has been the traditional go-to approach when suspicion is high but induced sputum is either unavailable or negative. Rarely, lung biopsy via transbronchial sampling during bronchoscopy, via video-assisted thoracoscopic surgery (VATS), or even an open lung procedure is used to establish the diagnosis [2, 70].

Multiple stains are helpful for identifying the organism. We recommend using a combination of stains that target both cysts and trophic forms [71]. The trophic forms, which make up over 90% of the organism burden, can be visualized with

Papanicolaou, Wright-Giemsa, or Gram-Weigert stains. The cyst forms, which usually make up about 5–10% of the organism burden, are best visualized by Gomori methenamine silver, cresyl echt violet, toluidine blue O, or calcofluor white stain cysts [21]. Hematoxylin-eosin staining is useful for demonstrating the characteristic foamy, eosinophilic material within the alveoli in lung tissue biopsy samples [14]. Direct or indirect immunofluorescence stains capture both forms and are especially helpful for induced sputum, where their sensitivity and specificity are superior to other stains; the difference is less apparent in BAL samples [2, 70]. However, microscopic exam can be difficult requiring highly skilled personnel, particularly when the fungal burden is low as may be the case in transplant patients. When the pretest probability is high, a negative microscopic exam does not rule out pneumocystis [71, 72].

Molecular Methods

Use of polymerase chain reaction (PCR) to detect *Pneumocystis* has greater sensitivity and specificity than conventional staining in both induced sputum and BAL samples. It is more frequently positive than microscopy in non-HIV patients where the organism burden is lower [72–77]. Traditional PCR positivity suggests presence of the fungus in the respiratory tract but cannot effectively differentiate whether it is causing disease or an innocent bystander. Positive results should be interpreted with particular care in asymptomatic individuals [14, 74, 78, 79]. Negative PCR can effectively rule out PJP with a negative predictive value close to 100% [23, 71, 72, 74, 76, 80]. Other specimens could be used for PCR testing, such as induced sputum, oral washings, nasopharyngeal aspirates, and nasal swabs, but these may result in false-negative tests as fungal burden is highest in the alveoli [71].

Attempts at using quantitative PJP PCR (qPCR) in BAL have been made to try to distinguish colonization versus infection; however, different PCR techniques, and the presence of both trophic (single genomes) and cyst (multiple genomes) forms with unknown ratios, make quantitation difficult to interpret [72, 80]. In addition, there might be a continuous from colonization to active infection which hinders discrimination between the two entities [71]. There are no clear PCR thresholds to distinguish infection versus colonization. Guidelines for the validation of qPCR assays may overcome some of this issues by creating more homogeneous and comparable testing that may allow an international standard [71, 81].

Serum samples can be used for PCR testing; however, sensitivity is very low at 6% [82]. A study using cell-free DNA PCR (cfPCR) in blood samples showed similar sensitivity but higher specificity than BAL and sputum samples; this technique may prove useful in those patients who cannot undergo invasive diagnostic procedures [83]. Specific PCR techniques have the capability of detecting mutations in genes encoding dihydropteroate synthase (DHPS, target for trimethoprim), which has been previously associated with possible prophylaxis and treatment failure [84, 85].

Beta-Glucan Testing

1,3-β-D-glucan (BDG) is a cell wall polysaccharide present in many pathogenic fungi including *P. jiroveci*. Serum levels are typically elevated in PJP. Several commercial tests have been developed, but only Fungitell (Associates of Cape Cod Inc., East Falmouth, MA, USA) is FDA approved. Studies in immunocompromised patients have shown high sensitivity and negative predictive value for PJP, with positive BDG values even 10 days prior to microbiological diagnosis, but low positive predictive values [82]. Elevated BDG results may be due to another fungal infection or a non-fungal trigger (e.g., *Pseudomonas aeruginosa*, severe mucositis, and recent intravenous immunoglobulin). Cutoffs for PJP should probably be higher than the 60–80 pg/mL used for other infections [84].

A meta-analysis including 13 studies found a sensitivity of 0.91 (95% CI 0.88–0.93) and specificity of 0.75 (95% CI, 0.8–0.81); studies included both HIV and non-HIV population. When only non-HIV patients were analyzed, sensitivity dropped to 0.85 (95% CI, 0.77–0.94) and specificity to 0.73 (95% CI, 0.61–0.85) [86]. Two other meta-analysis including 12 and 14 studies, respectively, found similar sensitivity and specificity (96%/84% and 95%/86%, respectively); only one of these looked grouped HIV and non-HIV patients but found no significant differences [87, 88].

Some studies have found that the level of BDG might reflect organism burden; it is therefore postulated that the lower sensitivity might be due to lower organism burden in the non-HIV population [86, 89]. In critically ill patients with PJP presenting as acute respiratory distress syndrome (ARDS), BDG testing was found to be highly reliable with generally high levels and a negative predictive value of 96% [90].

In summary, we recommend BDG testing as an adjunctive diagnostic test that can be used as part of a diagnostic/treatment algorithm along with PCR, but caution about lack of specificity and that such an approach has not been validated in clinical studies [23, 71, 91]. Additionally, we urge caution in overreliance on a negative BDG test in transplant recipient with clinical and radiographic findings consistent with PJP, as a negative result may not exclude the diagnosis due to its lower sensitivity; diagnosis in this setting should be ruled out by other testing.

Of note, BDG might be useful in predicting favorable clinical course by documenting decreasing BDG levels during treatment; however, increasing or persistently elevated BDG levels do not necessarily correlate with treatment failure and may persist long after clinical resolution [92, 93].

Other Tests

Elevated serum lactate dehydrogenase (LDH) (>300 IU) is usually present, although other pulmonary processes leading to lung injury may also cause elevated LDH. Antibody testing is generally not useful (IgM and IgG), as most asymptomatic adults will test positive [14].

Treatment (Table 3)

Timely initiation of treatment for PJP is particularly important in transplant recipients. As detailed above, such patients experience a faster progression of illness, more severe hypoxemia, greater need for intensive care and invasive mechanical ventilation, and higher prevalence of shock and have higher death rates. Delay in initiation of treatment is independently associated with death in such patients [18]. Without treatment, an immunocompromised patient's infection will continue to progress ultimately resulting in respiratory failure and death. Even with appropriate treatment, about 50% of transplant patients will go on to require intensive care unit level of care with mortality rates up to 78% [66].

Hence, while treatment should absolutely be initiated when there is definitive evidence for PJP, it must also be strongly considered before the diagnosis has been firmly established (i.e., empiric therapy). As detailed in the diagnostics section, definitive diagnosis requires direct visualization of the organism. However, therapy commonly needs to be started before a definitive diagnosis is established. This can become complicated as pulmonary edema, which can be a common issue in transplant patients, may mimic PJP. Moreover, patients are often too unstable to undergo bronchoscopy right away, and even when done, the results may be negative despite disease. Published guidelines suggest initiation of empiric therapy in hematological malignancy patients (including BMT) who have risk factors for PJP in conjunction with signs and symptoms and radiographic abnormalities compatible with disease

Table 3 Treatment options for moderate to severe disease

Drug	Comment
Trimethoprim and sulfamethoxazole (IV or PO) 15–20 mg/kg/day in three to four divided doses (based on TMP component)	Side effects as detailed in Table 2 Monitor renal function, electrolytes, CBC, liver function tests
Clindamycin (IV or PO) 600 mg 4 times/day (or 900 mg thrice daily) And Primaquine (PO) 30 mg/day	Second-line therapy. Efficacy may be suboptimal in severe disease Clindamycin side effects include GI intolerance, *Clostridium difficile* infection. Primaquine side effects include hemolysis with G6PD deficiency and methemoglobinemia Check G6PD level, monitor CBC
Pentamidine (IV) 4 mg/kg/day	Second-line therapy. Safety concerns (serious side effects in >50% of patients) including GI intolerance, hypoglycemia, pancreatitis, hyperglycemia, arrhythmias, hypotension, renal dysfunction Monitor cardiac rhythm, blood pressure, renal function, electrolytes, and pancreatic function

Note: Options for mild to moderate disease include atovaquone 750 mg twice daily and trimethoprim 5 mg/kg thrice daily + dapsone 100 mg daily

[94]. The length of treatment is usually 14–21 days, but longer may be required in more severe cases.

Specific Agents

Trimethoprim-sulfamethoxazole (TMP/SMX) is the first-line agent for treatment of PJP. The standard dose is 15–20 mg/kg of TMP component (adjusted as needed for renal function) daily in 6- to 8-h intervals [28]. The bioavailability of TMP/SMX is excellent, so it can be given either by IV route or by mouth providing the enteric absorption is good. We recommend initial therapy by the IV route in moderate to severe disease. In cases of milder disease, when therapy is given as an outpatient or during times of IV TMP/SMX shortages, treatment may be initiated by the oral route. Standard doses for oral therapy are two-double strengths tablets (TMP/SMX 160 mg/800 mg) 2–3 times daily. Another appealing feature of oral therapy is that unlike IV TMP/SMX, it does not require the infusion of large volume of fluids. This issue is particularly important in patients with propensity for fluid overload, which can be an issue for many solid organ and bone marrow transplant recipients.

As detailed earlier TMP/SMX is associated with multiple side effects. In addition to the allergic reactions and gastrointestinal symptoms common to many antibiotics, there are other potential toxicities that could be more relevant to transplant patients. Hyperkalemia is a potential problem for patients with abnormal renal function, which is a common situation in solid organ and bone marrow transplant recipients and when co-administered with drugs that impact potassium metabolism (e.g., angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and potassium-sparing diuretics). Elevations in serum creatinine are another issue. TMP/SMX can cause true renal dysfunction and/or mild elevations in creatinine that are not related to reduced glomerular filtration rate; it can lead to urinary crystal formation with ensuing obstruction leading to acute failure. This can be particularly challenging to interpret in renal transplant patients.

Cytopenias (e.g., leukopenia, anemia, and thrombocytopenia) are another problem that may be particularly relevant for bone marrow transplant recipients but also for patients on mycophenolate and other bone marrow suppressing agents (e.g., ganciclovir or valganciclovir). Drug-induced liver injury including cholestasis can be an issue. In patients with hematological malignancy who might be receiving methotrexate, TMP/SMX should be avoided because of potentially serious adverse drug effects [94]. Other side effects include aseptic meningitis, Steven-Johnson syndrome/toxic epidermal necrolysis, and drug fever. One approach to reduce the chance of side effects is to use a lower dose. This was evaluated in a retrospective study of non-HIV PJP. When compared to the typical regimen (15–20 mg/kg/d), a reduced dose (median 10.8 mg/kg) had similar efficacy and fewer side effects [95].

Clindamycin 600 mg IV or PO 4 times daily (or 900 mg thrice daily) and oral primaquin 30 mg daily are a second-line option for PJP [28]. Data for this regimen has largely been extrapolated from the HIV literature. The major side effects are GI in nature (including *Clostridium difficile* infection) and neutropenia. Screening for glucose-6-phosphate dehydrogenase (G6PD) deficiency is required prior to primaquine use.

Intravenous pentamidine given at a dose of 4 mg/kg/day is the other major second-line agent. IV pentamidine has multiple side effects. It should be given over 1–2 h to avoid infusion-related hypotension. Additional common toxicities include arrhythmias, electrolyte abnormalities, renal injury, bone marrow suppression, and pancreatitis. The latter can cause either low or high blood sugars and may lead to diabetes mellitus due to irreversible damage to islet cells. This drug is probably best avoided in pancreatic and islet cell transplant recipients.

Atovaquone 750 mg twice daily by mouth may be used for mild to moderate disease. This drug is only available as an oral suspension and is absorbed best with fatty foods. Aside from its unpleasant taste and some instances of rash, it is well tolerated, but efficacy in active diseases is uncertain and hence should be avoided in more serious cases. Other combinations such as pyrimethamine and sulfadiazine, a macrolide and SMX, and dapsone with trimethoprim have been used as well, but data is limited. Echinocandins do have activity in pneumocystis but are not effective when used. We do not recommend their use for treatment of PJP.

Resistance Issues

Resistance to TMP/SMX is rare but of concern. Widespread exposure to the drug may cause selective pressure for resistant isolates. These can then be transmitted between individuals such that prior exposure to TMP/SMX is not necessary for a resistant isolate to be present in a given patient. Assessing for drug resistance is a challenging issue in *Pneumocystis* owing to the absence of culture systems. Mutations in genes coding for dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), the targets of trimethoprim and sulfa, respectively, have been speculated to cause resistance to TMP/SMX. The clinical significance of such mutations is unclear. In one study, presence of such mutations was associated with longer duration of mechanical ventilation, but not mortality [96]. It appears that DHPS mutations represent a low-level resistance that can be overcome by higher doses of TMP-SMX [8]. Mutations in the gene coding for mitochondrial cytochrome b, which is the target of atovaquone, have been associated with resistance to that drug and have been linked to failure of prophylaxis.

Steroids

As a general rule, immunosuppression should be reduced in patients with PJP, although this is not always straightforward with corticosteroids. Clearly the pathway leading to PJP includes immunosuppression, and corticosteroids are often directly implicated. However, once infection has developed, particularly when it is severe, the immune response is a double-edged sword. Sometimes infection only becomes manifest when

high doses of corticosteroids are being tapered. Similarly, experience from HIV has shown that hypoxemia can actually worsen with treatment due to an overexuberant inflammatory response and that corticosteroids are necessary to avoid further respiratory decline. How this experience translates to non-HIV patients is a matter of ongoing debate. Recent data in non-HIV patients, some of which were transplant recipients, found no advantage in routine steroid use [97].

Published guidelines are somewhat conflicting. Guidelines from the European Conference on Infections in Leukemia (ECIL) recommend against routine adjunctive steroids and state that the decision in those with PJP and respiratory failure be made on an individual basis [94]. The American Society of Transplantation (AST) guidelines on the other hand recommend that patients with severe disease (i.e., hypoxemia; pAO2 $<$ 70 mmHg on room air) received adjunctive corticosteroids, ideally within 72 h of initiating antimicrobial therapy [28]. The recommended dose is 40–60 mg of prednisone twice daily for 5–7 days followed by a 7- to 14-day taper. With respect to those patients that have been on steroids prior to onset of PJP, it is unclear whether the appropriate approach is to maintain the current dose, escalate it, or even taper off. The approach typically has to be individualized.

Response to Therapy

Patients with PJP do not always improve right away. In cases of empiric therapy, it is tempting to abandon the diagnosis if the patient does not respond immediately. It should be expected that patients may take up to a week to respond, and sometimes there is clinical decline in the first few days. Conversely, an immediate response to therapy should not necessarily be interpreted as proof of the accuracy of the diagnosis as the improvement may be due to co-administration of corticosteroids. In cases of proven infection, a lack of response or worsening after a week of therapy should raise concern. The differential diagnosis includes coinfection due to an additional pathogen (e.g., CMV, other fungal organisms, bacteria), complication of PJP such as pneumothorax, pleural effusion or extensive pulmonary injury, noninfectious etiologies such as pulmonary edema due to fluid overload, and possibly treatment failure related to the anti-pneumocystis regimen selected. Resistance to TMP/SMX has been described as detailed above. However, this phenomenon is uncommon. The decision to replace TMP/SMX, which is the frontline treatment for pneumocystis, in favor of second-line agents with less proven efficacy and more potential toxicity (e.g., clindamycin/primaquine or IV pentamidine) should not be taken lightly (Fig. 2).

Conclusion

PJP is at once a classic infection of immunocompromised patients and an emerging infection in transplant patients. In the past two decades, there has been a major shift in the epidemiology away from it being a disease predominantly associated with

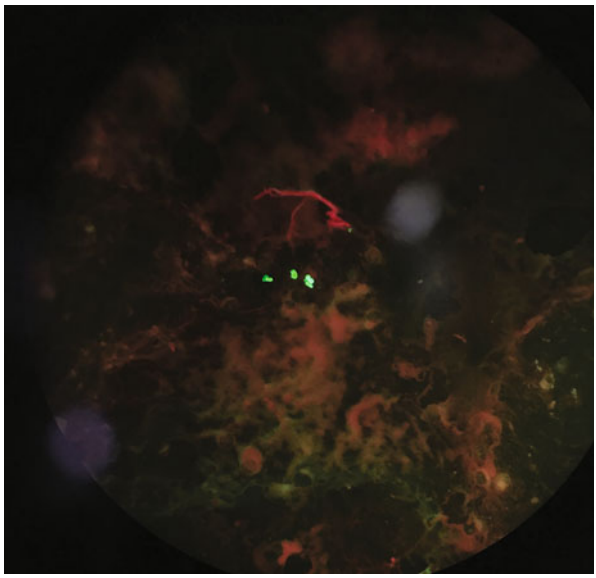


Fig. 2 PJP immunofluorescent staining (From Eunice L. Rogaishio, Melis Anahtar, and Camille N. Kotton, with permission)

HIV to one increasingly seen in non-HIV patients, including solid and bone marrow transplant recipients. It is important for clinicians to be aware of this shift in epidemiology, understand which patients are at particular risk, and how PJP presents in transplant recipients. Such knowledge is important in determining who should receive prophylaxis, where to place PJP on the differential diagnosis, and how to interpret results of diagnostic tests.

Key Points

- *Highest-risk periods*: The first 6–12 months after transplant and during times of intensification of immunosuppression for rejection of GVHD (e.g., with lymphocyte-depleting agents and corticosteroids at $\geq$20 mg prednisone equivalent/day $\geq$2 weeks).
- *Prevention*: Patients falling into the high-risk categories above should receive prophylaxis, ideally with TMP/SMX.
- *Diagnosis*: Stains of induced sputum or bronchoalveolar lavage fluid are helpful if positive. However, a negative stain does not rule out PJP in a transplant recipient with compatible symptoms. PCR has high sensitivity, especially if obtained from BAL. Serum beta glucan can be useful and may obviate need for more invasive procedure such as bronchoscopy but is nonspecific.
- *Treatment*: TMP/SMX is the mainstay of therapy with clindamycin/primaquine and IV pentamidine as alternative options. Adjunctive corticosteroids may have a role in severe disease, but evidence for their use is not as clear cut as in HIV.

References

1. Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML. Ribosomal RNA sequence shows Pneumocystis carinii to be a member of the fungi. Nature. 1988;334(6182):519–22.
2. Thomas CF Jr, Limper AH. Pneumocystis pneumonia. N Engl J Med. 2004;350(24):2487–98.
3. Durand-Joly I, Aliouat el M, Recourt C, Guyot K, Francois N, Wauquier M, et al. Pneumocystis carinii f. sp. hominis is not infectious for SCID mice. J Clin Microbiol. 2002;40(5):1862–5.
4. Skalski JH, Kottom TJ, Limper AH. Pathobiology of Pneumocystis pneumonia: life cycle, cell wall and cell signal transduction. FEMS Yeast Res. 2015;15(6)
5. Schildgen V, Mai S, Khalfaoui S, Lusebrink J, Pieper M, Tillmann RL, et al. *Pneumocystis jirovecii* can be productively cultured in differentiated CuFi-8 airway cells. MBio. 2014;5(3): e01186–14.
6. Gigliotti F, Limper AH, Wright T. Pneumocystis. Cold Spring Harb Perspect Med. 2014;4(12): a019828.
7. Sokulska M, Kicia M, Wesolowska M, Hendrich AB. *Pneumocystis jirovecii* – from a commensal to pathogen: clinical and diagnostic review. Parasitol Res. 2015;114(10):3577–85.
8. Ma L, Cisse OH, Kovacs JA. A molecular window into the biology and epidemiology of Pneumocystis spp. Clin Microbiol Rev. 2018;31(3):e00009-18.
9. McClarey A, Phelan P, O'Shea D, Henderson L, Gunson R, Laurenson I. Lessons learned from a Pneumocystis pneumonia outbreak at a Scottish renal transplant Centre. J Hosp Infect. 2019;
10. Medrano FJ, Montes-Cano M, Conde M, de la Horra C, Respaldiza N, Gasch A, et al. *Pneumocystis jirovecii* in general population. Emerg Infect Dis. 2005;11(2):245–50.
11. Szydlowicz M, Jakuszko K, Szymczak A, Piesiak P, Kowal A, Kopacz Z, et al. Prevalence and genotyping of *Pneumocystis jirovecii* in renal transplant recipients-preliminary report. Parasitol Res. 2019;118(1):181–9.
12. Fritzsche C, Riebold D, Fuehrer A, Mitzner A, Klammt S, Mueller-Hilke B, et al. *Pneumocystis jirovecii* colonization among renal transplant recipients. Nephrology (Carlton). 2013;18(5):382–7.
13. Vestereng VH, Bishop LR, Hernandez B, Kutty G, Larsen HH, Kovacs JA. Quantitative real-time polymerase chain-reaction assay allows characterization of pneumocystis infection in immunocompetent mice. J Infect Dis. 2004;189(8):1540–4.
14. Kovacs JA, Masur H. Evolving health effects of Pneumocystis: one hundred years of progress in diagnosis and treatment. JAMA. 2009;301(24):2578–85.
15. Gajdusek DC. Pneumocystis carinii; etiologic agent of interstitial plasma cell pneumonia of premature and young infants. Pediatrics. 1957;19(4 Pt 1):543–65.
16. Patterson L, Coyle P, Curran T, Verlander NQ, Johnston J. Changing epidemiology of Pneumocystis pneumonia, Northern Ireland, UK and implications for prevention, 1 July 2011–31 July 2012. J Med Microbiol. 2017;66(11):1650–5.
17. Wickramasekaran RN, Jewell MP, Sorvillo F, Kuo T. The changing trends and profile of pneumocystosis mortality in the United States, 1999–2014. Mycoses. 2017;60(9):607–15.
18. Roux A, Canet E, Valade S, Gangneux-Robert F, Hamane S, Lafabrie A, et al. *Pneumocystis jirovecii* pneumonia in patients with or without AIDS, France. Emerg Infect Dis. 2014;20(9):1490–7.
19. Yale SH, Limper AH. Pneumocystis carinii pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. Mayo Clin Proc. 1996;71(1):5–13.
20. Roblot F, Le Moal G, Kauffmann-Lacroix C, Bastides F, Boutoille D, Verdon R, et al. *Pneumocystis jirovecii* pneumonia in HIV-negative patients: a prospective study with focus on immunosuppressive drugs and markers of immune impairment. Scand J Infect Dis. 2014;46(3):210–4.
21. Rodriguez M, Fishman JA. Prevention of infection due to Pneumocystis spp. in human immunodeficiency virus-negative immunocompromised patients. Clin Microbiol Rev. 2004;17(4):770–82, table of contents.
22. Mansharamani NG, Garland R, Delaney D, Koziel H. Management and outcome patterns for adult Pneumocystis carinii pneumonia, 1985 to 1995: comparison of HIV-associated cases to other immunocompromised states. Chest. 2000;118(3):704–11.

23. Cooley L, Dendle C, Wolf J, Teh BW, Chen SC, Boutlis C, et al. Consensus guidelines for diagnosis, prophylaxis and management of *Pneumocystis jirovecii* pneumonia in patients with haematological and solid malignancies, 2014. Intern Med J. 2014;44(12b):1350–63.
24. Messiaen PE, Cuyx S, Dejagere T, van der Hilst JC. The role of CD4 cell count as discriminatory measure to guide chemoprophylaxis against *Pneumocystis jirovecii* pneumonia in human immunodeficiency virus-negative immunocompromised patients: a systematic review. Transpl Infect Dis. 2017;19(2)
25. Baulier G, Issa N, Gabriel F, Accoceberry I, Camou F, Duffau P. Guidelines for prophylaxis of Pneumocystis pneumonia cannot rely solely on CD4-cell count in autoimmune and inflammatory diseases. Clin Exp Rheumatol. 2018;36(3):490–3.
26. Neofytos D, Hirzel C, Boely E, Lecompte T, Khanna N, Mueller NJ, et al. *Pneumocystis jirovecii* pneumonia in solid organ transplant recipients: a descriptive analysis for the Swiss transplant cohort. Transpl Infect Dis. 2018;20(6):e12984.
27. Iriart X, Bouar ML, Kamar N, Berry A. Pneumocystis pneumonia in solid-organ transplant recipients. J Fungi (Basel). 2015;1(3):293–331.
28. Fishman JA, Gans H, Practice ASTIDCo. Pneumocystis jiroveci in Solid Organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;e13587
29. Werbel WA, Ison MG, Angarone MP, Yang A, Stosor V. Lymphopenia is associated with late onset *Pneumocystis jirovecii* pneumonia in solid organ transplantation. Transpl Infect Dis. 2018;20(3):e12876.
30. Hosseini-Moghaddam SM, Shokoohi M, Singh G, Dufresne SF, Boucher A, Jevnikar A, et al. A multi-center case-control study of the effect of acute rejection and Cytomegalovirus Infection on Pneumocystis Pneumonia (PCP) in solid organ transplant recipients. Clin Infect Dis. 2018;
31. Faure E, Lionet A, Kipnis E, Noel C, Hazzan M. Risk factors for Pneumocystis pneumonia after the first 6 months following renal transplantation. Transpl Infect Dis. 2017;19(5)
32. Yu Q, Jia P, Su L, Zhao H, Que C. Outcomes and prognostic factors of non-HIV patients with *Pneumocystis jirovecii* pneumonia and pulmonary CMV co-infection: a retrospective cohort study. BMC Infect Dis. 2017;17(1):392.
33. Korkmaz Ekren P, Toreyin ZN, Nahid P, Doskaya M, Caner A, Turgay N, et al. The association between Cytomegalovirus co-infection with Pneumocystis pneumonia and mortality in immunocompromised non-HIV patients. Clin Respir J. 2018;12(11):2590–7.
34. Park SY, Jung JH, Kwon H, Shin S, Kim YH, Chong YP, et al. Epidemiology and risk factors associated with *Pneumocystis jirovecii* pneumonia in kidney transplant recipients after 6-month trimethoprim-sulfamethoxazole prophylaxis: a case-control study. Transpl Infect Dis. 2020; e13245
35. Hosseini-Moghaddam SM, Krishnan RJ, Guo H, Kumar D. Cytomegalovirus infection and graft rejection as risk factors for pneumocystis pneumonia in solid organ transplant recipients: a systematic review and meta-analysis. Clin Transpl. 2018;32(8):e13339.
36. Fishman JA. Pneumocystis jiroveci. Semin Respir Crit Care Med. 2020;41(1):141–57.
37. Schuster MG, Cleveland AA, Dubberke ER, Kauffman CA, Avery RK, Husain S, et al. Infections in hematopoietic cell transplant recipients: results from the organ transplant infection project, a multicenter, prospective, cohort study. Open Forum Infect Dis. 2017;4(2):ofx050.
38. Williams KM, Ahn KW, Chen M, Aljurf MD, Agwu AL, Chen AR, et al. The incidence, mortality and timing of Pneumocystis jiroveci pneumonia after hematopoietic cell transplantation: a CIBMTR analysis. Bone Marrow Transplant. 2016;51(4):573–80.
39. Redjoul R, Robin C, Foulet F, Leclerc M, Beckerich F, Cabanne L, et al. *Pneumocystis jirovecii* pneumonia prophylaxis in allogeneic hematopoietic cell transplant recipients: can we always follow the guidelines? Bone Marrow Transplant. 2018;54:1082.
40. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.

41. Maertens J, Cesaro S, Maschmeyer G, Einsele H, Donnelly JP, Alanio A, et al. ECIL guidelines for preventing *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. J Antimicrob Chemother. 2016;71(9):2397–404.
42. Chamilos G, Lionakis MS, Kontoyiannis DP. Call for action: invasive fungal infections associated with Ibrutinib and other small molecule kinase inhibitors targeting immune signaling pathways. Clin Infect Dis. 2018;66(1):140–8.
43. Dioverti MV, Abu Saleh OM, Tande AJ. Infectious complications in patients on treatment with Ruxolitinib: case report and review of the literature. Infect Dis (Lond). 2018;50(5):381–7.
44. Jones JA, Robak T, Brown JR, Awan FT, Badoux X, Coutre S, et al. Efficacy and safety of idelalisib in combination with ofatumumab for previously treated chronic lymphocytic leukaemia: an open-label, randomised phase 3 trial. Lancet Haematol. 2017;4(3):e114–e26.
45. Yiannakis EP, Boswell TC. Systematic review of outbreaks of *Pneumocystis jirovecii* pneumonia: evidence that *P. jirovecii* is a transmissible organism and the implications for healthcare infection control. J Hosp Infect. 2016;93(1):1–8.
46. Veronese G, Ammirati E, Moioli MC, Baldan R, Orcese CA, De Rezende G, et al. Single-center outbreak of *Pneumocystis jirovecii* pneumonia in heart transplant recipients. Transpl Infect Dis. 2018;20(3):e12880.
47. White PL, Price JS, Backx M. Therapy and management of *Pneumocystis jirovecii* infection. J Fungi (Basel). 2018;4(4)
48. Vasconcelles MJ, Bernardo MV, King C, Weller EA, Antin JH. Aerosolized pentamidine as pneumocystis prophylaxis after bone marrow transplantation is inferior to other regimens and is associated with decreased survival and an increased risk of other infections. Biol Blood Marrow Transplant. 2000;6(1):35–43.
49. Branten AJ, Beckers PJ, Tiggeler RG, Hoitsma AJ. Pneumocystis carinii pneumonia in renal transplant recipients. Nephrol Dial Transplant. 1995;10(7):1194–7.
50. Olsen SL, Renlund DG, O'Connell JB, Taylor DO, Lassetter JE, Eastburn TE, et al. Prevention of Pneumocystis carinii pneumonia in cardiac transplant recipients by trimethoprim sulfamethoxazole. Transplantation. 1993;56(2):359–62.
51. Purdy BH, Philips DM, Summers RW. Desensitization for sulfasalazine skin rash. Ann Intern Med. 1984;100(4):512–4.
52. Leoung GS, Stanford JF, Giordano MF, Stein A, Torres RA, Giffen CA, et al. Trimethoprim-sulfamethoxazole (TMP-SMZ) dose escalation versus direct rechallenge for Pneumocystis Carinii pneumonia prophylaxis in human immunodeficiency virus-infected patients with previous adverse reaction to TMP-SMZ. J Infect Dis. 2001;184(8):992–7.
53. Green H, Paul M, Vidal L, Leibovici L. Prophylaxis of Pneumocystis pneumonia in immunocompromised non-HIV-infected patients: systematic review and meta-analysis of randomized controlled trials. Mayo Clin Proc. 2007;82(9):1052–9.
54. Green H, Paul M, Vidal L, Leibovici L. Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients. Cochrane Database Syst Rev. 2007;3:CD005590.
55. Souza JP, Boeckh M, Gooley TA, Flowers ME, Crawford SW. High rates of Pneumocystis carinii pneumonia in allogeneic blood and marrow transplant recipients receiving dapsone prophylaxis. Clin Infect Dis. 1999;29(6):1467–71.
56. Urbancic KF, Pisasale D, Wight J, Trubiano JA. Dapsone safety in hematology patients: pathways to optimizing *Pneumocystis jirovecii* pneumonia prophylaxis in hematology malignancy and transplant recipients. Transpl Infect Dis. 2018;20(6):e12968.
57. Sangiolo D, Storer B, Nash R, Corey L, Davis C, Flowers M, et al. Toxicity and efficacy of daily dapsone as Pneumocystis jiroveci prophylaxis after hematopoietic stem cell transplantation: a case-control study. Biol Blood Marrow Transplant. 2005;11(7):521–9.
58. Chan C, Montaner J, Lefebvre EA, Morey G, Dohn M, McIvor RA, et al. Atovaquone suspension compared with aerosolized pentamidine for prevention of Pneumocystis carinii pneumonia in human immunodeficiency virus-infected subjects intolerant of trimethoprim or sulfonamides. J Infect Dis. 1999;180(2):369–76.

59. El-Sadr WM, Murphy RL, Yurik TM, Luskin-Hawk R, Cheung TW, Balfour HH Jr, et al. Atovaquone compared with dapsone for the prevention of Pneumocystis carinii pneumonia in patients with HIV infection who cannot tolerate trimethoprim, sulfonamides, or both. Community Program for Clinical Research on AIDS and the AIDS Clinical Trials Group. N Engl J Med. 1998;339(26):1889–95.
60. Beck-Sague C, Dooley SW, Hutton MD, Otten J, Breeden A, Crawford JT, et al. Hospital outbreak of multidrug-resistant *Mycobacterium tuberculosis* infections. Factors in transmission to staff and HIV-infected patients. JAMA. 1992;268(10):1280–6.
61. Hardy WD, Northfelt DW, Drake TA. Fatal, disseminated pneumocystosis in a patient with acquired immunodeficiency syndrome receiving prophylactic aerosolized pentamidine. Am J Med. 1989;87(3):329–31.
62. Mahon FX, Sadoun A, Benz-Lemoine E, Roblot-Casenave F, Guilhot F, Tanzer J. Possible prevention of Pneumocystis carinii pneumonia by pentamidine aerosol after bone marrow transplantation. Bone Marrow Transplant. 1991;8(1):64–5.
63. Link H, Vohringer HF, Wingen F, Bragas B, Schwardt A, Ehninger G. Pentamidine aerosol for prophylaxis of Pneumocystis carinii pneumonia after BMT. Bone Marrow Transplant. 1993;11 (5):403–6.
64. Machado CM, Macedo MC, Medeiros RS, Massumoto C, Silva AC, Castelli JB, et al. Primary Pneumocystis carinii prophylaxis with aerosolized pentamidine after bone marrow transplantation. Acta Haematol. 1998;99(1):54–6.
65. Marras TK, Sanders K, Lipton JH, Messner HA, Conly J, Chan CK. Aerosolized pentamidine prophylaxis for Pneumocystis carinii pneumonia after allogeneic marrow transplantation. Transpl Infect Dis. 2002;4(2):66–74.
66. Schmidt JJ, Lueck C, Ziesing S, Stoll M, Haller H, Gottlieb J, et al. Clinical course, treatment and outcome of Pneumocystis pneumonia in immunocompromised adults: a retrospective analysis over 17 years. Crit Care. 2018;22(1):307.
67. Kanne JP, Yandow DR, Meyer CA. Pneumocystis jiroveci pneumonia: high-resolution CT findings in patients with and without HIV infection. AJR Am J Roentgenol. 2012;198(6): W555–61.
68. Ebner L, Walti LN, Rauch A, Furrer H, Cusini A, Meyer AM, et al. Clinical course, radiological manifestations, and outcome of *Pneumocystis jirovecii* pneumonia in HIV patients and renal transplant recipients. PLoS One. 2016;11(11):e0164320.
69. Turner D, Schwarz Y, Yust I. Induced sputum for diagnosing Pneumocystis carinii pneumonia in HIV patients: new data, new issues. Eur Respir J. 2003;21(2):204–8.
70. Kovacs JA, Ng VL, Masur H, Leoung G, Hadley WK, Evans G, et al. Diagnosis of Pneumocystis carinii pneumonia: improved detection in sputum with use of monoclonal antibodies. N Engl J Med. 1988;318(10):589–93.
71. Alanio A, Hauser PM, Lagrou K, Melchers WJ, Helweg-Larsen J, Matos O, et al. ECIL guidelines for the diagnosis of *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. J Antimicrob Chemother. 2016;71(9):2386–96.
72. Robert-Gangneux F, Belaz S, Revest M, Tattevin P, Jouneau S, Decaux O, et al. Diagnosis of *Pneumocystis jirovecii* pneumonia in immunocompromised patients by real-time PCR: a 4-year prospective study. J Clin Microbiol. 2014;52(9):3370–6.
73. Fan LC, Lu HW, Cheng KB, Li HP, Xu JF. Evaluation of PCR in bronchoalveolar lavage fluid for diagnosis of *Pneumocystis jirovecii* pneumonia: a bivariate meta-analysis and systematic review. PLoS One. 2013;8(9):e73099.
74. Bienvenu AL, Traore K, Plekhanova I, Bouchrik M, Bossard C, Picot S. Pneumocystis pneumonia suspected cases in 604 non-HIV and HIV patients. Int J Infect Dis. 2016;46:11–7.
75. Huh HJ, Lim KR, Ki CS, Huh K, Shim HJ, Song DJ, et al. Comparative evaluation between the RealStar *Pneumocystis jirovecii* PCR kit and the AmpliSens *Pneumocystis jirovecii* (carinii)-FRT PCR kit for detecting *P. jirovecii* in non-HIV immunocompromised patients. Ann Lab Med. 2019;39(2):176–82.

76. Botterel F, Cabaret O, Foulet F, Cordonnier C, Costa JM, Bretagne S. Clinical significance of quantifying *Pneumocystis jirovecii* DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol. 2012;50(2):227–31.
77. Montesinos I, Brancart F, Schepers K, Jacobs F, Denis O, Delforge ML. Comparison of 2 real-time PCR assays for diagnosis of *Pneumocystis jirovecii* pneumonia in human immunodeficiency virus (HIV) and non-HIV immunocompromised patients. Diagn Microbiol Infect Dis. 2015;82(2):143–7.
78. Morris A, Wei K, Afshar K, Huang L. Epidemiology and clinical significance of pneumocystis colonization. J Infect Dis. 2008;197(1):10–7.
79. Lee HY, Kang HS, Lee HY, Rhee CK, Lee SY, Kim SC, et al. Clinical significance of positive *Pneumocystis jirovecii* polymerase chain reaction in non-human immunodeficiency virus immunocompromised patients in a real practice. Korean J Intern Med. 2017;32(3):478–85.
80. Fauchier T, Hasseine L, Gari-Toussaint M, Casanova V, Marty PM, Pomares C. Detection of *Pneumocystis jirovecii* by quantitative PCR to differentiate colonization and pneumonia in immunocompromised HIV-positive and HIV-negative patients. J Clin Microbiol. 2016;54 (6):1487–95.
81. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611–22.
82. Held J, Koch MS, Reischl U, Danner T, Serr A. Serum (1 → 3)-beta-D-glucan measurement as an early indicator of *Pneumocystis jirovecii* pneumonia and evaluation of its prognostic value. Clin Microbiol Infect. 2011;17(4):595–602.
83. Wang D, Hu Y, Li T, Rong HM, Tong ZH. Diagnosis of *Pneumocystis jirovecii* pneumonia with serum cell-free DNA in non-HIV-infected immunocompromised patients. Oncotarget. 2017;8 (42):71946–53.
84. Yoon C, Subramanian A, Chi A, Crothers K, Meshnick SR, Taylor SM, et al. Dihydropteroate synthase mutations in Pneumocystis pneumonia: impact of applying different definitions of prophylaxis, mortality endpoints and mutant in a single cohort. Med Mycol. 2013;51 (6):568–75.
85. Montesinos I, Delforge ML, Ajjaham F, Brancart F, Hites M, Jacobs F, et al. Evaluation of a new commercial real-time PCR assay for diagnosis of *Pneumocystis jirovecii* pneumonia and identification of dihydropteroate synthase (DHPS) mutations. Diagn Microbiol Infect Dis. 2017;87(1):32–6.
86. Li WJ, Guo YL, Liu TJ, Wang K, Kong JL. Diagnosis of pneumocystis pneumonia using serum (1-3)-beta-D-Glucan: a bivariate meta-analysis and systematic review. J Thorac Dis. 2015;7 (12):2214–25.
87. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, et al. Diagnostic accuracy of serum 1,3-beta-D-glucan for pneumocystis jiroveci pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol. 2012;50(1):7–15.
88. Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. Accuracy of beta-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: a meta-analysis. Clin Microbiol Infect. 2013;19(1):39–49.
89. Nakamura H, Tateyama M, Tasato D, Haranaga S, Yara S, Higa F, et al. Clinical utility of serum beta-D-glucan and KL-6 levels in *Pneumocystis jirovecii* pneumonia. Intern Med. 2009;48 (4):195–202.
90. Lahmer T, da Costa CP, Held J, Rasch S, Ehmer U, Schmid RM, et al. Usefulness of 1,3 Beta-D-glucan detection in non-HIV immunocompromised mechanical ventilated critically ill patients with ARDS and suspected *Pneumocystis jirovecii* pneumonia. Mycopathologia. 2017;182 (7–8):701–8.
91. Damiani C, Le Gal S, Da Costa C, Virmaux M, Nevez G, Totet A. Combined quantification of pulmonary *Pneumocystis jirovecii* DNA and serum (1→3)-beta-D-glucan for differential diagnosis of pneumocystis pneumonia and Pneumocystis colonization. J Clin Microbiol. 2013;51 (10):3380–8.

92. Matsumura Y, Shindo Y, Iinuma Y, Yamamoto M, Shirano M, Matsushima A, et al. Clinical characteristics of Pneumocystis pneumonia in non-HIV patients and prognostic factors including microbiological genotypes. BMC Infect Dis. 2011;11:76.
93. Held J, Wagner D. Beta-d-glucan kinetics for the assessment of treatment response in *Pneumocystis jirovecii* pneumonia. Clin Microbiol Infect. 2011;17(7):1118–22.
94. Maschmeyer G, Helweg-Larsen J, Pagano L, Robin C, Cordonnier C, Schellongowski P, et al. ECIL guidelines for treatment of *Pneumocystis jirovecii* pneumonia in non-HIV-infected haematology patients. J Antimicrob Chemother. 2016;71(9):2405–13.
95. Kosaka M, Ushiki A, Ikuyama Y, Hirai K, Matsuo A, Hachiya T, et al. A Four-center retrospective study of the efficacy and toxicity of low-dose trimethoprim-sulfamethoxazole for the treatment of pneumocystis pneumonia in patients without HIV infection. Antimicrob Agents Chemother. 2017;61(12):e01173.
96. Ponce CA, Chabe M, George C, Cardenas A, Duran L, Guerrero J, et al. High prevalence of *Pneumocystis jirovecii* Dihydropteroate synthase gene mutations in patients with a first episode of pneumocystis pneumonia in Santiago, Chile, and clinical response to trimethoprim-sulfamethoxazole therapy. Antimicrob Agents Chemother. 2017;61(2):e01290.
97. Wieruszewski PM, Barreto JN, Frazee E, Daniels CE, Tosh PK, Dierkhising RA, et al. Early corticosteroids for Pneumocystis pneumonia in adults without HIV are not associated with better outcome. Chest. 2018;154(3):636–44.

Coccidioidomycosis in Transplantation

50

Brandon T. Nokes and Janis E. Blair

Contents

Introduction 1207
Coccidioidomycosis in Solid Organ and Hematologic Stem Cell Transplantation 1208
Epidemiology 1208
Mycology and Immunity 1209
Clinical Manifestations 1210
Laboratory Diagnosis of Coccidioidomycosis 1211
Prevention of Coccidioidomycosis in Transplantation 1212
Treatment of Coccidioidomycosis in Transplantation 1217
Hematopoietic Stem Cell (Blood and Marrow) Transplantation (HSCT) 1218
Conclusion 1220
Key Points 1221
Cross-References 1221
References 1221

Introduction

Coccidioidomycosis is a common fungal infection of the desert southwestern USA and has been the subject of much study among transplant programs in the endemic area. However, because of the ease and accessibility of travel to endemic

Brandon T. Nokes and Janis Blair contributed equally with all other contributors.

B. T. Nokes
Division of Pulmonary, Critical Care, and Sleep Medicine, University of California, San Diego, CA, USA
e-mail: bnokes@ucsd.edu

J. E. Blair (✉)
Division of Infectious Diseases, Mayo Clinic Arizona, Phoenix, AZ, USA
e-mail: blair.janis@mayo.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_49

areas, donors and recipients can bring transplant-related coccidioidomycosis to transplant programs in non-endemic areas. In this chapter, we will discuss the epidemiology, clinical manifestations, diagnostics, prophylaxis, and treatment strategies in those undergoing or who have undergone solid organ transplantation. We will also briefly discuss the limited available data pertaining to coccidioidomycosis in stem cell transplant patients.

Coccidioidomycosis in Solid Organ and Hematologic Stem Cell Transplantation

Epidemiology

Coccidioidomycosis is caused by the dimorphic soil fungi *Coccidioides immitis* and *C. posadasii*. These infections occur predominantly in the southwestern USA, adjacent northern Mexico, and parts of Central and South America, but cases of infections have also occurred in Northern California, Oregon [1], and Washington state [2], areas that are not normally considered to be regions endemic for *Coccidioides*. Primary pulmonary coccidioidomycosis is colloquially referred to as "valley fever" because of its original descriptions of clinical manifestations among patients in the San Joaquin Valley in California [3]. For years, nearly two-thirds of all reported symptomatic coccidioidomycosis occurred in Arizona, while roughly one-third of cases occurred in California [1, 4]. Recently, however, the numbers from Arizona and California are becoming similar. In 2018, Arizona reported 7626 cases [5] (approximately 111 per 100,000), and in 2017, California reported 7466 cases [6] (approximately 19 per 100,000).

Among the general population, those at risk for acquiring infection include those who reside or travel to *Coccidioides*-endemic areas. Within that population, persons who spend large amounts of time outdoors either for work or recreation, especially in the presence of soil disruption, have increased risk for acquiring coccidioidomycosis [7]. Risk factors for severe pulmonary infections and *Coccidioides*-related hospitalizations include age >65 years, male sex [8, 9], Black race and Hispanic ethnicity [8, 9], older ages [9], diabetes mellitus [8], tobacco use [8], and immunosuppressed states [8]. In one study of coccidioidomycosis-related hospitalizations, nearly half were for pulmonary disease, and the other half occurred in patients with a wide array of extrapulmonary manifestations [9]. Immunosuppression in a wide variety of forms (including individuals with HIV and CD4 counts <250 cells/ml, those receiving tumor necrosis factor-α (TNF-α) inhibition, persons with hematologic malignancies, those receiving high-dose steroids, or those receiving immunosuppressants for solid organ transplantation) substantially increases the likelihood of symptomatic coccidioidal infection in individuals residing in endemic areas [10, 11]. The presence of malignant solid tumors does not appear to increase risk for coccidioidal infection [12].

Extrathoracic infections, which most commonly manifest in the skin, lymph nodes and adjacent soft tissues, and musculoskeletal and central nervous systems

(CNS), occur very infrequently (less than 1% of all infections) in the general public [13]. Risk factors for extrathoracic coccidioidomycosis include male sex, infection acquired in the third trimester of pregnancy, various immunosuppressing illnesses and medications (especially those that impair the cellular immune pathway, including antirejection therapies for organ and hematologic stem cell transplantation), and alterations of critical immune pathways such as the interleukin-12/interferon-γ and STAT3 (a mediator of IL12 signaling) axes [14]. Additionally, extrathoracic infection is thought to be more common in individuals of Filipino and African American descent for reasons that are not clear [15], but perhaps related to inherited defects in immunity [16]. Risk factors for coccidioidomycosis-related death in the general population reflect the same risk factors for severe or extrapulmonary infection. Advanced age, especially individuals greater than 75 years of age, is at high risk of mortality [17].

Individuals who have undergone solid organ transplant are at increased risk for symptomatic coccidioidal infection if they live within or frequently travel to a *Coccidioides*-endemic area [10, 18–20]. Inexplicably, however, while positive coccidioidal serology at the time of transplantation is a risk factor for coccidioidomycosis following transplantation, the presence of old granuloma on chest imaging is not [21]. Coccidioidomycosis in transplant recipients can manifest as a result of reactivation of infection acquired prior to transplantation, infection acquired through the donor organ, or de novo acquisition following transplantation. Prior to the programmatic institution of routine screening and antifungal prophylaxis, coccidioidal infection among transplant recipients was seen in 4–9% of such patients within the endemic area [19, 20]. Since the initiation of screening and prophylaxis programs in these areas, rates of coccidioidal infections have improved (2–3%) [11].

Mycology and Immunity

Outside of an animal host, *Coccidioides* lives as mycelia in the top layers of desert soil. When the soil is disrupted, arthroconidia are released into the air, acting as spores. Once inhaled, arthroconidia morph in the lung microenvironment into mature spherules, containing thousands of endospores which, when released after spherule rupture, are capable of continuing local coccidioidal infection or disseminating disease outside the thorax. Released endospores beget mature spherules and can extend the infection [22]. Once an arthroconidium is inhaled, a complex cascade of immune activity is activated to control infection and includes both innate and adaptive immunities.

When the innate immune system is activated, polymorphonuclear leukocytes are recruited to ingest arthroconidia, followed by the influx of natural killer cells [23]. Human macrophages are recruited to phagocytose and kill *Coccidioides*, a function that depends on IL-12/IFN-γ signaling [14]. Recruited dendritic cells are potent antigen-presenting cells, which then signal and set off the complex cascade of inflammatory cytokines that initiate adaptive immunity. Immunity to

coccidioidomycosis is dependent on the subsequent induction of Th1-related immune responses and establishment of cellular immunity [23]. Controlled and quiescent infection is often associated with the presence of granulomas in the lung or lymphatic tissue. The antifungals used (such as triazoles or amphotericin) to control coccidioidal infections do not appear to be fungicidal for this organism. Therefore, when "old" granulomas are sampled in an immunocompetent host, and cultured in the microbiology laboratory, growth of *Coccidioides* is frequently observed, regardless of whether the patient was previously administered antifungal treatment. Thus, following solid organ or hematopoietic stem cell transplantation (HSCT), with subsequent impairment of cell-mediated immunity, dormant organisms can potentially reactivate from either donor organs or recipients in the absence of antifungal prophylaxis. That cellular immunity maintains control of coccidioidal infection is of critical importance in discussions of transplantation, because antirejection treatments used in transplantation impair the same cellular immune responses that keep coccidioidal infections under control.

Clinical Manifestations

Infections from *Coccidioides immitis* and *Coccidioides posadasii* appear to be clinically indistinguishable. Most individuals (~60%), once exposed to *Coccidioides*, will be asymptomatic. In those infected, 95% of coccidioidal infections are limited to the respiratory system, and the most commonly recognized manifestation is coccidioidal pneumonia, accounting for 15–29% of community-acquired pneumonias in Arizona [24–26]. Symptoms of primary pulmonary coccidioidomycosis can be variable, and include a wide array of nonspecific complaints, but typically include one or more of the following: constitutional symptoms (fever, chills, night sweats, anorexia, headache, malaise, or fatigue), pulmonary symptoms (dyspnea, chest pain or tightness, cough), and often symptoms of hypersensitivity (such as arthralgia, arthritis, and a variety of rashes). Among recently located residents into a *Coccidioides*-endemic area, nearly two-thirds with newly developed coccidioidin skin test (indicative of recent infection) had no symptoms [27].

The primary manifestations of coccidioidomycosis in the transplant recipient predominantly reflect the pulmonary infection, but there is a notably greater propensity toward multi-organ, extrapulmonary infection. The symptoms of such disseminated infection include the same systemic symptoms as noted, and there may or may not be pain resulting from inflammatory lesions or abscesses in the particular area of involvement. Early studies indicated that transplant recipients whose course was complicated by coccidioidomycosis had concurrent pulmonary and extrathoracic infection in 75%, with 63% associated with mortality [28, 29]. More contemporary studies from transplant programs in the endemic area showed that, even with high index of suspicion and early empiric antifungal treatment, patients with new, symptomatic post-transplant coccidioidomycosis still experienced extrathoracic dissemination in 30%, and mortality rates range from 13% to 29% [20, 21, 30–32].

With current screening, treatment, and a 1-year post-transplant universal prophylaxis strategy, one transplant program diagnosed no patients with coccidioidomycosis in the first transplant year [33, 34], and fewer patients overall have contracted coccidioidomycosis. Coccidioidomycosis may still occur after the first post-transplant year, when the level of immunosuppression is lower than the level in the first year, and the first year antifungal prophylaxis has been withdrawn [10]. In a review of 91 solid organ recipients infected with coccidioidomycosis after the first post-transplant year, 56% were asymptomatic (identified by routine serologic screening), while 41% had primary coccidioidal pneumonia. While far fewer patients had severe (22% hospitalized) or disseminated (5.5%) infection, death due to coccidioidomycosis still occurred (1.1%) [10].

Laboratory Diagnosis of Coccidioidomycosis

An important tenant in the recognition of coccidioidomycosis is to think about the diagnosis. To this end, clinical history and exposure status (travel or residence) are important, and there should always be a suspicion of coccidioidomycosis for unidentified febrile illnesses in the transplant patient.

Although early recognition, identification, and treatment are paramount, it can be difficult to assess for active infection with standard lab tests. While 90% of immunocompetent adults with symptomatic coccidioidomycosis have a positive EIA IgG within 4 weeks after infection [35], the routine positivity of these serologic assays in acute infection cannot be assured in those who have suppression of the humoral or cellular immune systems, and the use of multiple methodologies (for diagnosis or screening) is recommended [35]. In a study of 27 transplant recipients with acute symptomatic coccidioidomycosis, initial serology using 3 different methodologies [enzyme immunoassay (EIA) for immunoglobulins IgM and IgG, complement fixation (CF) IgG, and immunodiffusion (ID) IgM and IgG] had a diagnostic sensitivity of 77%, but when repeated, the sensitivity increased to 92% [36]. A complement fixation titer of greater than 1:16 reflects substantial disease burden, but any positive serologic finding indicates the need for continued infection management, follow-up, and monitoring with serial serologies. Cultures of clinical specimens such as respiratory secretions had a sensitivity of approximately 50%, and adjunctive studies were helpful; tissue biopsies, polymerase chain reaction, and cytology had sensitivities of 75%, 60%, and 33%, respectively [36]. Coccidioidal antigen has proven helpful for diagnosis in a small study that included a few transplant recipients [37]. Another non-serologic test of value is the serum (1,3)-beta-D-glucan (sensitivity 43%, specificity 91%; positive and negative predictive values of 82% and 64%, respectively) [38].

Antibodies to coccidioidomycosis develop between 2 weeks and 2 months (or longer) following the onset of coccidioidal infection and are present during active infection. As cell-mediated immunity controls the infection, antibodies wane and eventually disappear over time [39, 40]. Remote infection, completely controlled, is generally not associated with seropositivity [39, 40]. Therefore, antibodies to

coccidioidomycosis are not "once positive, always positive," and the presence of IgG does not imply that infection is controlled and immunity is complete.

Radiographically, acute coccidioidal pneumonia is heterogeneous and appears as a nonspecific consolidation or mass, with or without hilar or mediastinal lymphadenopathy. Miliary, cavitary, and destructive intrapleural forms can be seen. Although there are no set rules as to how pulmonary coccidioidomycosis can manifest, the presence of pneumonia with ipsilateral hilar adenopathy in an endemic area is highly suggestive of coccidioidal infection [41].

Prevention of Coccidioidomycosis in Transplantation

Individuals undergoing heart, lung, kidney, and liver transplant are at increased risk of coccidioidal infection in endemic areas [19–21, 30, 42]. Recipients of other solid organ transplants have not been systematically studied but are presumed to overlap in their risk given the similarities across solid organ transplant immunosuppressive regimens. To this end, there are three modes of coccidioidal infection worth considering in the transplant recipient: [1] recrudescence of old infection in the recent transplant recipient, [2] primary infection after transplant, and [3] donor-derived infection [43]. It is not known whether particular types of solid organ transplants or post-transplant immunosuppressive regimens increase risk of coccidioidal infection relative to others.

Prevention of coccidioidomycosis in transplant recipients begins prior to the transplantation process and starts with identification (screening history and physical examination, chest radiographs, serology) and treatment of patients with active or subclinical infection.

Screening. In one transplant center within the endemic area, all donors and recipients are screened for evidence of active (but not necessarily symptomatic) *Coccidioides* infection prior to (and day of) transplantation to identify and treat subclinical infection prior to initiation of antirejection therapy. Additional screening occurs at regular intervals thereafter (generally 2–3 times in the first post-transplant year and annually thereafter). The recommended screening protocol includes history and physical examination, a chest radiograph, and serologies by EIA for IgM and IgG, complement fixation for IgG, and immunodiffusion for IgM and IgG. The combined sensitivity for detecting subclinical infection for these assays is 95% [21]. Another transplant program in an area of lower *Coccidioides* endemicity screens potential recipients from high endemic areas with the EIA only [44]. A positive screening serologic test implies some level of active infection. Since seropositivity implies recent or current infection, screening for such antibodies is believed to be a point at which intervention can occur by the use of antifungal medications to suppress or treat infections, whether overt or subclinical.

When undertaking serologic screening for coccidioidomycosis, false-positive serologies or serological results of uncertain significance are problematic [45], and if these problematic serologies cannot be adjudicated at the time of history and physical examination, we repeat testing in 1 months' time to assess for evidence of

serologic conversion. Because of low pretest probability in non-endemic areas, it is not necessary (or advisable) to screen for coccidioidal infection unless there has been travel to (or residence within) an endemic area temporally associated with a febrile or respiratory illness or history of coccidioidomycosis [44].

Once screening has identified possible infection, an evaluation by an infectious diseases specialist with knowledge of this infection is recommended to assess the activity of infection and whether treatment of active infection or suppression of subclinical infection is warranted. Computed tomography of the chest is helpful to assess the presence of early findings (such as infiltrate or mass) versus later findings of nodules, cavities, or granulomas.

When screening identifies active coccidioidal infection in recipients or donors, the infections should be treated before transplantation (Table 3) if clinically feasible. Once there has been symptom resolution, marked serologic improvement (our program requires at least 2 dilution lowering of the CF and a titer no higher than 1:2 or 1:4 on 2 occasions), and improved and stable radiographs, patients may proceed to transplant surgery and immunosuppression. Six to 12 months (or more) of antifungal treatment may be required to achieve these clearance goals. Our program requires a minimum of 6-month antifungal treatment for clearance of active infection, even if other clearance goals (i.e., symptoms, radiographs, and serology) have been reached. Following transplantation of these patients, 1 year of full-strength azole (equivalent to 400 mg daily of fluconazole) should be provided, followed by an annual evaluation by an infectious disease specialist to assess candidacy for lowering the dose of antifungal medication to a suppression dose. At the present time, for patients with any serologic evidence of active coccidioidomycosis at the time of transplantation, our program requires antifungal suppression for as long as the patient is receiving an immunosuppressive regimen. Whether there are specific groups who can discontinue antifungal suppression is unknown.

Prevention of primary coccidioidal infection following solid organ transplantation. Table 1 summarizes potential prophylaxis strategies to prevent coccidioidomycosis. Within the first year following transplantation, recipients without prior infection, who are living in the endemic area, appear to benefit from primary anti-coccidioidomycosis prophylaxis strategies. The risk of primary infection after transplant is greatest during the first 6–12 months, and this increased risk is thought to reflect the greater relative immunosuppression during this time [46]. During this time, immunosuppressive strategies aim to deplete T-cell populations and abrogate lymphocyte recruitment through direct and indirect blockade of cytokine-mediated lymphocyte proliferation and trafficking (particularly interleukin-2). This immunosuppressive therapy minimizes the risk of both cellular and humoral forms of rejection but also creates an increased risk for infection (including coccidioidal infection) [46].

With this relative increased risk of post-transplant infection, one transplant program in the *Coccidioides*-endemic area implemented a practice of universal antifungal primary prophylaxis with fluconazole 200 mg daily (or its triazole equivalent) during the first 6–12 months post-transplant in all solid organ recipients. This decision was based on the observation that targeted antifungal prophylaxis did

not eliminate de novo coccidioidomycosis, which occurred most frequently in the first 6–12 months after transplantation [32], and was associated with high morbidity and mortality [46]. Accordingly, there has been a substantial reduction in post-transplant infection [33].

Following the first post-transplant year, most organ recipients no longer require routine or long-term anti-*Coccidioides* prophylaxis. Since antirejection treatment is an additional risk factor for coccidioidomycosis following transplantation [19], some programs prescribe 3 months of fluconazole 200 mg daily (or its equivalent) following antirejection treatment, especially in transplant recipients at high risk for infection. With such prophylaxis, no coccidioidomycosis has been acquired within this time frame following treatment for acute rejection [10].

Prophylaxis of Recipient of Organs from a Coccidioides-Infected Donor

Finally, a recipient may receive an organ from a *Coccidioides*-infected donor and may require prophylaxis even if they do not reside in or travel to the

Table 1 Proposed coccidioidomycosis prophylaxis strategies for transplant recipients

Patient group	Prophylaxis strategy
Strategies for patients within *Coccidioides*-endemic regions	
All patients living in endemic region, who do not fit into a category below	Fluconazole 200 mg daily × 1 year beginning on the day of transplantation For treatment of acute rejection, after primary prophylaxis is completed: Consider fluconazole 200 mg/day × 3 months
Coccidioidomycosis prior to transplant; proceed with transplantation if all criteria met: Symptoms have resolved Cocci CF titer low or negative Radiographs resolved/stable	If seropositive at transplant: Fluconazole 400 mg/day × 1 year post-transplantation After 1 year, continue fluconazole 200 mg/day if seronegative If seronegative at transplant: Fluconazole 200 mg/day × 1 year
Primary coccidioidomycosis after transplantation, after treatment AND Symptoms resolved Serology negative Radiographs stable	Secondary prophylaxis: Fluconazole 200 mg daily for the duration of immunosuppression
Strategies for patients outside the endemic area	
Patients with history of prior residence or travel to endemic area	Screening serology positive at transplant: Fluconazole 400 mg/day × 1 year, then reassess at 1 year for further prophylaxis 200–400 mg/day Screening serology negative: No coccidioidal prophylaxis indicated
Patients without history of prior residence or travel to endemic area No screening serology recommended	No prophylaxis indicated
Post-transplantation relocation to *Coccidioides*-endemic area	Lung: consider fluconazole 200 mg/day Other organs: no prophylaxis indicated

Coccidioides-endemic region. Living donors with exposure to the *Coccidioides*-endemic area should undergo evaluation to assess history of known prior infection or potential infection and then be assessed serologically and radiographically for evidence of coccidioidomycosis [47]. How to best operationalize such assessments for the deceased donor from an endemic area is an unsettled issue [48].

Organ donors from *Coccidioides*-endemic regions may have experienced acute, chronic, asymptomatic, or resolved coccidioidal infection, all of which may harbor live organisms in granulomas, and the potential to transmit infection to the transplant recipient. The risk of transmission likely depends on the organ infected in the donor and the organ received. For example, a lung recipient of a patient with prior pulmonary coccidioidomycosis with residual coccidioidal granulomas in the lung is much more likely to acquire post-transplant coccidioidomycosis [49] than the kidney recipient of the same donor. However, a study of the Organ Procurement and Transplantation Network data identified 6 donors with coccidioidomycosis who donated various organs to 21 recipients, with a transmission rate of 43% and overall mortality of 28% [48]. Among those with preemptive azole treatment, infection was prevented or mitigated, whereas five out of the six recipients who did not receive antifungal prophylaxis died [48].

A number of case reports and case series document the severity of early, donor-derived coccidioidomycosis, which has high mortality [48, 50]. When one recipient is identified, the physicians of the other recipients must be notified, and anti-coccidioidal prophylaxis begun for all recipients of the infected donor. Such prophylaxis is effective in preventing coccidioidomycosis acquired from the donor organs [48, 50]. In the absence of clinical trials, guidance recommendations have been published outlining the approach to such prophylaxis, as summarized in Table 2 [47]. The duration of antifungal prophylaxis for these situations is uncertain, and is often lifelong, especially for lung recipients. Alternatively, in recipients of organs that are less likely to transmit coccidioidal infection, discontinuation of triazole prophylaxis could be considered after the period of highest immunosuppression, if patients are observed closely and understand that the risk of coccidioidal infection after discontinuation of prophylaxis is unknown. All recipients of infected donors should be periodically monitored for evidence of seroconversion.

Effective prophylaxis requires the use of a triazole. If the recipient is already receiving systemic itraconazole, voriconazole, posaconazole, isavuconazole, or amphotericin for prophylaxis or treatment of another fungal agent, additional fluconazole is not needed for prophylaxis of coccidioidomycosis. However, if a recipient is receiving *Aspergillus* prophylaxis with an echinocandin or inhaled amphotericin, additional (or switch) prophylaxis with a triazole will be required [47]. A more complete discussion of issues of prophylaxis for donor-derived coccidioidomycosis has been published [47]. The safety and efficacy of chronic suppressive triazole prophylaxis for endemic fungal infections were studied in 18 organ recipients over a 10-year period; among these, none had reactivated infection, and 1 experienced an adverse drug reaction (itraconazole-related symptomatic bradycardia) requiring medication discontinuation [51].

Table 2 Suggested prophylaxis strategies for organ recipients of a *Coccidioides*-infected donor[a]

Donor with documented coccidioidomycosis		
	Lung recipient	Non-lung recipient
Donor with documented pulmonary coccidioidomycosis but no evidence of extrapulmonary coccidioidomycosis, seropositive, or culture positive	Fluconazole 400 mg daily lifelong	Fluconazole 400 mg daily × 12 months, then 200 mg daily or stop and watch
Donor with documented pulmonary coccidioidomycosis but no evidence of extrapulmonary coccidioidomycosis, seronegative, culture negative	Fluconazole 400 mg daily lifelong	Fluconazole 400 mg daily for 3–6 months, then consider decreased dose 200 mg daily or stop and observe without ongoing prophylaxis
Documented extrapulmonary coccidioidomycosis	Fluconazole 400 mg daily lifelong	Fluconazole 400 mg daily lifelong
Donor with positive serology, but no clear focus of infection	Fluconazole 400 mg daily lifelong	Fluconazole 400 mg daily for 12 months, then consider decreased dose 200 mg daily thereafter

Modified from Singh et al. [47], with permission, endorsed by the American Society of Transplantation Infectious Diseases Community of Practice [60]

[a]All recipients should be monitored periodically for evidence of seroconversion; if discontinuation of prophylaxis is contemplated, regular follow-up clinically, radiographically, and serologically is recommended

Can transplant recipients visit the endemic area, and if so, will they require prophylaxis? The answer to this question is not well-studied. Among the general population in Arizona, the risk of coccidioidomycosis is estimated to be 2–3% per year [52]. Certainly there are published cases of immunosuppressed transplant recipients who have briefly visited the endemic area, and subsequently acquired coccidioidomycosis, some of which had severe manifestations [19]. However, in a small study of 37 liver transplant recipients who relocated to the endemic area, did not receive antifungal prophylaxis, and had at least 1-year follow-up, 1 (2.7%) acquired new coccidioidomycosis [53]. Therefore, we believe the risk of such infection is low, but not zero. When patients are receiving high-level immunosuppression, the risk of symptomatic or disseminated infection is likely higher. However, given drug-drug interactions of triazoles and antirejection treatments such as tacrolimus, there are risks of starting and stopping such prophylaxis. One author suggested the use of such prophylaxis for lung recipients only [42]. Whether prophylaxis should be given for brief visits to the endemic area is an unsettled question.

Non-pharmacologic prevention strategies. Vaccination for the prevention of coccidioidomycosis has been the subject of much study [54]. At the present time, however, there is no vaccine for clinical use. We strongly support current efforts to push this effort forward. In the meantime, avoidance of the endemic area may be possible for some, but not for others, and is not advocated. Theoretically, use of masks to avoid exposure during situations or activities with known or perceived risk

(such as being outside in a dust storm or during digging or landscaping or traversing unpaved roads, etc.) seems logical, but has no proven efficacy.

Treatment of Coccidioidomycosis in Transplantation

Treatment of coccidioidomycosis in transplantation requires the identification of the infection, assessment for the presence of extrathoracic dissemination, and classification of disease severity. An extensive history and physical examination and adjunctive testing will guide this classification.

Recognition of coccidioidomycosis. The symptoms of coccidioidomycosis overlap substantially with community-acquired pneumonia. In addition to respiratory symptoms, patients often complain of significant fatigue, myalgias, and night sweats. Rashes can appear and are highly varied (diffuse macular rash, erythema nodosum, erythema multiforme, or toxic erythema) [55]. Careful attention to symptoms suggesting involvement of the skin, lymph nodes, bones and joints, and/or meninges should help identify extrapulmonary infection.

Antifungal treatment of infection before, during, and after solid organ transplant. As noted above, pre-transplant infection should be treated if possible per standard Infectious Diseases Society of America (IDSA) guidelines (Table 3) [56]. Treatment of de novo, donor-derived, and recrudescent coccidioidal infections is the same as in immunocompetent hosts. Fluconazole and itraconazole are first-line agents, and amphotericin is used (added or substituted) if the infection is rapidly progressing or

Table 3 Initial approach to treatment of coccidioidomycosis [56][a]

Situation	Treatment
Primary pulmonary coccidioidomycosis	Fluconazole 400 mg daily or itraconazole 200 mg BID or TID Lipid-formulated amphotericin if severe or rapidly progressing
Extrapulmonary coccidioidomycosis	
Musculoskeletal Skin/soft tissue Meningitis/CNS	Itraconazole 200 mg BID or TID or fluconazole 400 mg daily Fluconazole 400 mg daily or itraconazole 200 mg BID or TID Fluconazole 800 mg daily
Pregnancy	Triazoles contraindicated, especially in the first trimester Lipid-formulation amphotericin
Other agents for intolerance or salvage	Voriconazole 200–300 mg BID Posaconazole extended release 300 mg daily

Duration of treatment in each case should be individualized, but not less than 12 months. Patients with meningitis/CNS disease should be treated for life

[a]For patients prior to transplantation or following transplantation

BID, twice daily

TID, thrice daily

so unusually severe that management requires the patient to be in the intensive care unit [56]. The level of immunosuppression may need to be decreased to gain control of infection [19].

For patients who do not respond to (or are intolerant of) fluconazole or itraconazole, voriconazole or posaconazole can be used as salvage therapy (Table 4). There is limited experience with isavuconazole in the treatment of coccidioidomycosis [57].

Amphotericin B products (Table 4) have predictable activity versus *Coccidioides* species, and the deoxycholate formulation was used for decades prior to the advent of triazole antifungal agents. However, the amphotericin deoxycholate formulation is fraught with toxicity, including infusional toxicity (fever, rigors, and phlebitis) and acute and chronic kidney injury with nephrogenic losses of potassium and magnesium. The newer lipid formulations of amphotericin have fewer (but still present) toxicities and, although never studied head-to-head with amphotericin deoxycholate, appear to work well in patients who are rapidly deteriorating. Amphotericin formulations are not associated with fetal malformations and are safe for pregnant patients. Intrathecal administration of amphotericin deoxycholate is rarely used anymore, but can be considered for patients with coccidioidal meningitis recalcitrant to triazole therapy (Table 4).

Once the infection is controlled clinically, radiographically, and serologically, secondary suppression is recommended for the duration of immunosuppression. For such suppression (secondary prophylaxis), fluconazole is typically dosed at 200–400 mg daily. Ideally, patients with coccidioidal infection of the central nervous system should not have their triazole dose lowered. Daily azole dosing should not be lowered in patients who have not yet achieved clinical, radiographic, and serologic resolution.

Drug interactions in transplant recipients. Table 4 summarizes selected important drug-drug interactions with azoles in the transplant recipient. Azoles vary in their pharmacologic characteristics, but typically interact with metabolism of tacrolimus and cyclosporine, raising the serum levels of these antirejection medications and risk of related nephrotoxicity. Similarly, azoles variably increase levels and toxicity of sirolimus and, to a lesser extent, everolimus. Therefore, teamwork and close communication with the transplant team are necessary to optimally initiate and discontinue these antifungal regimens.

Follow-up. Depending on the severity of illness in patients with acute coccidioidomycosis, patients may need to be followed closely (e.g., daily while hospitalized and then weekly or monthly as outpatients), but after improvement, follow-up with clinical examination, radiographs, and serology can take place every 2–3 months and then every 3–6 months.

Hematopoietic Stem Cell (Blood and Marrow) Transplantation (HSCT)

Compared to coccidioidomycosis in solid organ transplantation, far less is known about coccidioidal infections in patients who have undertaken HSCT.

Table 4 Antifungal agents for the treatment of coccidioidomycosis in transplant recipients

Agent	Typical dosing in patients with normal kidney function	Notations	Transplantation-relevant drug interactions
First-line agents for coccidioidomycosis			
Fluconazole[b]	400 mg daily for most 800 mg daily for most meningitis	Teratogenic, especially in the first trimester ↑Risk miscarriage	Increases levels of tacrolimus, cyclosporine, and perhaps sirolimus
Itraconazole[b]	200 mg BID or TID	Requires gastric acid for absorption Serum levels required Strong CYP3a inhibitor Teratogenic, especially in the first trimester Black box warning: congestive heart failure and numerous drug interactions	Increased serum levels of tacrolimus, cyclosporine, sirolimus, everolimus
Salvage/second-line agents for coccidioidomycosis			
Voriconazole	200–300 mg BID	Monitor serum trough levels Levels may decline over time Strong CYP3a inhibitor Numerous drug interactions	Increases levels of tacrolimus, cyclosporine, sirolimus, everolimus,
Posaconazole	Liquid formulation: 300 mg BID Extended release capsules: 3 × 100 mg capsules daily	Monitor serum trough levels	Increases levels of tacrolimus, cyclosporine, sirolimus, everolimus,
Agents used in specific circumstances			
Lipid-associated amphotericin products[a]	3–5 mg/kg/day IV for patients with severe/rapidly progressing illness or for control of infection in pregnancy	For patients with severe or rapidly progressing coccidioidomycosis No teratogenicity Nephrotoxicity is less than deoxycholate formulation ↓K, ↓Mg	No drug interactions with any antirejection treatment

(continued)

Table 4 (continued)

Agent	Typical dosing in patients with normal kidney function	Notations	Transplantation-relevant drug interactions
Amphotericin B deoxycholate	Systemic protocol: 0.25–1 mg/kg/day intravenously Intrathecal protocol for recalcitrant meningitis:0.1–1.5 mg/dose IT	For salvage of coccidioidomycosis, if lipid-associated formulations unavailable For salvage of coccidioidal meningitis (IT) No teratogenicity Nephrotoxicity, ↓K, ↓Mg Local and infusional toxicity	No drug interactions with any antirejection treatment

Mg, milligrams
QD, daily
BID, twice daily
TID, thrice daily
IV, intravenous infusion
IT, intrathecal administration
[a]liposomal amphotericin, amphotericin lipid complex, amphotericin B cholesteryl sulfate complex
[b]Will require dosage adjustments in patients with kidney disease

Unlike those undergoing solid organ transplantation, in the absence of relapsed hematologic malignancy or prolonged graft-versus-host disease, the immunosuppression of allogeneic HSCT is more intensive early on, but has a shorter duration than solid organ recipients, and most patients are felt to have normal immunity 2 years following stem cell transplantation. In two back-to-back studies of patients in our HSCT program spanning 20 years (1993–2013), we reviewed the courses of 1318 HSCT patients, of which 439 (33%) were allogeneic HSCT recipients, whose graft-versus-host disease-based immunosuppression was tacrolimus. Among the 439 allogeneic HSCT recipients, 12 (2.7%) had coccidioidomycosis, and 2/12 (16.7%) had extrapulmonary dissemination. Eight of the 12 (66.7%) contracted coccidioidomycosis within 2 years following HSCT, and 6 of these 8 (75%) died with evidence of active coccidioidomycosis [11, 58, 59]. Therefore, we recommend that, for HSCT patients in endemic areas, antifungal prophylaxis with anti-*Coccidioides* activity be continued for 2 years following HSCT.

Conclusion

Organ transplantation can be a successful undertaking within the *Coccidioides*-endemic region. Careful attention to screening and treatment of infection prior to transplantation is important to prevent activation or reactivation following

transplantation. Prophylaxis strategies have undergone study and revision and should target the prevention of coccidioidomycosis in the first post-transplantation year, during acute organ rejection, and in recipients of *Coccidioides*-infected donors. Early recognition and treatment of organ recipients with coccidioidomycosis is key to minimizing morbidity and mortality for patients within and outside the endemic area.

Key Points

- Coccidioidomycosis is an important cause of morbidity in transplant programs in endemic areas.
- Coccidioidomycosis causes infection that ranges from asymptomatic to severe and disseminated infections; among the symptomatic, primary pulmonary infections are most common.
- Immunity, especially cell-mediated immunity, plays an important role in control of coccidioidomycosis in healthy populations. Following transplantation, impaired cellular immunity makes control of infection more difficult.
- Screening potential transplant recipients for active infection prior to transplantation allows treatment and suppression without reactivation following transplantation.
- Post-transplant coccidioidomycosis is complicated by high rates of extra-pulmonary infection and death. A high index of suspicion and early treatment is needed.

Cross-References

► Transplant Tourism and the Traveling Transplant Recipient: Infection Mitigation and Treatment Strategies

References

1. Benedict K, Ireland M, Weinberg MP, Gruninger RJ, Weigand J, Chen L, Perez-Lockett K, Bledsoe C, Denny L, Cibulskas K, Gibbons-Burgener S, Kocharian A, DeBess E, Miller TK, Lepp A, Cronquist L, Warren K, Serrano JA, Loveland C, Turabelidze G, McCotter O, Jackson BR. Enhanced surveillance for coccidioidomycosis, 14 US states, 2016. Emerg Infect Dis. 2018;24:1444–52.
2. Marsden-Haug N, Goldoft M, Ralston C, Limaye AP, Chua J, Hill H, Jecha L, Thompson GR 3rd, Chiller T. Coccidioidomycosis acquired in Washington state. Clin Infect Dis. 2013;56:847–50.
3. Hirschmann JV. The early history of coccidioidomycosis: 1892–1945. Clin Infect Dis. 2007;44:1202–7.
4. Blair JE, Chang YH, Cheng MR, Vaszar LT, Vikram HR, Orenstein R, Kusne S, Ho S, Seville MT, Parish JM. Characteristics of patients with mild to moderate primary pulmonary coccidioidomycosis. Emerg Infect Dis. 2014;20:983–90.

5. Summary of selected reportable diseases. 2018. [cited 2019 4/08/2019]. Available from: https://www.azdhs.gov/documents/preparedness/epidemiology-disease-control/disease-data-statistics-reports/data-statistics-archive/2018/2018-ytd-communicable-disease-summary.pdf.
6. Health CDo. Epidemiologic summary of coccidioidomycosis in California. 2017. [cited 2019 4/08/2019]. Available from: https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/CocciEpiSummary2017.pdf.
7. Freedman M, Jackson BR, McCotter O, Benedict K. Coccidioidomycosis outbreaks, United States and worldwide, 1940–2015. Emerg Infect Dis. 2018;24:417–23.
8. Rosenstein NE, Emery KW, Werner SB, Kao A, Johnson R, Rogers D, Vugia D, Reingold A, Talbot R, Plikaytis BD, Perkins BA, Hajjeh RA. Risk factors for severe pulmonary and disseminated coccidioidomycosis: Kern County, California, 1995–1996. Clin Infect Dis. 2001;32:708–15.
9. Sondermeyer G, Lee L, Gilliss D, Tabnak F, Vugia D. Coccidioidomycosis-associated hospitalizations, California, USA, 2000–2011. Emerg Infect Dis. 2013;19:1590–7.
10. Asbury K, Blair JE, August J, Beatty NL, Mi L, Carey EJ, Huskey JL, LeMond LM, Zangeneh TT. De novo coccidioidomycosis among solid organ transplant recipients 1 or more years after transplant. Am J Transplant. 2019;19(9):2517.
11. Blair JE, Ampel NM, Hoover SE. Coccidioidomycosis in selected immunosuppressed hosts. Med Mycol. 2019;57:S56–63.
12. Fitterer C, Berg Z, Halfdanarson TR, Vikram HR, Kusne S, Orenstein R, Seville MT, Blair JE. Coccidioidomycosis in patients with selected solid organ cancers: a case series and review of medical literature. Mycopathologia. 2016;181:787–98.
13. Parish JM, Blair JE. Coccidioidomycosis. Mayo Clin Proc. 2008;83:343–8; quiz 348–349
14. Odio CD, Marciano BE, Galgiani JN, Holland SM. Risk factors for disseminated coccidioidomycosis, United States. Emerg Infect Dis. 2017;23:308–311.
15. Ruddy BE, Mayer AP, Ko MG, Labonte HR, Borovansky JA, Boroff ES, Blair JE. Coccidioidomycosis in African Americans. Mayo Clin Proc. 2011;86:63–9.
16. Hung CY, Hsu AP, Holland SM, Fierer J. A review of innate and adaptive immunity to coccidioidomycosis. Med Mycol. 2019;57:S85–92.
17. Sondermeyer GL, Lee LA, Gilliss D, Vugia DJ. Coccidioidomycosis-associated deaths in California, 2000–2013. Public Health Rep. 2016;131:531–5.
18. Blair JE, Mulligan DC. Coccidioidomycosis in healthy persons evaluated for liver or kidney donation. Transpl Infect Dis. 2007;9:78–82.
19. Blair JE, Logan JL. Coccidioidomycosis in solid organ transplantation. Clin Infect Dis. 2001;33:1536–44.
20. Chaudhary S, Meinke L, Ateeli H, Knox KS, Raz Y, Ampel NM. Coccidioidomycosis among persons undergoing lung transplantation in the coccidioidal endemic region. Transpl Infect Dis. 2017;19:e12713.
21. Braddy CM, Heilman RL, Blair JE. Coccidioidomycosis after renal transplantation in an endemic area. Am J Transplant. 2006;6:340–5.
22. Kirkland TN, Fierer J. Coccidioidomycosis: a reemerging infectious disease. Emerg Infect Dis. 1996;2:192–9.
23. Cox RA, Magee DM. Coccidioidomycosis: Host response and vaccine development. Clin Microbiol Rev. 2004;17:804–39.
24. Valdivia L, Nix D, Wright M, Lindberg E, Fagan T, Lieberman D, Stoffer T, Ampel NM, Galgiani JN. Coccidioidomycosis as a common cause of community-acquired pneumonia. Emerg Infect Dis. 2006;12:958–62.
25. Kim MM, Blair JE, Carey EJ, Wu Q, Smilack JD. Coccidioidal pneumonia, Phoenix, Arizona, USA, 2000–2004. Emerg Infect Dis. 2009;15:397–401.
26. Chang DC, Anderson S, Wannemuehler K, Engelthaler DM, Erhart L, Sunenshine RH, Burwell LA, Park BJ. Testing for coccidioidomycosis among patients with community-acquired pneumonia. Emerg Infect Dis. 2008;14:1053–9.

27. Smith C, Beard R, Whiting E, Rosenberger H. Varieties of coccidioidal infection in relation to the epidemiology and control of the diseases. Am J Public Health. 1946;36:1394–402.
28. Cohen IM, Galgiani JN, Potter D, Ogden DA. Coccidioidomycosis in renal replacement therapy. Arch Intern Med. 1982;142:489–94.
29. Holt C, Winston D, Kubak B, Imagawa D, Martin P, Goldstein L, et al. Coccidioidomycosis in liver transplant patients. Clin Infect Dis. 1997;24:216–21.
30. Blair JE. Coccidioidomycosis in liver transplantation. Liver Transpl. 2006;12:31–9.
31. Blair JE, Douglas DD, Mulligan DC. Early results of targeted prophylaxis for coccidioidomycosis in patients undergoing orthotopic liver transplantation within an endemic area. Transpl Infect Dis. 2003;5:3–8.
32. Vucicevic D, Carey EJ, Blair JE. Coccidioidomycosis in liver transplant recipients in an endemic area. Am J Transplant. 2011;11:111–9.
33. Kahn A, Carey EJ, Blair JE. Universal fungal prophylaxis and risk of coccidioidomycosis in liver transplant recipients living in an endemic area. Liver Transpl. 2015;21:353–61.
34. Lohrmann GM, Vucicevic D, Lawrence R, Steidley DE, Scott RL, Kusne S, Blair JE. Single-center experience of antifungal prophylaxis for coccidioidomycosis in heart transplant recipients within an endemic area. Transpl Infect Dis. 2017;19:e12744.
35. Blair JE, Coakley B, Santelli AC, Hentz JG, Wengenack NL. Serologic testing for symptomatic coccidioidomycosis in immunocompetent and immunosuppressed hosts. Mycopathologia. 2006;162:317–24.
36. Mendoza N, Blair JE. The utility of diagnostic testing for active coccidioidomycosis in solid organ transplant recipients. Am J Transplant. 2013;13:1034–9.
37. Kuberski T, Myers R, Wheat LJ, Durkin M, Connolly P, Kubak BM, Bruckner D, Pegues D. Diagnosis of coccidioidomycosis by antigen detection using cross-reaction with a Histoplasma antigen. Clin Infect Dis. 2007;44:e50–4.
38. Thompson GR 3rd, Bays DJ, Johnson SM, Cohen SH, Pappagianis D, Finkelman MA. Serum (1→3)-beta-d-glucan measurement in coccidioidomycosis. J Clin Microbiol. 2012;50:3060–2.
39. Saubolle MA, McKellar PP, Sussland D. Epidemiologic, clinical, and diagnostic aspects of coccidioidomycosis. J Clin Microbiol. 2007;45:26–30.
40. Pappagianis D, Zimmer BL. Serology of coccidioidomycosis. Clin Microbiol Rev. 1990;3:247–68.
41. Thompson GR 3rd. Pulmonary coccidioidomycosis. Semin Respir Crit Care Med. 2011;32:754–63.
42. Vikram HR, Dosanjh A, Blair JE. Coccidioidomycosis and lung transplantation. Transplantation. 2011;92:717–21.
43. Vikram HR, Blair JE. Coccidioidomycosis in transplant recipients: a primer for clinicians in nonendemic areas. Curr Opin Organ Transplant. 2009;14:606–12.
44. Phonphok K, Beaird O, Duong T, Datta N, Schaenman J, Bunnapradist S. Screening coccidioides serology in kidney transplant recipients: a 10-year cross-sectional analysis. Transpl Infect Dis. 2018;20:e12932.
45. Blair JE, Mendoza N, Force S, Chang YH, Grys TE. Clinical specificity of the enzyme immunoassay test for coccidioidomycosis varies according to the reason for its performance. Clin Vaccine Immunol. 2013;20:95–8.
46. Blair JE. Coccidioidomycosis in patients who have undergone transplantation. Ann N Y Acad Sci. 2007;1111:365–76.
47. Singh N, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, Infectious Diseases Community of Practice. Am J Transplant. 2012;12:2414–28.
48. Kusne S, Taranto S, Covington S, Kaul DR, Blumberg EA, Wolfe C, Green M. Coccidioidomycosis transmission through organ transplantation: a report of the OPTN Ad Hoc disease transmission advisory committee. Am J Transplant. 2016;16:3562–7.

49. Miller M, Hendren R, Gilligan P. Posttransplantation disseminated coccidioidomycosis acquired from donor lungs. J Clin Microbiol. 2004;42:2347–9.
50. Dierberg KL, Marr KA, Subramanian A, Nace H, Desai N, Locke JE, Zhang S, Diaz J, Chamberlain C, Neofytos D. Donor-derived organ transplant transmission of coccidioidomycosis. Transpl Infect Dis. 2012;14:300–4.
51. Trinh SA, Echenique IA, Penugonda S, Angarone MP. Safety and efficacy of chronic suppressive azole therapy for endemic fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2018;20:e12963.
52. Dodge RR, Lebowitz MD, Barbee R, Burrows B. Estimates of *c. Immitis* infection by skin test reactivity in an endemic community. Am J Public Health. 1985;75:863–5.
53. Blair JE, Douglas DD. Coccidioidomycosis in liver transplant recipients relocating to an endemic area. Dig Dis Sci. 2004;49:1981–5.
54. Kirkland TN. The quest for a vaccine against coccidioidomycosis: a neglected disease of the Americas. J Fungi (Basel). 2016;2:1–10.
55. Carpenter JB, Feldman JS, Leyva WH, DiCaudo DJ. Clinical and pathologic characteristics of disseminated cutaneous coccidioidomycosis. J Am Acad Dermatol. 2010;62:831–7.
56. Galgiani JN, Ampel NM, Blair JE, Catanzaro A, Geertsma F, Hoover SE, Johnson RH, Kusne S, Lisse J, MacDonald JD, Meyerson SL, Raksin PB, Siever J, Stevens DA, Sunenshine R, Theodore N. 2016 Infectious Diseases Society of America (IDSA) clinical practice guideline for the treatment of coccidioidomycosis. Clin Infect Dis. 2016;63:e112–46.
57. Thompson GR 3rd, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F, Ostrosky-Zeichner L, Azie N, Maher R, Lee M, Kovanda L, Engelhardt M, Vazquez JA, Cornely OA, Perfect JR. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. Clin Infect Dis. 2016;63:356–62.
58. Glenn TJ, Blair JE, Adams RH. Coccidioidomycosis in hematopoietic stem cell transplant recipients. Med Mycol. 2005;43:705–10.
59. Mendoza N, Noel P, Blair JE. Diagnosis, treatment, and outcomes of coccidioidomycosis in allogeneic stem cell transplantation. Transpl Infect Dis. 2015;17:380–8.
60. Miller R, Assi M. Endemic fungal infections in solid organ transplant recipients-guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13553.

Emerging Epidemics in Transplant: Histoplasmosis, Blastomycosis, and Paracoccidioidomycosis

51

Jeremy Nel, Anne Lachiewicz, and David van Duin

Contents

Introduction 1226
Histoplasmosis 1227
Disease Epidemiology 1227
Clinical Impact on Transplant Patients 1228
Pathogenesis 1229
Clinical Manifestations 1229
Diagnosis 1230
Treatment 1231
Prophylaxis 1232
Blastomycosis 1233
Disease Epidemiology 1233
Clinical Impact in Transplant Patients 1234
Pathogenesis and Clinical Manifestations 1234
Diagnosis 1235
Treatment 1236
Prophylaxis 1237
Paracoccidioidomycosis 1237
Disease Epidemiology 1237
Clinical Impact in Transplant Patients 1238
Pathogenesis and Clinical Manifestations 1238
Diagnosis 1239
Treatment 1240
Prophylaxis 1241

J. Nel
University of the Witwatersrand, Johannesburg, South Africa
e-mail: jeremynel@hotmail.com

A. Lachiewicz · D. van Duin (✉)
Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, NC, USA
e-mail: anne_lachiewicz@med.unc.edu; david_vanduin@med.unc.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_50

Common Antifungal Drugs Used to Treat the Endemic Mycoses 1242
Itraconazole 1242
Fluconazole 1242
Amphotericin B 1244
Conclusions 1244
Key Points 1244
References 1244

Introduction

The endemic fungi are a group of thermally dimorphic fungi that occupy specific defined environmental ranges (Fig. 1). They count *Histoplasma*, *Blastomyces*, *Paracoccidioides*, *Coccidioides*, *Talaromyces*, *Emergomyces*, and *Sporothrix* among their number. In the environment they exist as molds, and at body temperature, they transform into their pathogenic yeast form (or, in the case of coccidioidomycosis, into a specialized structure called a spherule). *Histoplasma*, *Blastomyces*, and *Paracoccidioides* are covered in this chapter. The main form of acquiring these diseases is by inhaling the mold form's conidia, and this frequently happens after the soil or decaying vegetation that houses the fungus is disturbed. After the conidia are inhaled, the fungus undergoes one of three fates. If the host's innate immunity is strong enough, or the inoculum small enough, the conidia and/or yeasts may be killed outright. If this defense fails, the infection may be able to be controlled by the host's T-cell-mediated immunity, often in a quiescent state within granulomas, from which it may subsequently escape if the host's immunity wanes – as, for example, following a transplant. For this reason, disease from one of the endemic mycoses can occur years, sometimes decades, after the patient visited an endemic area. Lastly, if the infection is not able to be sufficiently controlled by the

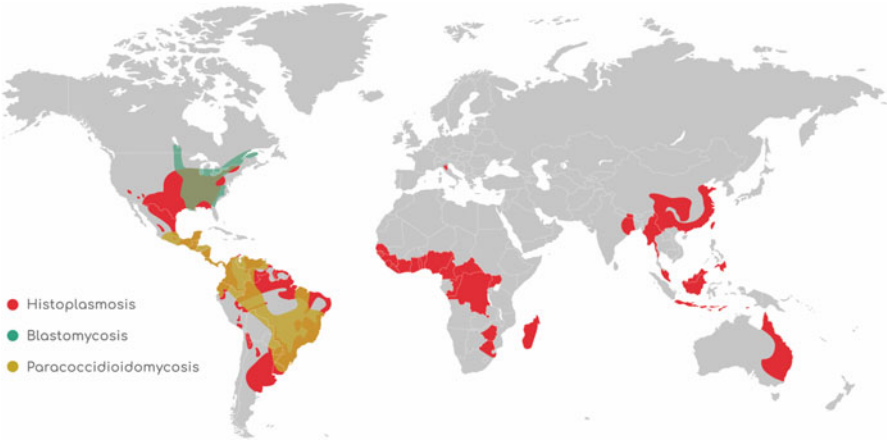


Fig. 1 Approximate areas of endemicity for histoplasmosis, blastomycosis, and paracoccidioidomycosis [6, 86, 87]

patient's immune system initially, symptomatic disease may occur more immediately, either within one organ system (often the lung) or disseminated more broadly.

The microbiologic diagnosis of endemic mycoses is limited by a relative lack of sensitivity of cultures, difficulty in obtaining the most appropriate samples from patients, and the slow growth of the organism in culture. Therefore, non-culture-based methods of diagnosis such as direct microscopic exam of specimens, antigen assays, and antibody tests are used in conjunction with cultures. Antibody assays often have decreased sensitivity in transplant recipients however. Polymerase chain reaction (PCR) assays are an emerging diagnostic modality, albeit one that is often not commercially available or well studied.

The treatments of choice for all the endemic mycoses are liposomal amphotericin B (given up front for severe disease) and the azoles. While the newer azoles such as posaconazole or isavuconazole show some promise, the bulk of the evidence resides with the older azoles, such as itraconazole, fluconazole, and voriconazole, and there are few direct comparisons available by which to dislodge the latter drugs as frontline agents. In general, treatment must be given for a prolonged period (frequently a year or more) in order to prevent a relapse.

Histoplasmosis

Disease Epidemiology

Histoplasmosis is caused by the fungus *Histoplasma capsulatum*. Mycelial growth in histoplasmosis is favored in soil in climates with moderate temperatures (between 15 °C and 40 °C), high relative humidity, and a soil pH >5.5 [1]. Growth and sporulation of *Histoplasma capsulatum* are especially enhanced by the presence of bird and bat guano, which provides a high nitrogen and phosphorus content that can on occasion permit mycelial growth even in the absence of soil [2]. Activities that disturb soil and aerosolize *Histoplasma* spores can place patients at risk of acquiring histoplasmosis. These activities classically include spelunking, digging in soil where there are bird or bat droppings, cleaning bird coops, and renovating or demolishing buildings that have been colonized by bats or birds [1, 3, 4]. However, *Histoplasma* conidia may be carried for miles on air currents, and many patients do not have a history of a specific exposure.

The highest incidence of histoplasmosis worldwide appears to be in North America, in an area surrounding the Ohio and Mississippi River Valleys [5, 6]. However, patches of histoplasmosis endemicity are spread throughout the world. A large number of endemic cases have been reported from Mexico, Central America, and the majority of South American countries (Fig. 1). In areas of Brazil, skin reactivity to histoplasmin antigens (reflecting prior exposure to the fungus) has been reported in nearly 90% of inhabitants tested in some areas of the country [5]. Cases of histoplasmosis have also been identified from the majority of African countries, though the picture is somewhat clouded by the existence of two variants there, with an "African" variant, *Histoplasma capsulatum* var. *duboisii*, prevalent

in the western parts of the continent. *Histoplasma capsulatum* var. *capsulatum*, the variant present elsewhere in the world, is found in Africa's southern parts, and the two variants coexist in the intervening areas [6, 7]. Parts of India and Southeast Asia also appear to be endemic for histoplasmosis, with Malaysia, Thailand, Taiwan, China, and the eastern parts of Australia all reporting endogenously acquired cases and substantial rates of histoplasmin skin hypersensitivity [6]. Histoplasmosis is not generally considered endemic in Europe, with a near 0% rate of positive skin hypersensitivity assays there and reported cases from Europe generally having been imported from endemic areas elsewhere in the world. However, a small number of potentially autochthonous cases have been described from Italy, Germany, and Turkey [8]. This worldwide distribution of histoplasmosis stands in contrast with the much more geographically restricted ranges of other endemic fungi, such as coccidioidomycosis, paracoccidioidomycosis, and blastomycosis. It is possible that naturally infected bats and birds with long-range migrations may be responsible for *Histoplasma capsulatum*'s wide dispersal to disparate regions of the globe [5].

Surveys of histoplasmin skin hypersensitivity that have identified the traditional endemic areas of the world have been performed in only a small minority of countries, and many such maps are now decades old [9]. Factors like climate change and population migration may have changed these areas since the surveys. Indeed, recent evidence suggests that the preferred soil environments in the USA might now include the Missouri River basin, in addition to the Ohio and Mississippi River basins [10]. In addition, soil and climatic conditions that support *Histoplasma* growth likely exist in many "microenvironments" throughout the world, and thus histoplasmosis should be considered in any patient with a compatible clinical picture, even without travel to one of the classic endemic areas [6]. This dictum is all the more important considering that histoplasmosis has been reported to reactivate up to 50 years after initial exposure, which further clouds the association between the disease and a history of exposure to an endemic area [8].

Clinical Impact on Transplant Patients

Histoplasmosis causes the greatest burden of disease of all the endemic fungi seen in immunocompromised hosts. The Transplant-Associated Infection Surveillance Network (TRANSNET) reported 48 histoplasmosis cases in solid organ transplant (SOT) recipients and 4 histoplasmosis cases in in hematopoietic cell transplant (HSCT) recipients, respectively, from 15 surveillance sites over 5 years from 2001 to 2006 [11]. The cumulative incidence in SOT recipients was 0.1% for the 12-month period following their transplants. In a high-volume SOT center in Ohio, incidence of post-SOT histoplasmosis was 1 case per 1000 transplant-person-years (95% confidence interval, 0.6–1.7) [12]. The risk of histoplasmosis is highest in the first year after transplantation, though an increased posttransplant risk is likely to persist indefinitely compared to immunocompetent patients. By way of example, the incidence of histoplasmosis among SOT recipients in Ohio, a known endemic area, was 16 times higher than that of the general population >65 years of age in the same

state [13]. Mortality from histoplasmosis arising after SOT is approximately 10%, with the bulk of deaths occurring within the first month after diagnosis [11, 14]. In the TRANSNET cohort, there were no deaths at 3 months among the four HSCT recipients who developed histoplasmosis.

Pathogenesis

Aerosolized *Histoplasma capsulatum* conidia may be inhaled, and microconidia between the sizes of 2 and 4 micrometers are small enough to reach the alveolar spaces. Microconidia that evade the lung's innate defenses develop into the yeast phase of the organism. Macrophages are initially capable of phagocytosing but not killing the organisms, as it takes approximately 10–14 days for the development of the required CD4 T-lymphocyte-mediated immune response [15]. If this immune response is robust enough, the spread of the organism is arrested. Most immunocompetent patients are thus either asymptomatic or have mild, self-limiting respiratory symptoms [4]. Immunocompromised patients, including SOT and HSCT recipients, may fail to stop the spread of the organism and present with progressive disseminated disease [15].

Even those patients who do control the infection frequently fail to completely eradicate it. Rather, viable *Histoplasma capsulatum* organisms may be walled off inside granulomas within the body. Patients who have had prior histoplasmosis exposure, even decades before, are at risk of suffering a reactivation of the disease if their immunity subsequently declines, whether from SOT, HSCT, immunosuppressant medications, old age, HIV, or other immunosuppressing conditions [15]. In many cases, whether a case of histoplasmosis represents disease reactivation or a new acquisition can be difficult to determine, although some evidence suggests that new acquisition may predominate [13, 16, 17].

SOT recipients may acquire the disease from an infected allograft. To date this method of transmission remains extremely rare, with only a handful of cases in the literature [18, 19].

Clinical Manifestations

Consistent with *Histoplasma capsulatum*'s pulmonary route of acquisition and subsequent engulfment by macrophages, the predominant organs involved are the lungs and the lymphoid-rich tissues. The better the patient's cell-mediated immunity, the greater the chance that disease remains confined to the lungs. Those with a modest exposure to *Histoplasma capsulatum* may develop a subacute or chronic pneumonia or one or more pulmonary nodules that may be incidentally picked up on chest X-ray. Those with a larger exposure can develop an acute pneumonia and acute respiratory distress syndrome. Other less common presentations such as broncholithiasis and fibrosing mediastinitis are also possible [3]. SOT and HSCT recipients may develop any of the above pulmonary syndromes, but disease more

frequently disseminates beyond the lungs; disseminated disease is the rule rather than the exception in these patient subgroups. In the TRANSNET cohort of HSCT and SOT recipients mentioned above, >71% of cases had demonstrable dissemination at the time of diagnosis [11].

Extrapulmonary disease can occur with or without concomitant pulmonary disease in immunocompromised hosts. Commonly infected sites include the liver, spleen, lymph nodes, bone marrow, gastrointestinal tract, and skin (Fig. 2). Systemic symptoms such as fever, loss of weight, anorexia, and fatigue are common. Clinical signs seen with disseminated disease include hepatomegaly, splenomegaly, lymphadenopathy (which may sometimes be massive), and the effects of cytopenias [3]. Hemophagocytic lymphohistiocytosis is also a recognized complication of disseminated histoplasmosis.

Diagnosis

No diagnostic test for histoplasmosis has adequate sensitivity to be used in isolation. Rather, multiple tests should be performed in parallel, guided by the sites of involvement in the individual patient. Urine histoplasma antigen assays have the highest sensitivity of any single diagnostic test in disseminated histoplasmosis, with an estimated sensitivity of ~93% in SOT recipients. Serum antigen testing is somewhat less sensitive (estimated 80% sensitivity). Antigen testing may also be positive in bronchoalveolar lavage (BAL) fluid and cerebrospinal fluid (CSF), when these areas are involved. However, the specificity of the antigen test is limited

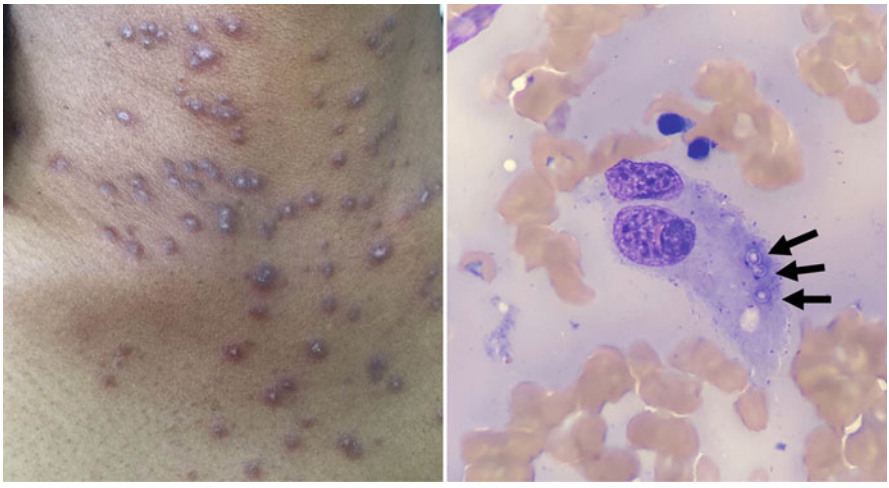


Fig. 2 Histoplasmosis. Left, typical papular rash sometimes seen with disseminated disease. Right, yeast form of histoplasmosis visible on this bone marrow aspirate as small yeasts (arrows) clustered within a macrophage. The pseudocapsule that provides the organism's species name ("capsulatum") is clearly visible but is an artifact of cytoplasmic shrinkage (Wright-Giemsa stain)

Table 1 Role of diagnostic tests in histoplasmosis, blastomycosis, and paracoccidioidomycosis in transplant recipients

	Histoplasmosis	Blastomycosis	Paracoccidioidomycosis
Microscopy	Small (2–4 μm) yeasts clustered within macrophages, with pseudocapsule	Large (10–15 μm) yeasts with thick walls and broad-based budding	Variably sized yeasts (4–60 μm) arranged as a central parent yeast with multiple other yeasts budding off
Cultures	Sensitive but growth typically takes 2–4 weeks	Sensitive but growth typically takes 2–4 weeks	Sensitive but growth typically takes 2–4 weeks
Serology	Limited value – poor sensitivity	Limited value – very poor sensitivity	Moderate sensitivity in transplant patients. Serial measurements can be used to follow treatment response
Antigen test	Urine antigen test has sensitivity >90%. Blood antigen test has sensitivity of ~80%. Some cross-reaction with other fungi	Urine antigen test has sensitivity of 76–93%. Cross-reaction with other fungi seen	Not commercially available

by cross-reaction with similar antigens from other endemic fungi [20]. Cross-reaction with *Aspergillus* antigens has also been reported in solid organ transplant patients [21]. Antibody assays are insensitive in histoplasmosis in general and particularly so in transplant recipients. Cultures from blood and any other infected site (e.g., bronchoalveolar lavage fluid, skin, liver, lymph nodes) are generally positive in >60% of cases [22]. However, the utility of cultures is further limited by the fact that the organism typically takes 2–4 weeks for adequate growth to permit identification [3]. Histologic specimens taken from the lungs, lymph nodes, liver, bone marrow, skin, or other involved organs may reveal typical clusters of small (2–4 μm) yeasts intracellularly within macrophages and other phagocytes (Fig. 2) [23]. This may permit a provisional diagnosis. Polymerase chain reaction (PCR) is a promising emerging diagnostic modality that offers the potential to obtain rapid and highly specific results. However, trials to date have used a variety of methodologies and technologies, with correspondingly varying results, preventing a firm recommendation for the use of PCR-based diagnostics [24–26]. Diagnostic considerations are outlined in Table 1.

Treatment

Transplant recipients with histoplasmosis should generally be considered to have disseminated disease unless proven otherwise. In most cases, amphotericin B is advised for 1–2 weeks initially, followed by itraconazole for at least 12 months [27]. Lipid formulations of amphotericin are preferred over amphotericin B deoxycholate, primarily because of decreased toxicity. In addition, histoplasmosis remains the only condition for which the liposomal form of amphotericin has also been shown to be

more efficacious than the deoxycholate form [28]. Severely ill patients may have attendant acute kidney injury, but this should not dissuade clinicians from using lipid formulations of amphotericin initially in such cases. Indeed, renal dysfunction may improve if the infection is rapidly brought under control with amphotericin.

Following an initial 1–2 weeks of amphotericin therapy, the patient may be transitioned to azole therapy to complete a minimum of 12 months of therapy. Itraconazole is the preferred azole for use in histoplasmosis. In mild to moderate cases of disease, itraconazole alone, without amphotericin, may even be sufficient as therapy [29]. Measuring blood levels of itraconazole is recommended, as its bioavailability may often be impaired by gastric conditions, and itraconazole is also frequently both a cause and target of drug-drug interactions (see section "Common Antifungal Drugs Used to Treat the Endemic Mycoses") [27].

Itraconazole appears superior to fluconazole for the treatment of disseminated histoplasmosis. In vitro data shows a greater potency for the former drug, and acquired immunodeficiency syndrome (AIDS) patients with mild-moderate disseminated disease who were treated with itraconazole had a greater response rate than those treated with fluconazole in a similar trial (85% vs. 70%) [29–31]. The relapse rate with fluconazole was also substantially higher than that seen with itraconazole in these trials, driven by the development of resistance while on therapy [30]. This phenomenon was not seen with itraconazole.

The role of other azoles in the treatment of histoplasmosis has not been well studied. On the basis of in vitro data, posaconazole seems more potent and less prone to developing resistance than voriconazole [32]. Other in vitro data suggest that isavuconazole is an extremely potent drug, and like posaconazole, its potency does not appear to be affected by the development of fluconazole resistance. Furthermore, isavuconazole offers excellent bioavailability that may preclude the need for routinely measured drug levels. However, clinical experience with posaconazole, voriconazole, or isavuconazole in treating histoplasmosis patients is decidedly limited to date, and thus their role is currently relegated to salvage therapy in patients who have failed or are intolerant of itraconazole therapy [27, 33].

Of note, the yeast form of *H. capsulatum* is not susceptible to the echinocandins, which therefore have no role in therapy [34].

Symptoms of histoplasmosis may take several weeks to fully resolve, though some overall clinical improvement is usually seen within a week. Histoplasma antigen levels may be monitored serially to assess response to therapy. A failure of the antigen level to decline on treatment may represent treatment failure. Antigen levels should also be checked for 6–12 months after completing therapy to check for a relapse. In general, antigen levels should be negative at the end of treatment, though some patients with persistent low-level antigenuria without evidence of active disease have had good clinical outcomes when their treatment was stopped [27, 35].

Prophylaxis

SOT or HSCT recipients should avoid activities known to be high risk for acquiring histoplasmosis, such as those mentioned previously. Primary prophylaxis has only been

studied in patients with advanced HIV, where a daily dose of itraconazole more than halved the incidence of histoplasmosis over a median follow-up period of 16 months in an endemic area [36]. Based on expert opinion however, the Infectious Diseases Society of America (IDSA) recommends prophylaxis only in situations where the incidence of histoplasmosis is greater than 10 cases per 100 patient years.

The role of secondary prophylaxis after transplant is also somewhat unclear. Patients with a history of previous histoplasmosis, or radiologic or serologic evidence of past disease, are at risk of reactivation in the posttransplantation period due to their profound immunosuppression. However, the risk of reactivation appears low. Not a single case of histoplasmosis was diagnosed in over 550 HSCT and SOT recipients over a mean follow-up of 16 months in a hyperendemic area. This cohort included patients with radiographic and serologic evidence of past disease. Another important consideration is that the urine histoplasma antigen is a useful screening test for disease reactivation. Thus, instead of itraconazole prophylaxis, an alternative strategy is to measure urine histoplasma antigen levels every 2–3 months during the intensive phase of the patient's immunosuppression regimen [27]. Similarly, patients who develop histoplasmosis for the first time after transplantation probably do not require secondary prophylaxis and should instead have serial urine histoplasma antigen levels mentioned after their treatment course has been completed. Notably, the risk of disease reactivation after appropriate treatment is low, at $<5\%$ [14].

A related problem involves organ donors with a remote history of histoplasmosis. Though little data exists to guide decision-making in this scenario, a suggested approach is outlined in Table 2.

Blastomycosis

Disease Epidemiology

Recent phylogenetic work has revealed that blastomycosis is caused by one of two species, *Blastomyces dermatitidis* and *Blastomyces gilchristii*. The natural habitat of the organism has been far harder to elucidate than that of the other endemic fungi, for two reasons. Firstly, it has proven very difficult to isolate the fungus from environmental sources, and secondly, there are no adequately sensitive and specific serologic tests or skin test reagents [37]. Despite these limitations, autochthonously acquired cases have historically clustered around the Great Lakes area on both the US and Canadian sides, as well as the Ohio, Mississippi, and St Lawrence River basins (Fig. 1) [37]. Indeed, two outbreak studies where *Blastomyces* was ultimately cultured suggest that soil and wooded areas surrounding rivers and lakes may be the organism's favored habitat [38, 39]. The blastomycosis-endemic areas overlap extensively with those for histoplasmosis, though the former are more extensive. Outside of North America, endogenously acquired cases have been reported from India and several African countries [40, 41].

Similar to histoplasmosis, risk factors for acquiring the disease include activities that disturb the soil and thereby aerosolize *Blastomyces* conidia. Known associations

Table 2 Suggested prophylactic regimens for histoplasmosis, blastomycosis, and paracoccidioidomycosis in transplant recipients

	Recipient peri-transplant (primary) prophylaxis	Secondary prophylaxis for recipients after completion of treatment course
Histoplasmosis	Donor with localized pulmonary disease: itraconazole 200 mg once or twice daily for 3–6 months Donor with disseminated disease: itraconazole 200 mg once or twice daily for 12 months [84]	Not routinely indicated. Can monitor urine antigen level every 3 months to determine need. If required, consider itraconazole 200 mg daily [27, 84]
Blastomycosis	Donor with localized pulmonary disease: itraconazole 200 mg once or twice daily for 3–6 months Donor with disseminated disease: itraconazole 200 mg once or twice daily for 12 months	Not routinely indicated. If required, consider itraconazole 200 mg daily [54]
Paracoccidioidomycosis	Itraconazole 200 mg once daily for 12 months [77]. Trimethoprim-sulfamethoxazole may be sufficient if already part of the posttransplant prophylaxis regimen	Not routinely indicated. Can follow with serial serology titers. If required, consider itraconazole 200 mg daily

include camping, forestry work, fishing, cutting trees, or clearing brush in endemic areas [16, 38, 39].

Clinical Impact in Transplant Patients

The TRANSNET surveillance network identified just 9 cases of blastomycosis in transplant recipients (compared to 52 cases of histoplasmosis) over a 5-year period [11]. All nine cases were seen in SOT recipients, though rare cases of blastomycosis in HSCT recipients have been described elsewhere [42]. Similarly to histoplasmosis, the majority of cases occur within the first year after transplantation [11]. Mortality estimates for SOT recipients with blastomycosis have ranged from 0% to 36% [11, 43, 44].

Pathogenesis and Clinical Manifestations

Blastomyces conidia, possibly aerosolized during soil disturbances, are inhaled and reach the alveoli. If they survive destruction by the lung's neutrophils, monocytes, and macrophages, the shift in ambient temperature signals to the conidia to develop

into the yeast form. As with histoplasmosis, this form is harder to kill due to its size (which prevents phagocytosis by neutrophils), its thicker cell wall, and the expression of various virulence factors. In the case of blastomycosis, the latter include BAD-1, which mediates attachment to macrophages and also shifts the cell-mediated immune response away from the Th1 response that is required to eliminate the infection [45]. If the patient's immune response is not adequate to contain the organism within the lungs, disseminated infection may occur. In addition, the organism may be held in check in a dormant state by the patient's immune system, allowing reactivation should the patient's immune competency decline, such as what occurs in SOT or HSCT recipients. Remote reactivation as long as 40 years after initial infection has been described [46]. No cases of graft-derived blastomycosis have been reported to date.

As with all the endemic fungi, clinical manifestations depend on the interplay between host immunity and the inoculum size. Patients with a robust immune response may clear the organism before any symptoms develop. Indeed, approximately half of immunocompetent patients are asymptomatic. However, transplant recipients are more likely to develop symptomatic pulmonary disease – the lungs are involved in 80–100% of cases [43, 44]. Transplant recipients also seem predisposed to have a more severe pulmonary course than immunocompetent patients. Rates of acute respiratory distress syndrome (ARDS) and early mortality are higher, and patients who do recover may have substantial residual lung damage [11, 43, 44].

Transplant patients may also have greater rates of disseminated disease [43]. When the disease spreads beyond the lungs, the next most commonly involved site is the skin, though multiple pustules or ulcers are more common than the classic verrucous lesions seen in immunocompetent patients (Fig. 3) [47]. Other clinical manifestations include lytic bone lesions, prostatitis, epididymitis, meningitis, or cerebral abscesses. Interestingly, CNS disease with blastomycosis appears to be far less common among SOT recipients than it is among patients with AIDS [48]. Importantly, at the time when extrapulmonary symptoms appear, pulmonary findings can be minimal or even absent [49].

Diagnosis

Blastomycosis is often not considered in a patient's differential diagnosis. In one study in Mississippi, USA, only 5% of patients with blastomycosis were correctly diagnosed at presentation, and 43% had a delay in diagnosis of over 1 month, despite the fact that Mississippi is a hyperendemic area [50].

Blastomyces grows readily in appropriate culture media, though, like all dimorphic fungi, it may take up to 4–6 weeks of incubation before it shows adequate growth to permit a diagnosis [49]. The yield from pulmonary specimens is often excellent, with sputum and bronchoalveolar lavage fluid cultures having a sensitivity of approximately 75% and 92%, respectively [51]. Cultures from the skin and other involved areas are also frequently positive.

Direct examination of culture or biopsies taken from the lung, skin, bone, and other sites is less sensitive but when positive allows for a rapid diagnosis. *Blastomyces* has a characteristic appearance, namely, that of a large yeast (10–15 μ) with thick "double-contoured" walls and distinctive broad-based budding (Fig. 3) [23].

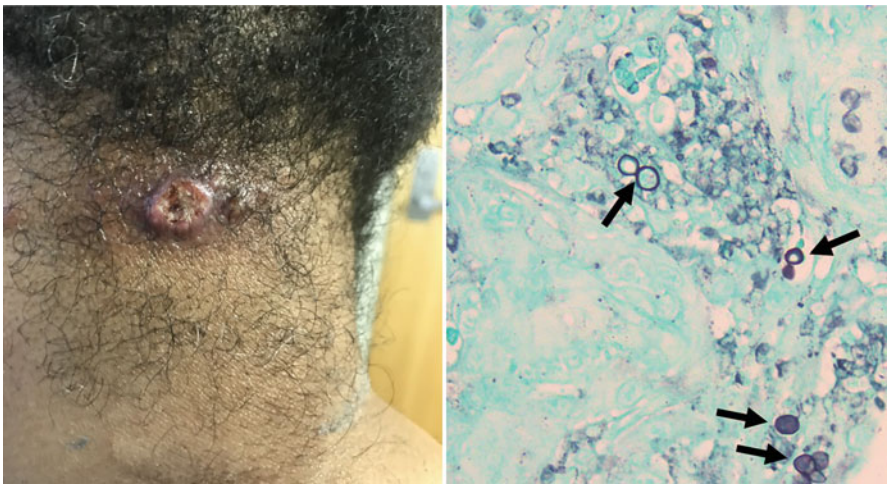


Fig. 3 Blastomycosis. Left, typical skin lesions of disseminated blastomycosis, with ulcerating nodules and papules. Right, histopathologic image of the same skin lesion, showing large yeasts with broad-based budding (arrows) (Grocott-Gomori methenamine silver stain)

A urinary antigen test for blastomycosis is available and has a reported sensitivity of 76–93%. In contrast to histoplasmosis, the test may be more sensitive for pulmonary (83–100%) than disseminated disease (67–89%) [52, 53]. However, because they share similar galactomannans, this assay may significantly cross-react in the presence of other endemic dimorphic fungi [52]. For this reason, simultaneously performing the blastomycosis urinary antigen in conjunction with the histoplasma antigen may be useful [23].

Serology in blastomycosis is of extremely limited value, owing to very poor sensitivity of both the complement fixation and immunodiffusion assays (as low as 9% and 28%, respectively). Enzyme immunoassays (EIAs) have better diagnostic accuracy but are not commercially available [23].

PCR offers some promise, but the technique has not been studied in large-scale trials, and so its place in the diagnostic algorithm for blastomycosis remains unclear.

Treatment

While itraconazole therapy alone may be sufficient to treat mild disease in patients with normal immunity, initial therapy with amphotericin B is recommended for all immunocompromised patients [54]. Lipid amphotericin formulations are preferred over the deoxycholate form due to the former's better side-effect profile, though unlike histoplasmosis there is no evidence that any of them are more efficacious.

Following 1–2 weeks of amphotericin and once the patient has demonstrated clinical improvement, the treatment regimen can be switched to an azole. Itraconazole is the preferred azole in most circumstances. No head-to-head

comparison trials have been done within this class of drugs, but clinical trials using itraconazole to treat blastomycosis have generally shown better treatment responses than trials using fluconazole or ketoconazole [54, 55]. If fluconazole has to be used, it should be at doses of 400–800 mg/day, as outcomes appear significantly better than if a dose of 200–400 mg/day is prescribed [56]. *Blastomyces* is susceptible to voriconazole, and the drug has been used successfully in small numbers of blastomycosis cases, often when there has been CNS involvement since, like fluconazole, voriconazole can achieve therapeutic concentrations within the brain [57]. Both posaconazole and isavuconazole also have activity against *Blastomyces*, but in vitro data and clinical experience are too limited for either drug to be recommended for anything beyond salvage therapy [33, 58]. Extrapolating from data in AIDS patients with histoplasmosis, it is recommended that transplant recipients with blastomycosis should receive azole therapy for at least 12 months. The extent of the patient's immunosuppression is also an important factor to consider when deciding when to discontinue therapy [54]. The role of serial urine antigen titers is less well established for blastomycosis than for histoplasmosis, but clearance of the urinary antigen does appear to be correlated with treatment success [59].

Central nervous system involvement by *Blastomyces* is not common in transplant recipients. When it does occur, the optimal treatment strategy is unknown. Current recommendations are to give lipid formulations of amphotericin B for a prolonged period initially (4–6 weeks) and then to follow this with either itraconazole, fluconazole, or voriconazole [54]. While itraconazole is the azole of choice in general, its CNS penetration is far less than either fluconazole or voriconazole. The lack of head-to-head data thus means that the optimal azole to choose with CNS blastomycosis is unclear.

Prophylaxis

There is limited data to guide decisions regarding prophylaxis in transplant recipients with a history of blastomycosis. Secondary prophylaxis may be reasonable in cases where the patients' immunosuppression regimen renders the patient profoundly immunosuppressed.

There is equally little information regarding the optimal therapy for recipients of organs from donors with a history of blastomycosis. Active disease in the donor is generally a contraindication to organ donation. Remote disease is not, however, and a suggested approach is outlined in Table 2.

Paracoccidioidomycosis

Disease Epidemiology

Paracoccidioidomycosis is caused by one of five species: the *Paracoccidioides lutzii* and the *P. brasiliensis* species complex, consisting of species 1 (S1) and phylogenetic species 2, 3, and 4 (PS2, PS3, PS4) [60]. The disease is limited

to Central and South America, and within this region, it is the most common of the endemic mycoses. Brazil accounts for >80% of cases, but the disease is also known to many other countries in the region from Mexico to Argentina (Fig. 1) [61]. Sensitivity surveys using intradermal paracoccidioidin antigen showed a median exposure rate of approximately 25–35% in Brazil, rising as high as 50–75% among adults in highly endemic areas [61, 62]. *Paracoccidioides* has not often been cultured from environmental sources, but its favored environment appears to be tropical and subtropical rainforest areas, in regions with mild temperatures and good annual precipitation. These conditions also favor crops such as tobacco or coffee, and agricultural work in endemic regions is a known risk factor for acquiring the disease [61].

Clinical Impact in Transplant Patients

A study of pretransplant serologies of liver transplant recipients in an endemic area of Brazil revealed that 0.8% were seropositive for *P. brasiliensis* [63]. There are very few actual cases in the literature of paracoccidioidomycosis in SOT recipients however. A recent review counted a total of just ten, nine of which were in renal transplant recipients [64]. Furthermore, no case of paracoccidioidomycosis has ever been described in a HSCT recipient. The low burden of this disease in transplant recipients is surprising and could reflect a combination of underdiagnosis, relatively limited access to transplants in endemic areas, and the use of trimethoprim-sulfamethoxazole, a drug with known activity against *Paracoccidioides*, as part of routine posttransplant prophylaxis regimens. For a similar reason, most cases of paracoccidioidomycosis reported in SOT recipients have occurred after the first year of transplant, when trimethoprim-sulfamethoxazole prophylaxis is withdrawn [64]. Mortality in SOT recipients is hard to define given the small numbers involved, but the mortality rate seen to date (44%) appears to be far higher than the 5–8% mortality seen in the general population [65]. This would comport with what is known about paracoccidioidomycosis in human immunodeficiency virus (HIV)-infected patients, in whom fungal burden, relapse rate, and overall mortality were all significantly greater than in patients without HIV coinfection [64].

Pathogenesis and Clinical Manifestations

In common with the other endemic mycoses, exposure occurs when the fungus' conidia are inhaled. Within the lung, those conidia that escape destruction by the local immune defenses develop into the organism's yeast form. As few as 2% of patients may develop symptomatic disease overall, reflecting the fact that host immunity is usually sufficient to quickly eliminate the infection [62]. A T-helper cell 1 (Th1) response seems necessary for this, with granulomas being the histological correlates of disease control [66]. By contrast, those patients who develop symptomatic disease may have an overtly Th2 immunologic profile, manifested by nonreactive paracoccidioidin skin tests but detectable antibodies,

or an immunologically intermediate state lying between a Th1 and Th2 response [66].

When symptomatic disease does occur, it occurs as one of two syndromes. An **acute/subacute form** may develop within the first few weeks to months after exposure and is seen almost exclusively in children, adolescents, and adults <30 years old (hence its other name, "juvenile paracoccidioidomycosis"). This form is characterized by disseminated disease that predominantly involves the reticuloendothelial system, with lymphadenopathy, fever, anorexia, and weight loss being the most prominent symptoms. The lymph nodes are often massively enlarged, and the condition must be distinguished from lymphomas and disseminated tuberculosis. Anemia, hypergammaglobulinemia, and eosinophilia are also common, and hepatosplenomegaly occurs in just over one third of cases [67]. Unlike the chronic form, symptomatic pulmonary disease is very uncommon, despite the lungs being the route of entry and respiratory cultures often being positive.

The **chronic form** is much more common, accounting for 90% of symptomatic disease [62]. This represents a reactivation of previously controlled disease that can occur up to decades after the exposure [65]. There is a striking male predominance in the chronic form, with a male-female ratio as high as 22:1. This is possibly explained by the fact that β-estradiol inhibits the conversion of the *Paracoccidioides* conidia into the yeast form [62]. Although disease dissemination is also evident in the chronic form, the lungs are usually the chief focus. The most common symptoms are nonproductive cough and dyspnea, but the underlying pulmonary process is highly destructive over the long term, often leaving the patients with long-term pulmonary insufficiency. Despite this, symptoms and signs may be deceptively minimal. Chest X-ray or CT shows nodular and alveolar infiltrates, with a predilection for involvement of the mid- and lower zones [65]. The oral mucosa is also commonly affected, manifesting as painful ulcers that are most prominently seen around the gingiva and palate. The skin may be seeded by hematogenous dissemination, in which case verrucous or ulcerating papulonodular lesions are seen (Fig. 4). The adrenal glands are involved histologically in approximately 85% of cases, though overt Addison's disease is only seen in roughly 3% [64, 68]. Lytic bone lesions are also sometimes seen, as is lymphadenopathy in a similar manner to the acute/subacute form.

Diagnosis

The yeast form of *Paracoccidioides* has a distinctive microscopic appearance that when visualized allows for a direct diagnosis from sputum, scrapings of skin or mucosal lesions, tissue biopsy specimens, or abscess aspirates [62]. Characteristically, the microscopic appearance is of a central parent cell with multiple other daughter yeasts budding off it [23]. When just two daughter yeasts are present, the complex resembles a "Mickey Mouse head," and when the daughter yeasts surround the parent, a "ship's wheel" appearance is seen (Fig. 4). The yeasts are variably sized, at between 4 and 60 μ [23].

The other principal method by which paracoccidioidomycosis can be diagnosed is by culture. In keeping with the other dimorphic mycoses, a positive result typically

Fig. 4 Paracoccidioidomycosis. Left, typical skin lesions, consisting of nodules with central ulceration. Right, microscopy showing a classic "ship's wheel" configuration with a central yeast, and numerous daughter yeasts budding out circumferentially (methenamine silver stain). (Image credit: CDC/Dr. Martins Castro, Dr. Lucille K. Georg)

takes several weeks. Serology can also assist with the diagnosis. Quantitative immunodiffusion testing is the most widely used assay. Compared with many other types of *Paracoccidioides* serologic tests, this method has a lower rate of cross-reactivity with other organisms such as *Histoplasma*, *Aspergillus*, and *Leishmania* [69]. However, its sensitivity for *P. lutzii* is far lower than that for *P. brasiliensis* [70]. In addition, the sensitivity of serologic tests in general is also lower in immunosuppressed patients. This phenomenon has been documented in patients with paracoccidioidomycosis who were coinfected with HIV and may be expected in transplant recipients as well [64]. Serologic titers usually decline with successful treatment to levels of 1:2 or below, and thus following the titers sequentially is a useful adjunct to monitor treatment response [69].

Antigen tests targeting specific *Paracoccidioides* glycoproteins have been developed for use on serum, BAL fluid, and CSF, and these show great promise. In one diagnostic accuracy study that used the gp43 antigen, the test was 95% sensitive and 97% specific [71]. Unfortunately, these antigen tests are not yet commercially available. PCR assays have been developed but are similarly restricted to research rather than clinical settings [72].

Treatment

Paracoccidioides is susceptible to a wider range of drugs than any of the other dimorphic fungi. In addition to the azoles and polyenes, the sulfonamides and even terbinafine have substantial activity. Equally remarkably, no cases of resistance to

any of the antifungals have yet been documented [69]. The choice of antifungal agent is therefore based on what clinical experience exists in the literature, taking into account the side-effect profiles and pharmacokinetic considerations of the various therapeutic options.

For mild to moderate disease, itraconazole is recommended [69]. The drug has been widely used and demonstrates good efficacy [73, 74]. For severe disease, initial treatment with amphotericin B is recommended, with a transition to itraconazole once the patient's clinical condition has begun to improve (usually after 2–4 weeks) [69]. Lipid formulations of amphotericin are preferred due to their lower nephrotoxicity. The duration of treatment is not well established and should be guided by improvement in clinical, serological, and radiological criteria. Durations of 9–18 months are typically used in the general population [69]. However, it is probably best to err toward the upper end of this range in transplant recipients, since immunocompromised patients tend to have higher rates of relapse [64].

When the above drugs are unavailable or contraindicated, trimethoprim-sulfamethoxazole (co-trimoxazole) is a second-line option [69]. The drug has excellent bioavailability and an acceptable side-effect profile. There have been no randomized trials comparing this drug to any of the other treatment modalities, but most (though not all) retrospective data show equivalent rates of cure. However, side effects are more frequent, and a longer treatment duration (typically $\geq$2 years) is required to prevent relapse [75, 76]. Trimethoprim-sulfamethoxazole may also be the treatment of choice for paracoccidioidomycosis involving the central nervous system due to superior CNS penetration when compared to itraconazole.

The other azoles, such as fluconazole, posaconazole, voriconazole, and isavuconazole, all have activity against *Paracoccidioides*. In a small randomized trial, voriconazole was shown to be approximately as effective as itraconazole, albeit at the cost of slightly more side effects [74]. Fluconazole has not been adequately studied, though it appears to be significantly less potent in vitro as judged by MIC [74]. Posaconazole and isavuconazole have in vitro activity, though clinical experience is limited. In one trial, 8/10 patients with paracoccidioidomycosis had at least a partially successful outcome when treated with isavuconazole as primary therapy [33]. As for other dimorphic fungi, the echinocandins have no therapeutic role.

Prophylaxis

Recipients should avoid travel to rural areas in *Paracoccidioides* endemic regions and should especially be counseled to avoid contact with agricultural activities there [77]. Potential donors with active disease should be excluded until they have been treated for at least 6 months. So far, there have not been any reported cases of transmission via infected allografts.

Recipients of organs from donors with previously treated disease should receive prophylaxis for 12 months, though this is based on expert opinion rather than clinical trial data [77]. Itraconazole can be used, as per Table 2. However, most patients will receive trimethoprim-sulfamethoxazole as part of their routine posttransplant

prophylaxis, and although there is little data to guide clinicians in this scenario, this drug may be sufficient prophylaxis alone without adding itraconazole.

Given that only a handful of paracoccidioidomycosis cases have ever been described in transplant recipients, it is not clear whether posttreatment secondary prophylaxis is required. Provided the patient's immunosuppressant regimen is not too potent at the time of treatment completion, it may be reasonable to withhold secondary prophylaxis and instead monitor the patient both clinically and with serial serology titers, keeping in mind that serology likely has a lower sensitivity in transplant recipients.

Common Antifungal Drugs Used to Treat the Endemic Mycoses

Itraconazole

Itraconazole belongs to the azole family and comes in two forms, as a solution and as capsules. The solution form is preferred due to its better bioavailability and should be taken on an empty stomach. However, this form of the drug is associated with greater gastrointestinal side effects than the capsule. In addition to poorer bioavailability, the capsule also requires an acidic stomach pH for optimal drug absorption, precluding administration of proton pump inhibitors, histamine-2 blockers, and antacids for several hours on either side of taking the capsule [78]. Furthermore, the capsule should be taken with food, rather than without food like the solution, and with an acidic beverage (e.g., soft drinks, or orange juice) to aid absorption [78, 79]. If the solution is used, a lower dose of itraconazole is required, due to its much greater bioavailability.

Side effects include gastrointestinal upset and occasionally transaminitis. Itraconazole also has negative inotropic effects and in rare instances can cause heart failure, especially at high doses. Particular care should be taken in patients with underlying ventricular dysfunction. Despite poor CSF penetration, the drug has proven efficacy in CSF disease. Lastly, itraconazole is a both potent inhibitor and a substrate of the important CYP3A4 enzyme, meaning that it can be the cause or effect of multiple drug-drug interactions [80]. The enzymatic inhibition reaches its peak approximately 1 week after commencing itraconazole, and the effects can last up to a month after stopping the drug [81]. Therefore, any drugs whose levels are affected by itraconazole (e.g., the calcineurin inhibitors) should have their dosages adjusted and/or their levels checked around these times (Table 3).

The usual dose of itraconazole is 200 mg three times a day for 2 days, followed by 200 mg twice daily thereafter. However, care should be taken to make allowances for any drug-drug interactions. Checking drug levels is strongly recommended once the drug is at steady state (after 5–7 days).

Fluconazole

Fluconazole is an azole that is available in both intravenous and oral formulations. The oral form has excellent bioavailability (~90%), and its absorption is not

Table 3 Drug-drug interactions between antifungal drugs and common immunosuppressants and suggested dose immunosuppressant dose modifications

Drug	Tacrolimus dose adjustment	Cyclosporine dose adjustment	Mycophenolate dose adjustment	Sirolimus dose adjustment	Prednisone dose adjustment
Liposomal amphotericin B	No significant change	No significant change	No significant change	No significant change	No significant change
Fluconazole	Tacrolimus levels increased. Decrease dose by 50%	Cyclosporine levels increased. Decrease dose by 50%	No significant change required	No significant change	No significant change
Itraconazole	Tacrolimus levels increased. Monitor level	Cyclosporine level increased. Monitor level	No significant change required	Sirolimus level increased. Monitor level	Unclear; monitor for side effects
Voriconazole	Tacrolimus levels increased. Decrease dose by 66%	Cyclosporine levels increased. Decrease dose by 50%	No significant change	*Coadministration contraindicated*	Unclear; monitor for side effects
Posaconazole	Tacrolimus levels increased. Decrease dose by 66%	Cyclosporine levels increased. Decrease dose by 25%	No significant change	*Coadministration contraindicated* [85]	Unclear; monitor for side effects
Isavuconazole	Tacrolimus levels increased. Decrease dose 25%	Cyclosporine levels increased slightly. Monitor levels	Mycophenolate levels increased mildly. Monitor for clinical effects	Sirolimus levels increased. Decrease dose by 50% and monitor level	No significant change

significantly affected by food or gastric pH. It is generally well-tolerated, with gastrointestinal side effects and occasional transaminitis being the chief complaints.

Fluconazole is a potent inhibitor of CYP2C9 and CYP2C19. However, it is only a moderate inhibitor of the important CYP3A4 isoenzyme, which means that drug-drug effects are often less troublesome than is seen with other azoles [80]. Nonetheless, like itraconazole, the same warnings apply with regard to drugs whose levels are affected by fluconazole (Table 3). Fluconazole is also a substrate of CYP3A4. CSF penetration is good (>50%), which sometimes results in its selection to treat CNS disease despite its relatively weaker potency in most of the endemic mycoses.

The usual dose of fluconazole is 400 mg daily. Because the drug is reliably well absorbed, drug levels do not need to be routinely checked.

Amphotericin B

Amphotericin B belongs to the polyene family and is only available as an intravenous drug. It comes in three formulations – a "conventional" deoxycholate form and two lipid formulations (liposomal amphotericin B and amphotericin B lipid complex). Nephrotoxicity is a major adverse effect of amphotericin, though the lipid forms are substantially less nephrotic than the deoxycholate formulation [82]. Amphotericin also commonly causes hypokalemia, hypomagnesemia, anemia, and phlebitis. A febrile infusion reaction is sometimes noted, especially if the drug is administered more quickly than recommended. This is not a true allergic reaction, and the patient can continue with subsequent doses of the regimen. However, slowing the infusion rate and administering acetaminophen, ibuprofen, hydrocortisone, or diphenhydramine prior to the infusion can help prevent subsequent similar febrile reactions [83]. Resistance to amphotericin B is exceptionally rare among the endemic fungi.

The usual doses of amphotericin vary by preparation. For liposomal amphotericin B, the conventional dose is 3–5 mg/kg, whereas for amphotericin B lipid complex, it is 5 mg/kg. "Conventional" (deoxycholate) amphotericin is administered at the lower dose of 1 mg/kg.

Conclusions

Histoplasmosis, blastomycosis, and paracoccidioidomycosis are important threats to transplant recipients. The risk for these infections is primarily determined by geographic exposure. Diagnosis is made by combining various diagnostic modalities, with tissue biopsy often being needed for confirmation.

Treatment usually includes polyenes and azoles for prolonged treatment durations.

Key Points

- Endemic mycoses are restricted geographically.
- Transplant recipients are at increased risk for dissemination of endemic mycoses.
- Diagnosis of histoplasmosis, blastomycosis, and paracoccidioidomycosis is difficult, and often delayed.
- Combining various diagnostic modalities is often needed for accurate diagnosis of endemic mycoses.
- Treatment of severe disease should start with an intravenous polyene preparation.
- Azoles are the primary choice for maintenance regimens.

References

1. Mott DF. The opposite end of the spectrum – managing nongame species that are prospering. In: Proceedings of the workshop on management of nongame species and ecological communities. 1985. p. 151–8.

2. Krzysik AJ 1989 Birds in Human Modified Environments and Bird Damage Control Social Economic and Health Implications Technical Report N 90 03 US Army Construction Engineering Research Laboratory USACERL
3. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. Histoplasmosis. Infect Dis Clin North Am. 2016;30(1):207–27.
4. Benedict K, Mody RK. Epidemiology of histoplasmosis outbreaks, United States, 1938–2013. Emerg Infect Dis. 2016;22(3):370–8.
5. Teixeira Mde M, Patane JS, Taylor ML, Gomez BL, Theodoro RC, de Hoog S, et al. Worldwide phylogenetic distributions and population dynamics of the genus histoplasma. PLoS Negl Trop Dis. 2016;10(6):e0004732.
6. Bahr NC, Antinori S, Wheat LJ, Sarosi GA. Histoplasmosis infections worldwide: thinking outside of the Ohio River valley. Curr Trop Med Rep. 2015;2(2):70–80.
7. Oladele RO, Ayanlowo OO, Richardson MD, Denning DW. Histoplasmosis in Africa: an emerging or a neglected disease? PLoS Negl Trop Dis. 2018;12(1):e0006046.
8. Ashbee HR, Evans EG, Viviani MA, Dupont B, Chryssanthou E, Surmont I, et al. Histoplasmosis in Europe: report on an epidemiological survey from the European Confederation of Medical Mycology Working Group. Med Mycol. 2008;46(1):57–65.
9. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. Am Rev Respir Dis. 1969; 99(4 Suppl):1–132.
10. Maiga AW, Deppen S, Scaffidi BK, Baddley J, Aldrich MC, Dittus RS, et al. Mapping *Histoplasma capsulatum* exposure, United States. Emerg Infect Dis. 2018;24(10):1835–9.
11. Kauffman CA, Freifeld AG, Andes DR, Baddley JW, Herwaldt L, Walker RC, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the Transplant-Associated Infection Surveillance Network (TRANSNET). Transpl Infect Dis. 2014;16(2):213–24.
12. Cuellar-Rodriguez J, Avery RK, Lard M, Budev M, Gordon SM, Shrestha NK, et al. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. Clin Infect Dis. 2009;49(5):710–6.
13. Gajurel K, Dhakal R, Deresinski S. Histoplasmosis in transplant recipients. Clin Transplant. 2017;31(10):e13087.
14. Assi M, Martin S, Wheat LJ, Hage C, Freifeld A, Avery R, et al. Histoplasmosis after solid organ transplant. Clin Infect Dis. 2013;57(11):1542–9.
15. Woods JP. Revisiting old friends: developments in understanding *Histoplasma capsulatum* pathogenesis. J Microbiol. 2016;54(3):265–76.
16. Nel JS, Bartelt LA, van Duin D, Lachiewicz AM. Endemic mycoses in solid organ transplant recipients. Infect Dis Clin North Am. 2018;32(3):667–85.
17. Wheat LJ, Smith EJ, Sathapatayavongs B, Batteiger B, Filo RS, Leapman SB, et al. Histoplasmosis in renal allograft recipients. Two large urban outbreaks. Arch Intern Med. 1983; 143(4):703–7.
18. Limaye AP, Connolly PA, Sagar M, Fritsche TR, Cookson BT, Wheat LJ, et al. Transmission of *Histoplasma capsulatum* by organ transplantation. N Engl J Med. 2000;343(16):1163–6.
19. Schwenk HT, Vo P, Moffitt K, Kehoe E, Blume E, Sharma T, et al. Allograft-transmitted *Histoplasma capsulatum* infection in a solid organ transplant recipient. J Pediatric Infect Dis Soc. 2013;2(3):270–3.
20. Wheat J, Wheat H, Connolly P, Kleiman M, Supparatpinyo K, Nelson K, et al. Cross-reactivity in *Histoplasma capsulatum* variety capsulatum antigen assays of urine samples from patients with endemic mycoses. Clin Infect Dis. 1997;24(6):1169–71.
21. Vergidis P, Walker RC, Kaul DR, Kauffman CA, Freifeld AG, Slagle DC, et al. False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis. Transpl Infect Dis. 2012;14(2):213–7.
22. Assi MA, Sandid MS, Baddour LM, Roberts GD, Walker RC. Systemic histoplasmosis: a 15-year retrospective institutional review of 111 patients. Medicine (Baltimore). 2007;86(3):162–9.
23. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev. 2011;24(2):247–80.

24. Ohno H, Tanabe K, Umeyama T, Kaneko Y, Yamagoe S, Miyazaki Y. Application of nested PCR for diagnosis of histoplasmosis. J Infect Chemother. 2013;19(5):999–1003.
25. Muraosa Y, Toyotome T, Yahiro M, Watanabe A, Shikanai-Yasuda MA, Kamei K. Detection of *Histoplasma capsulatum* from clinical specimens by cycling probe-based real-time PCR and nested real-time PCR. Med Mycol. 2016;54(4):433–8.
26. Koepsell SA, Hinrichs SH, Iwen PC. Applying a real-time PCR assay for *Histoplasma capsulatum* to clinically relevant formalin-fixed paraffin-embedded human tissue. J Clin Microbiol. 2012;50(10):3395–7.
27. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. Clin Infect Dis. 2007;45(7):807–25.
28. Johnson PC, Wheat LJ, Cloud GA, Goldman M, Lancaster D, Bamberger DM, et al. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. Ann Intern Med. 2002;137(2):105–9.
29. Wheat J, Hafner R, Korzun AH, Limjoco MT, Spencer P, Larsen RA, et al. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trial Group. Am J Med. 1995;98(4):336–42.
30. Wheat LJ, Connolly P, Smedema M, Brizendine E, Hafner R, AIDS Clinical Trials Group and the Mycoses Study Group of the National Institute of Allergy and Infectious Diseases, et al. Emergence of resistance to fluconazole as a cause of failure during treatment of histoplasmosis in patients with acquired immunodeficiency disease syndrome. Clin Infect Dis. 2001;33(11): 1910–3.
31. Wheat J, MaWhinney S, Hafner R, McKinsey D, Chen D, Korzun A, et al. Treatment of histoplasmosis with fluconazole in patients with acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Acquired Immunodeficiency Syndrome Clinical Trials Group and Mycoses Study Group. Am J Med. 1997;103(3):223–32.
32. Wheat LJ, Connolly P, Smedema M, Durkin M, Brizendine E, Mann P, et al. Activity of newer triazoles against *Histoplasma capsulatum* from patients with AIDS who failed fluconazole. J Antimicrob Chemother. 2006;57(6):1235–9.
33. Thompson GR 3rd, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F, Ostrosky-Zeichner L, Azie N, et al. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. Clin Infect Dis. 2016;63(3):356–62.
34. Hage CA, Connolly P, Horan D, Durkin M, Smedema M, Zarnowski R, et al. Investigation of the efficacy of micafungin in the treatment of histoplasmosis using two North American strains of *Histoplasma capsulatum*. Antimicrob Agents Chemother. 2011;55(9):4447–50.
35. Goldman M, Zackin R, Fichtenbaum CJ, Skiest DJ, Koletar SL, Hafner R, et al. Safety of discontinuation of maintenance therapy for disseminated histoplasmosis after immunologic response to antiretroviral therapy. Clin Infect Dis. 2004;38(10):1485–9.
36. McKinsey DS, Wheat LJ, Cloud GA, Pierce M, Black JR, Bamberger DM, et al. Itraconazole prophylaxis for fungal infections in patients with advanced human immunodeficiency virus infection: randomized, placebo-controlled, double-blind study. National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1999;28(5):1049–56.
37. Saccente M, Woods GL. Clinical and laboratory update on blastomycosis. Clin Microbiol Rev. 2010;23(2):367–81.
38. Klein BS, Vergeront JM, DiSalvo AF, Kaufman L, Davis JP. Two outbreaks of blastomycosis along rivers in Wisconsin. Isolation of *Blastomyces dermatitidis* from riverbank soil and evidence of its transmission along waterways. Am Rev Respir Dis. 1987;136(6): 1333–8.
39. Klein BS, Vergeront JM, Weeks RJ, Kumar UN, Mathai G, Varkey B, et al. Isolation of *Blastomyces dermatitidis* in soil associated with a large outbreak of blastomycosis in Wisconsin. N Engl J Med. 1986;314(9):529–34.
40. Chakrabarti A, Slavin MA. Endemic fungal infections in the Asia-Pacific region. Med Mycol. 2011;49(4):337–44.

41. Baily GG, Robertson VJ, Neill P, Garrido P, Levy LF. Blastomycosis in Africa: clinical features, diagnosis, and treatment. Rev Infect Dis. 1991;13(5):1005–8.
42. Linder KAKC, Miceli M. Pulmonary blastomycosis in a hematopoietic cell transplant recipient. Infect Dis Clin Pract. 2016;24(6):e71–3.
43. Grim SA, Proia L, Miller R, Alhyraba M, Costas-Chavarri A, Oberholzer J, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. Transpl Infect Dis. 2012;14(1):17–23.
44. Gauthier GM, Safdar N, Klein BS, Andes DR. Blastomycosis in solid organ transplant recipients. Transpl Infect Dis. 2007;9(4):310–7.
45. Wuthrich M, Finkel-Jimenez B, Brandhorst TT, Filutowicz HI, Warner T, Klein BS. Analysis of non-adhesive pathogenic mechanisms of BAD1 on *Blastomyces dermatitidis*. Med Mycol. 2006;44(1):41–9.
46. Ehni W. Endogenous reactivation in blastomycosis. Am J Med. 1989;86(6 Pt 2):831–2.
47. Kauffman CA, Miceli MH. Histoplasmosis and blastomycosis in solid organ transplant recipients. J Fungi (Basel). 2015;1(2):94–106.
48. Smith JA, Gauthier G. New developments in blastomycosis. Semin Respir Crit Care Med. 2015;36(5):715–28.
49. Smith JA, Kauffman CA. Blastomycosis. Proc Am Thorac Soc. 2010;7(3):173–80.
50. Chapman SW, Lin AC, Hendricks KA, Nolan RL, Currier MM, Morris KR, et al. Endemic blastomycosis in Mississippi: epidemiological and clinical studies. Semin Respir Infect. 1997;12(3):219–28.
51. Martynowicz MA, Prakash UB. Pulmonary blastomycosis: an appraisal of diagnostic techniques. Chest. 2002;121(3):768–73.
52. Durkin M, Witt J, Lemonte A, Wheat B, Connolly P. Antigen assay with the potential to aid in diagnosis of blastomycosis. J Clin Microbiol. 2004;42(10):4873–5.
53. Frost HM, Novicki TJ. Blastomyces antigen detection for diagnosis and management of blastomycosis. J Clin Microbiol. 2015;53(11):3660–2.
54. Chapman SW, Dismukes WE, Proia LA, Bradsher RW, Pappas PG, Threlkeld MG, et al. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. Clin Infect Dis. 2008;46(12):1801–12.
55. Pappas PG, Bradsher RW, Kauffman CA, Cloud GA, Thomas CJ, Campbell GD Jr, et al. Treatment of blastomycosis with higher doses of fluconazole. The National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1997;25(2):200–5.
56. Pappas PG, Bradsher RW, Chapman SW, Kauffman CA, Dine A, Cloud GA, et al. Treatment of blastomycosis with fluconazole: a pilot study. The National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1995;20(2):267–71.
57. Bariola JR, Perry P, Pappas PG, Proia L, Shealey W, Wright PW, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. Clin Infect Dis. 2010;50(6):797–804.
58. Sugar AM, Liu XP. In vitro and in vivo activities of SCH 56592 against *Blastomyces dermatitidis*. Antimicrob Agents Chemother. 1996;40(5):1314–6.
59. Mongkolrattanothai K, Peev M, Wheat LJ, Marcinak J. Urine antigen detection of blastomycosis in pediatric patients. Pediatr Infect Dis J. 2006;25(11):1076–8.
60. Theodoro RC, Bagagli E, Oliveira C. Phylogenetic analysis of PRP8 intein in *Paracoccidioides brasiliensis* species complex. Fungal Genet Biol. 2008;45(9):1284–91.
61. Martinez R. Epidemiology of paracoccidioidomycosis. Rev Inst Med Trop Sao Paulo. 2015; 57(Suppl 19):11–20.
62. Travassos LR, Taborda CP, Colombo AL. Treatment options for paracoccidioidomycosis and new strategies investigated. Expert Rev Anti Infect Ther. 2008;6(2):251–62.
63. Boin ID, Boteon YL, Stucchi RS, Pereira MI, Portugal TC, Udo EY. Serological profile of pretransplantation liver patients. Transplant Proc. 2010;42(2):491–3.
64. de Almeida JN Jr, Pecanha PM, Colombo AL. Paracoccidioidomycosis in immunocompromised patients: a literature review. J Fungi (Basel). 2018;5(1).

65. Brummer E, Castaneda E, Restrepo A. Paracoccidioidomycosis: an update. Clin Microbiol Rev. 1993;6(2):89–117.
66. Fortes MR, Miot HA, Kurokawa CS, Marques ME, Marques SA. Immunology of paracoccidioidomycosis. An Bras Dermatol. 2011;86(3):516–24.
67. Pereira RM, Bucaretchi F, Barison Ede M, Hessel G, Tresoldi AT. Paracoccidioidomycosis in children: clinical presentation, follow-up and outcome. Rev Inst Med Trop Sao Paulo. 2004;46(3):127–31.
68. Onate JM, Tobon AM, Restrepo A. Adrenal gland insufficiency secondary to paracoccidioidomycosis. Biomedica. 2002;22(3):280–6.
69. Shikanai-Yasuda MA, Mendes RP, Colombo AL, Queiroz-Telles F, Kono ASG, Paniago AMM, et al. Brazilian guidelines for the clinical management of paracoccidioidomycosis. Rev Soc Bras Med Trop. 2017;50(5):715–40.
70. Batista J Jr, de Camargo ZP, Fernandes GF, Vicentini AP, Fontes CJ, Hahn RC. Is the geographical origin of a *Paracoccidioides brasiliensis* isolate important for antigen production for regional diagnosis of paracoccidioidomycosis? Mycoses. 2010;53(2):176–80.
71. Marques da Silva SH, Colombo AL, Blotta MH, Lopes JD, Queiroz-Telles F, Pires de Camargo Z. Detection of circulating gp43 antigen in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid of patients with paracoccidioidomycosis. J Clin Microbiol. 2003;41(8):3675–80.
72. Gomes GM, Cisalpino PS, Taborda CP, de Camargo ZP. PCR for diagnosis of paracoccidioidomycosis. J Clin Microbiol. 2000;38(9):3478–80.
73. Naranjo MS, Trujillo M, Munera MI, Restrepo P, Gomez I, Restrepo A. Treatment of paracoccidioidomycosis with itraconazole. J Med Vet Mycol. 1990;28(1):67–76.
74. Queiroz-Telles F, Goldani LZ, Schlamm HT, Goodrich JM, Espinel-Ingroff A, Shikanai-Yasuda MA. An open-label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis. Clin Infect Dis. 2007;45(11):1462–9.
75. Cavalcante Rde S, Sylvestre TF, Levorato AD, de Carvalho LR, Mendes RP. Comparison between itraconazole and cotrimoxazole in the treatment of paracoccidiodomycosis. PLoS Negl Trop Dis. 2014;8(4):e2793.
76. Borges SR, Silva GM, Chambela Mda C, Oliveira Rde V, Costa RL, Wanke B, et al. Itraconazole vs. trimethoprim-sulfamethoxazole: a comparative cohort study of 200 patients with paracoccidioidomycosis. Med Mycol. 2014;52(3):303–10.
77. Abdala E, Miller R, Pasqualotto AC, Munoz P, Colombo AL, Cuenca-Estrella M. Endemic fungal infection recommendations for solid-organ transplant recipients and donors. Transplantation. 2018;102(2S Suppl 2):S52–9.
78. Van Peer A, Woestenborghs R, Heykants J, Gasparini R, Gauwenbergh G. The effects of food and dose on the oral systemic availability of itraconazole in healthy subjects. Eur J Clin Pharmacol. 1989;36(4):423–6.
79. Jaruratanasirikul S, Kleepkaew A. Influence of an acidic beverage (Coca-Cola) on the absorption of itraconazole. Eur J Clin Pharmacol. 1997;52(3):235–7.
80. Venkatakrishnan K, von Moltke LL, Greenblatt DJ. Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. Clin Pharmacokinet. 2000;38(2):111–80.
81. Dodds-Ashley E. Management of drug and food interactions with azole antifungal agents in transplant recipients. Pharmacotherapy. 2010;30(8):842–54.
82. Wong-Beringer A, Jacobs RA, Guglielmo BJ. Lipid formulations of amphotericin B: clinical efficacy and toxicities. Clin Infect Dis. 1998;27(3):603–18.
83. Gigliotti F, Shenep JL, Lott L, Thornton D. Induction of prostaglandin synthesis as the mechanism responsible for the chills and fever produced by infusing amphotericin B. J Infect Dis. 1987;156(5):784–9.
84. Singh N, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ, et al. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, infectious diseases community of practice. Am J Transplant. 2012;12(9):2414–28.

85. Merck & Co. Inc. Noxafil® (posaconazole) injection 18 mg/mL Noxafil® (posaconazole) delayed-release tablets 100 mg Noxafil® (posaconazole) oral suspension 40 mg/mL. 2015.
86. Centers for Disease Control and Prevention. Fungal infections – protect your health. Centers for Disease Control and Prevention; 2019. https://www.cdc.gov/features/fungalinfections/index.html
87. Schwartz S, Kontoyiannis DP, Harrison T, Ruhnke M. Advances in the diagnosis and treatment of fungal infections of the CNS. Lancet Neurol. 2018;17(4):362–72.

Part V

Parasitic Infections Introduction

Toxoplasma gondii in Solid Organ and Stem Cell Transplant: Prevention and Treatment

52

Hélène Guegan and Florence Robert-Gangneux

Contents

Introduction 1254
Burden of Toxoplasmosis in Transplant Patients 1254
Toxoplasmosis in HSCT Transplant Patients 1256
Toxoplasmosis in SOT Transplant Patients 1256
Clinical Signs 1257
Diagnosis of Toxoplasmosis in Transplant Patients 1258
Serology 1259
Parasite Detection 1259
Histopathologic Examination 1260
Imaging 1261
Patient Management 1262
Treatment 1264
Prevention of Toxoplasmosis in Transplant Patients 1268
Pretransplant Serologic Screening for Toxoplasmosis 1268
Anti-*Toxoplasma* Chemoprophylaxis 1269
Patient Counseling and Hygienic Measures 1272
Diagnostic Follow-Up After Transplantation 1273
Conclusion 1274
Key Points 1274
References 1275

H. Guegan
Univ Rennes, CHU Rennes, Inserm, EHESP, Irset UMR_S 1085, Rennes, France

Centre Hospitalier Universitaire de Rennes, Rennes, France
e-mail: helene.guegan@chu-rennes.fr

F. Robert-Gangneux (✉)
Univ Rennes, CHU Rennes, Inserm, EHESP, Irset UMR_S 1085, Rennes, France

Centre Hospitalier Universitaire de Rennes, Rennes, France

Study Group for Clinical Parasitology (ESGCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Basel, Switzerland
e-mail: florence.robert-gangneux@univ-rennes1.fr

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_51

Introduction

Toxoplasmosis is a zoonosis infecting humans and warm-blooded animals worldwide, with variable prevalence and clinical burden according to geographical areas [1]. After primary infection, it can disseminate to all organs, as it can infect virtually all nucleated cells. *Toxoplasma gondii* has the capacity to interfere with host immune response, which allows it to persist lifelong within dormant tissue cysts, as long as the host is immunocompetent. Transmission to humans mainly occurs by ingestion of food or water contaminated with oocysts present in feces of infected felids (i.e., the cat family) or by undercooked meat containing cysts [2]. Although largely asymptomatic in adults, toxoplasmosis is a life-threatening opportunistic infection in immunocompromised patients, whether from primary acquisition or due to cyst reactivation. In developed countries, toxoplasmosis is more frequently seen in patients receiving immunosuppressive therapy than in HIV-infected patients, as observed with *Pneumocystis* pneumonia [3, 4]. Indeed, the growing number of grafts makes transplant patients an increasing population at risk, not only because immunosuppressive therapy can reactivate quiescent cysts but also because the parasite can be transmitted through cyst-containing organs from a seropositive donor [5]. In immunocompromised patients, reactivation infection often results in cerebral toxoplasmosis or disseminated toxoplasmosis and leads to death in the absence of treatment. Although prevention measures can be implemented to prevent severe toxoplasmosis, they are not applied in all countries, due to a lack of consensus. This chapter will review the burden of toxoplasmosis according to graft type and the diagnostic methods and discuss prevention measures and treatment.

Burden of Toxoplasmosis in Transplant Patients

In contrast to HIV-infected patients, the incidence of toxoplasmosis in transplant recipients is poorly documented. Published studies reporting patient series are scarce [6–11], and literature mostly consists of case reports [2, 12–14]. Overall, the incidence of toxoplasmosis in a given transplantation center or region depends on many factors: the *Toxoplasma* seroprevalence in the general population, the number of transplant procedures, the immunosuppressive protocols used, the serologic screening guidelines for donor and recipient, the chemoprophylaxis protocol, the diagnostic follow-up (serial PCR and/or serology) after transplantation, and other contributory factors.

The risk for reactivation of chronic infection varies according to the depth of immune suppression induced by immunosuppressive protocol, thus according to the type of graft, with allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients being the most at-risk patients [15]. By contrast, the risk of acquiring infection through transplantation is virtually solely due to solid organ transplantation (SOT), usually due to an organ from a seropositive donor to a seronegative recipient (D+/R-, i.e., “Toxo- mismatch”), and risk depends on organ type (Table 1).

Table 1 Patient management according to organ type and *Toxoplasma* serology

Type of transplant	Risk	Hygienic prevention measures	Chemoprophylaxis	Follow-up
HSCT				
Toxo+	High risk of reactivation	NA	Necessary (preferably 12 months) or according to CD4+ counts	Periodic blood PCR recommended
Toxo-	No graft-related risk[a] Risk of foodborne infection	Recommended	Recommended (6 months) as for *Pneumocystis* prevention	
Heart				
Toxo+	Moderate risk of reactivation	NA	Recommended (3–6 months)	Serology (2/yr)
Toxo-	High risk of organ-related infection if D + Risk of foodborne infection	Recommended	Necessary (6–12 months)	Serology (2/yr) + PCR (1/wk., first month if D+/R-)
Liver				
Toxo+	Low risk of reactivation	NA	3-6 months as for *Pneumocystis* prevention, recommended if organ rejection	None
Toxo-	High risk of organ-related infection if D + Risk of foodborne infection	Recommended	Necessary (6–12 months)	Optional
Kidney				
Toxo+	Low risk of reactivation	NA	3-6 months as for *Pneumocystis* prevention, recommended if organ rejection	None
Toxo-	High risk of organ-related infection if D + Risk of foodborne infection	Recommended	Necessary 6 months	Optional

NA: not applicable

[a]Provided that donor serologic screening is performed and preventive measures applied

Toxoplasmosis in HSCT Transplant Patients

The contamination of HSCT with *Toxoplasma* tachyzoites is theoretically possible, in the setting of acute infection in the donor at the time of sampling. The bone marrow and the blood are not sites for parasite encystation; thus, chronically infected people are not a source for parasite transmission. Cord blood cells could also be a vector of toxoplasmosis if they contain replicating parasites, i.e., when the newborn has been congenitally infected. In both instances, serologic screening of the donor is an efficient barrier to prevent HSCT-acquired infection (see below) [16]. Therefore, in HSCT patients, toxoplasmosis results primarily from reactivation of latent tissue *Toxoplasma* cysts in the *Toxoplasma*-seropositive recipient (R+). Most cases are observed in pretransplant R+ who are grafted with a HSCT from a *Toxoplasma*-seronegative donor (D-) [11, 17]. It is intuitively explained by the loss of memory T-cell repertoire following immunosuppressive chemotherapy and the lack of protection conferred by acquired T cells from the D-. Toxoplasmosis onset mainly occurs early, within the first 6 months after transplantation [11, 15, 17, 18], although it can be observed up to 1 year or more [3, 11, 19]. In a recent French series of 419 HSCT recipients, 17 (4%) cases were reported, of which 7 occurred within the first 30 days, before the prophylaxis was started [20]; these cases of early reactivation have a higher mortality rate. Later onset of toxoplasmosis can be associated with etanercept, usually introduced during steroid-refractory graft-versus-host disease (GVHD) when immunosuppressive regimen intensification is needed [18]. In the study by Conrad et al. [18], the origin of the HSCT (bone marrow, peripheral blood, or cord blood) had no clear impact on the onset of toxoplasmosis, as no significant differences were observed between cases and controls, but in view of the low number of patients included (23 cases et 46 controls), this question should be further explored.

Data on toxoplasmosis incidence are scarce and greatly vary according to the geographical area [21], but also on the diagnostic tools used. The incidence also greatly differs according to the care management practices and guidelines, as shown in a recent European study, where it ranged from 0 to 92.3 cases per 1000 grafts over a 5-year period [11].

Toxoplasmosis in SOT Transplant Patients

Due to the preferential localization of *Toxoplasma* in muscles, the incidence of *Toxoplasma* transmission via the graft is highest in heart transplant compared with other SOT. D+/R- heart recipients have a 57–75% risk of early posttransplant toxoplasmosis unless they receive prophylaxis [22, 23].

The incidence of toxoplasmosis was reported to be 0.08% in kidney and liver transplant patients, thus much less frequent by comparison to that observed in heart transplant patients (0.61%, $p < 0.001$), in a Spanish study including 7709 renal, 4872 liver, and 1979 heart transplant patients, respectively [6]. About half (10/22) cases occurred in kidney or liver transplant patients; the remaining 12

cases were heart transplant patients. No cases were diagnosed in the 1240 pancreas, lung, or small bowel recipients. All but two patients with toxoplasmosis who had documented serologic results before transplantation (20/22) were seronegative.

The overall incidence of toxoplasmosis in non-heart SOT patients is assumed to be below 1% [24], and the vast majority of cases are reported in D+/R- [6], although D/R serologic status is not always known in published reports [2].

Although kidneys are organs at low risk for parasite transmission, the very high number of transplantation procedures explains that the number of toxoplasmosis cases reported in kidney transplant patients is similar to that reported in liver recipients. By contrast, the liver is a frequent site for parasite encystation [25] and thus carries a high risk for the recipient in case of toxoplasmosis mismatch.

Unlike allo-HSCT, SOT (and particularly non-heart SOT) patients seem to be at low risk of reactivation toxoplasmosis well after transplant (>12 months), in the absence of rejection or unusual immunosuppression [24, 26] (Table 1). The Spanish case-control study [6] showed that a negative serostatus prior to transplantation was the only independent risk factor for toxoplasmosis in SOT patients, confirming that primary infection is more frequently observed than reactivation in these patients.

Clinical Signs

Common clinical manifestations may include neurological signs, which can be pleomorphic (such as headache, seizures, confusion, vertigo, language disorders, vision alteration, paralysis, coma), pulmonary signs (from moderate interstitial pneumonitis to acute respiratory distress), myocarditis, and ocular signs, but other organs can be involved in case of disseminated toxoplasmosis (including skin, kidney, and liver). Pulmonary toxoplasmosis is the result of disseminated toxoplasmosis, as no transmission of toxoplasmosis has been reported after lung transplantation, meaning that *Toxoplasma* cysts are virtually absent from this organ; thus, it cannot be the primary site of reactivation. In a recent European study, cerebral, disseminated, and pulmonary toxoplasmosis accounted for 19%, 17%, and 10% of cases, respectively, whereas in a French multicenter study, cerebral and disseminated toxoplasmosis were observed in 19% and 42%, respectively [3]. The overall survival is usually around 50% or lower, except when infection is limited to the eye compartment [11]. However, among transplant patients, HSCT have a poorer survival rate than SOT patients, regardless of chemoprophylaxis, probably because of deeper immune suppression [11]. In the study by Schmidt et al. [17], the mortality rate associated with disseminated toxoplasmosis was 69% in allo-HSCT, thus much higher than in HIV-infected patients (17%), underlining the need for rapid diagnosis and treatment in these patients. Additionally, data obtained from 13 French centers showed that frequency of disseminated toxoplasmosis was higher in transplant patients than in HIV-infected patients [3] (Fig. 1); this observation could be due to the more rapid evolution in transplant recipients or delay of diagnosis.

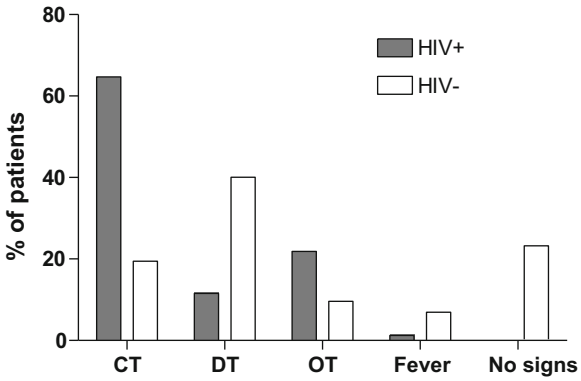


Fig. 1 Frequency of clinical setting of toxoplasmosis, according to a French multicenter study [3]. CT, cerebral toxoplasmosis; DT, disseminated toxoplasmosis; OT, ocular toxoplasmosis

Diagnosis of toxoplasmosis is sometimes difficult in HSCT recipients and may be delayed or missed, as clinical signs can be limited to fever or nearly absent at onset of reactivation, as observed in 65% (30/58), 65% (11/17), and 39% (28/72) of cases in three recent studies [3, 11, 20]. These cases are mostly reported in France, where follow-up by serial PCR after transplantation is common practice (see below), thus allowing early diagnosis.

Although nonspecific, hemophagocytic syndrome (HPS) can be associated with acute toxoplasmosis in SOT patients [27]. Therefore, diagnostic investigations targeting toxoplasmosis should be included in the workup following HPS diagnosis.

Furthermore, toxoplasmosis has been described in the setting of immune reconstitution inflammatory syndrome (IRIS) in a HSCT [28]. Although previously described in HIV-infected patients, HPS- and IRIS-associated toxoplasmosis in transplant recipients could be observed more frequently, due to the increasing number of transplantation procedures [27, 29–32].

Diagnosis of Toxoplasmosis in Transplant Patients

Although prophylaxis significantly reduces the risk of developing toxoplasmosis, the efficacy of such drugs can be hampered by pharmacokinetic issues as gastrointestinal intolerance or impaired gut absorption due to GVHD or due to the early interruption because of myelotoxicity. Patient compliance must be always queried. Therefore, toxoplasmosis should not be excluded on the sole knowledge that trimethoprim-sulfamethoxazole (cotrimoxazole, TMP/SMX) has been prescribed.

Given the potential severity of toxoplasmosis in immunosuppressed patients, urgent diagnosis of a primary *Toxoplasma* infection in a seronegative recipient (R-) or a reactivation in a seropositive recipient (R+) is crucial. This diagnosis remains challenging due to the nonspecificity of clinical and radiological signs, which can delay *Toxoplasma*-specific diagnostic exams, i.e., IgG/IgM serology and parasite detection.

Serology

Serologic techniques are essential to estimate the risk of toxoplasmosis in patients with clinical signs, which can be attributed to any other opportunistic infection. Indeed, if the serology is positive, a *Toxoplasma* reactivation should be considered as a possible etiology. Detection of specific IgM antibodies can result from either a primary infection or from a parasite transmission through the solid organ graft, but it must be confirmed by the rise of specific IgG. If the serology is negative, a primary infection cannot be excluded in a HSCT transplant or a SOT within the first months after the transplant, as antibody synthesis can be attenuated, delayed (SOT), or nearly abolished (HSCT). Thus, when negative results are obtained initially, serologic testing should be performed serially and periodically (every 2–4 weeks) after symptom onset, until at least 3 months before primary infection can be ruled out. Besides, serologic tests may miss a *Toxoplasma* reactivation, especially in HSCT patients in whom the humoral response can be reduced or abolished. The use of adequate serologic technique is required, and several different commercial assays should be performed when there is a low titer of IgG. A recent French study compared the performances of nine marketed immunoenzymatic assays in sera with low or negative IgG titers from immunosuppressed patients, showing the highly variable performances of the assays. They emphasized the need to confirm any results in the gray zone using a confirmation method based on Western blot [33].

Serology can be useful a tool for patient follow-up [34] (see below), but its interpretation should be done with caution in transplant patients, taking into account the pretransplant serologic status and the occurrence of blood transfusions which make the interpretation of anti-*Toxoplasma* antibody levels more complicated. When pretransplant serology is not available, IgG avidity testing can help to distinguish transfusion-acquired IgG (high avidity) from recent infection (low avidity), but is of no use if the patient was seropositive (high avidity) before transplant.

Finally, a significant increase of IgG level is not necessarily associated with a clinical reactivation, and thus, the IgG titer has only an indicative value.

In ocular toxoplasmosis, aqueous humor or vitreous fluid can be sampled to detect a neosynthesis of specific IgG, using Western blot. However, the weak antibody production in patients receiving immunosuppressant agentSerologic techniques usually makes serologic techniques of reduced sensitivity, compared to molecular detection of *Toxoplasma*.

Parasite Detection

While serologic screening is essential to estimate the risk of reactivation, the definitive diagnosis of acute infection is based on the detection of *Toxoplasma* tachyzoites and DNA in various fluids (such as bronchoalveolar lavages (BAL), cerebrospinal fluids (CSF), aqueous humor, blood, or bone marrow) or biopsies, either by microscopic examination or using PCR. Optical immersion microscopy on Giemsa or May-Grünwald-Giemsa-stained smears is a rapid and inexpensive exam but offers low

sensitivity and requires trained operators, given the small size of the parasite. Over the past 20 years, real-time PCRs have been developed and gradually replaced end point PCRs as more sensitive and more specific methods [35–37], with a lower detection limit of approximately one parasite genomes/mL [35]. The most sensitive techniques target the repeated 529 pb sequence (rep529) [38] which has been shown to provide a higher sensitivity than B1 PCR [39, 40].

Most clinical studies assessing the diagnostic performances of PCR in immunocompromised patients focused on HIV-infected patients, reporting sensitivities from 35% to 72% and from 15% to 80%, in CSF and the blood, respectively [41]. Of note, sensitivity on CSF was shown to be higher when cellularity was ≥4 nucleated cells/μL and in patients with multiple brain lesions (≥4) detected with MRI [42]. The delay between the start of curative treatment and CSF sampling also strongly impacted PCR sensitivity; as expected, early sampling after the beginning of anti-*Toxoplasma* therapy (<1 week) led to higher PCR performances [43].

There are very scarce clinical studies assessing PCR for the diagnosis of pulmonary toxoplasmosis in immunocompromised patients [7, 44]. The very low incidence of pulmonary toxoplasmosis (2/336 BAL [7]) did not allow the authors to study the sensitivity of PCR against microscopy. Nevertheless, the value of molecular methods remains indisputable for pulmonary toxoplasmosis, as PCR provided the diagnosis in 9/10 pulmonary cases versus only 1/10 by microscopy, as described in a recent European cohort [11].

PCR on peripheral blood has been evaluated in the diagnosis of ocular toxoplasmosis, but its sensitivity was shown to be lower than in ocular samples (25% and 36%, in blood and ocular specimens, respectively) [45]. Overall, PCR has a better sensitivity in ocular samples from immunocompromised patients than from immunocompetent patients, especially when it is performed early after the onset of symptoms [46, 47], and on vitreous fluid rather than on aqueous humor puncture [48]. PCR sensitivity in ocular samples also varies according to the size of lesions and is higher in case of large atypical foci of retinitis, as commonly observed in immunocompromised patients [49, 50]. Overall, when possible, combining both PCR and IgG testing in ocular samples will yield better performances.

PCR in peripheral blood is part of the diagnostic algorithm in transplant patients with any symptoms suggestive of systemic infection of unknown etiology, as an alternative or in complement to invasive sampling, although with lower sensitivity [3].

Finally, quantitative PCRs can also be of interest to monitor treatment efficacy, as parasite loads are expected to decrease rapidly in the blood, BAL, or CSF within the 3 first weeks of treatment [51, 52]. Given the lack of standardization of such testing, it would be important to use the same sample type and diagnostic platform so as to be able to trend and compare results over time.

Histopathologic Examination

Given the small size (7 μm × 2 μm) of tachyzoites, the parasite stage associated with acute disease progression histopathologic examination is difficult. It may

be contributive, albeit mainly used for postmortem diagnosis. Parasites can be seen in tissue sections after staining with hematoxylin-eosin-safran (HES) or May-Grünwald-Giemsa (MGG), provided that slides are observed by a trained microscopist. Immunoperoxidase staining can be also used. Parasite multiplication can be associated with cell lysis or necrosis, but such observation is nonspecific, and tissue reaction can be modified by immunosuppression. Infrequently, tachyzoites can be spotted in brain or heart biopsies, but qPCR has far better sensitivity.

Imaging

Radiographic imaging provides substantial diagnostic input, although with variable sensitivity depending on the clinical toxoplasmosis form, ranging from 92% (12/13), 70% (7/10), and 42% (8/19), respectively, in cerebral, pulmonary, and disseminated toxoplasmosis [11]. Clinical presentation guides selection of imaging. A cerebral computed tomography (CT) scan or a magnetic resonance imagery (MRI) should be performed if there is evidence of any neurological signs, best done with contrast. Imaging generally reveals solitary or multiple brain abscesses, of variable size, homogeneously nodular, or with a typical peripheral enhancement surrounding a central necrosis zone. The "asymmetric target sign" is another feature suggestive of toxoplasmosis; it is a small eccentric nodule along the wall of the enhancing ring, reflecting a vein thrombosis [53]. Edema is often present and can induce a deviation of the midline. Most lesions are observed in the white matter, followed by peripheral location (cortex and corticomedullary junction) and basal ganglia but also in the posterior fossa, thalamus, and brainstem [54]. These features are suggestive of toxoplasmosis but are not specific and may differ according to the type of immunosuppression. Atypical imaging can be observed in HSCT recipients, without any contrast enhancement and even any significant lesion, related to leukopenia and the missing inflammatory response [55, 56]. In this context, MRI should be preferentially performed as it can demonstrate additional abnormalities in gray matter or hemorrhagic lesions [55].

In pulmonary toxoplasmosis, chest x-ray or thoracic CT scan usually shows a diffuse interstitial pneumonitis with nonspecific alveolar condensations or ground-glass opacities [57].

Ultrasound scan can evidence an organ involvement as myocarditis, hepatitis, etc.

Funduscopic examination should be performed with any ocular symptoms. The eye fundus enables the diagnosis of toxoplasmosis in 50% of cases, when hyperpigmented old scars residual from previous retinochoroiditis foci are present. Typical acute lesions are intensely white with overlying vitreous inflammatory infiltrates, retinal vasculitis, and hemorrhages [58]. When observed in a *Toxoplasma*-seropositive patient, this type of lesions may be sufficient to ensure the diagnosis, but atypical lesions are frequently observed in immunosuppressed patients, and further diagnostics may be indicated (see above).

Patient Management

The European group for Blood and Marrow Transplantation Infectious Diseases Working Party (EBMT-IDWP) [59] has proposed a classification of toxoplasmosis according to clinical signs and diagnostic workup as follows:

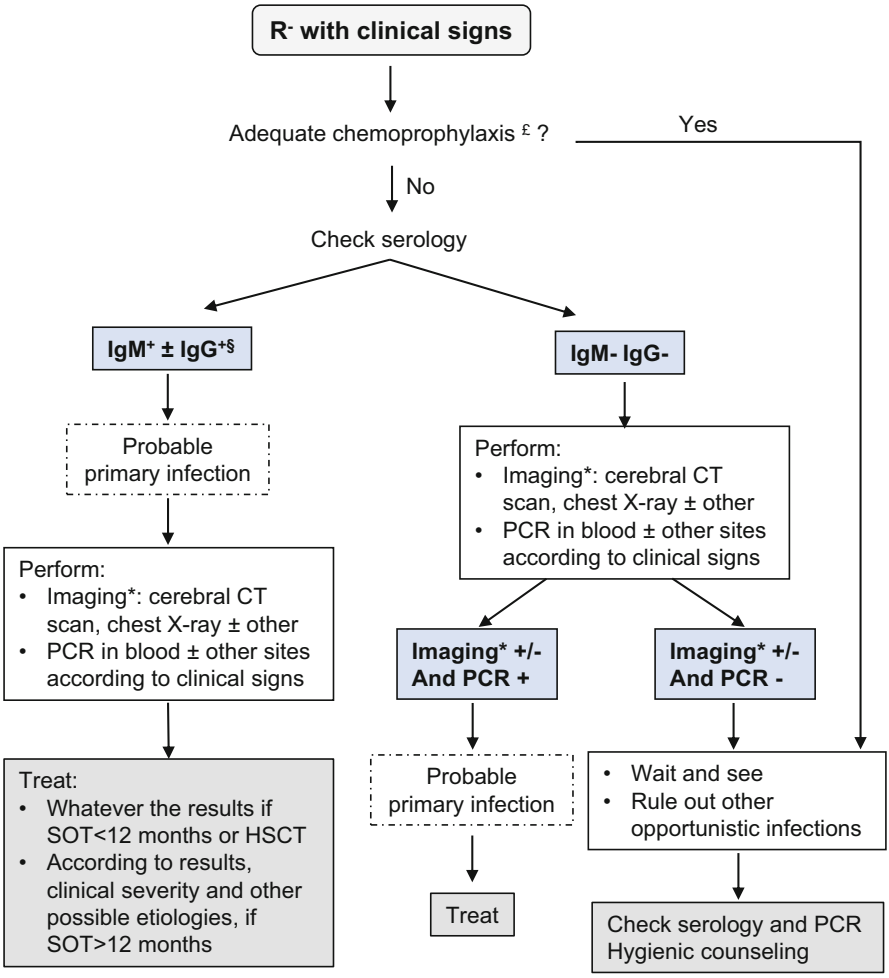
Toxoplasma infection is defined as a posttransplant seroconversion in R- or a significant increase of IgG titer in R+ or a positive *Toxoplasma* PCR in the blood, without any evidence of organ involvement. *Toxoplasma* disease requires demonstration of organ involvement and can be further classified as possible (radiological + clinical signs consistent with organ involvement), probable (radiological + clinical signs + detectable *T. gondii* DNA in any type of sample), and definite (microscopic evidence for tachyzoites in tissue or body fluids) [24, 59].

Probable and definite toxoplasmosis will require curative treatment, but it is sometimes less clear for *Toxoplasma* infection, when a positive PCR result or a seroconversion is found in a patient with no apparent clinical signs or with only fever. As it can lead to *Toxoplasma* disease in about one third of HSCT patients [59], leaving a patient untreated would be risky. In this setting, patients are usually given chemoprophylaxis if they had none previously, or are switched from TMP/SMX prophylaxis to curative therapy, as observed in recent reports [20].

Therefore, we propose diagnostic algorithms (Figs. 2 and 3) according to the patient background, clinical signs, imaging, and diagnostic findings. Overall, the diagnostic workup must be guided by clinical signs and adapted to various parameters, e.g., the pretransplant serologic status, and whether chemoprophylaxis has been administered.

In a previously seronegative recipient presenting with suggestive symptoms (Fig. 2), chemoprophylaxis should be checked, to verify the patient compliance to and/or correct absorption of TMP/SMX, and plasma levels of the drug should be measured when needed. In parallel, a specific serology should be performed. The detection of specific IgM antibodies and/or IgG is usually the result of primary infection (provided that the patient received no recent transfusions, see above) and needs specific treatment if transplant is <1 year, as well as further diagnostics according to clinical signs and symptoms (Fig. 2). If the time from transplant to diagnosis is >1 year, the decision to treat will depend on the PCR results, the graft type, the severity of clinical signs, and/or the diagnosis of organ rejection or GVHD. Even if the serology remains negative, any positive PCR result (in blood or other body fluids) can be suggestive of recently acquired toxoplasmosis and should prompt treatment. Conversely, if there is no evidence of *Toxoplasma* infection, the patient should be closely followed, through careful clinical, serologic, and molecular monitoring, without excluding the diagnosis of other opportunistic infections (Fig. 2).

The diagnostic interpretation of a symptomatic seropositive recipient will be different (Fig. 3). A positive PCR in the blood or in any other sample usually signals reactivation and requires starting a curative treatment. If both imaging and PCR are uninformative, serologic testing could help characterize a possible reactivation (significant increase of IgG level $\pm$ IgM recurrence). If antibody titers are steady, the interpretation of the results may differ according to the time post transplantation



£ Check compliance to medication, monitor plasmatic drug concentration
§ Check the absence of recent transfusions
* Imaging is non-specific, and cerebral CT scan can be normal in the first months after TP

Fig. 2 Management of clinical and diagnostic explorations in *Toxoplasma*-seropositive recipients with clinical signs

and the type of graft (HSCT, SOT), keeping in mind that SOT patients with organ rejection and HSCT patients with GVHD are prone to reactivation events even when >1 year after transplant. Besides, serologic tests may miss a *Toxoplasma* reactivation, especially in HSCT patients in whom the humoral response is highly reduced. Therefore, the decision to treatment must take into account all these parameters, and other options, such as restarting chemoprophylaxis, can be considered (Fig. 3).

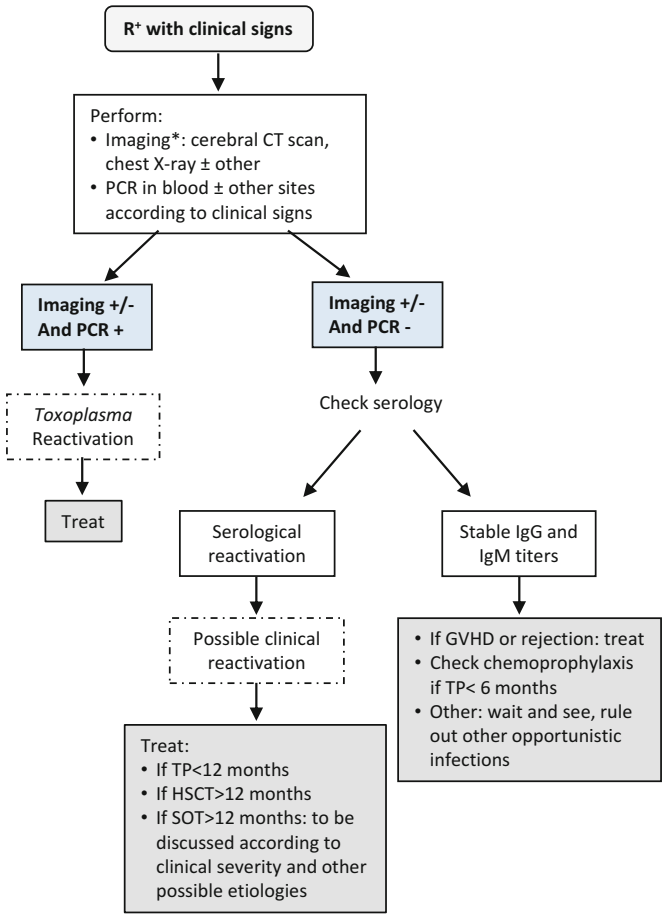


Fig. 3 Management of clinical and diagnostic explorations in *Toxoplasma*-seronegative recipients with clinical signs

Treatment

The severity of toxoplasmosis in immunocompromised hosts requires prompt initiation of effective therapy, including choices with effective penetration through the blood-brain barrier, as the delayed initiation can result in a poorer outcome [60]. It is particularly crucial in HSCT patients in whom toxoplasmosis-related mortality remains extremely high, from 38% to 67%, despite treatment [15]. In patients at high risk (HSCT R+, D+/R- SOT), either because chemoprophylaxis had been stopped or because of organ rejection or GVHD, empiric curative therapy should

be started without waiting for diagnostic confirmation and stopped in absence of improvement or if another etiology was subsequently documented.

In the absence of therapeutic guidelines based on comparative trials including transplant patients, the current treatment protocols follow recommendations established for HIV-infected patients. The therapeutic arsenal for toxoplasmosis is poor and relies on a few molecules [61, 62], as depicted in Table 2. Effective drugs interfere with parasite replication, i.e., on tachyzoites responsible for disease manifestation, but they do not act on cysts, which contain quiescent forms.

The standard protocol for toxoplasmosis curative treatment relies on combination therapy with two drugs for at least a 6-week induction period, i.e., until clinical and radiological improvement, and is followed by maintenance therapy. According to HIV American and European guidelines for toxoplasma encephalitis [63, 64], the recommended first-line treatment relies on the combination of pyrimethamine-sulfadiazine (PYR/SDZ), a synergistic association which offers the highest rapidity of action, starting with a loading dose on day 1 (Table 2). PYR displays an efficient penetration in the brain parenchyma, even in the absence of inflammation. Folic acid should be added to decrease the myelotoxicity attributed to PYR. Alternative regimen for patients who do not tolerate sulfonamides or who fail to respond to first-line therapy is PYR combined with clindamycin (CLD) [65, 66] or trimethoprim-sulfamethoxazole [67]. A marginally better survival with PYR-SDZ has been reported in a retrospective analysis of toxoplasmosis in HSCT, but other factors impacting on mortality such as underlying disease or delay between onset of symptoms and therapy were not introduced in the analysis [68]. Clinical reports raise the possible use of other combinations, including atovaquone (AVQ) combined with PYR or SDZ [69] and azithromycin (AZT) with PYR [70, 71]. Side effects, but also price and availability of those molecules, guide the choice of therapy. PYR is not available and affordable in many countries, thus explaining the preferential use of TMP/SMX in these areas [72, 73].

For severe cases, particularly in HSCT patients with reactivation, the addition of a third drug has been suggested, e.g., CLD combined with PYR-SDZ or TMP-SMX [61, 68].

In the specific context of cerebral toxoplasmosis, brain edema can be reduced through the use of a short course of high-dose corticosteroid. However, the risk of worsening the immune suppression should be considered carefully, as it has been sometimes shown to be detrimental [74].

For ocular toxoplasmosis, the adjunction of corticosteroids [75, 76] a few days after initiation of systemic anti-*Toxoplasma* therapy [77, 78] is recommended and usually continued for about 1 month with gradual tapering [62]. Despite the lack of clinical trials in immunocompromised patients, the classic PYR/SDZ regimen associated with corticosteroids is considered the treatment of choice [75, 77, 79]. As extensively reviewed by Dunay et al. [62], other regimens have been proposed for their potential efficacy in ocular toxoplasmosis in immunocompetent patients: TMP/SMX, intravitreal CLD, and more anecdotally AZT [80], or atovaquone alone or combined with PYR, but data supporting their use in immunocompromised patients are lacking.

Table 2 Main drugs used in curative treatment of toxoplasmosis in transplant recipients

Drug	Plasma half-life time/diffusion	Dose regimen[a]	Route	Combination	IDSA recommendations[b] (grade)	Other possible associations
Pyrimethamine (PYR)	Long (4 days) Brain diffusion ++	200 mg on day 1, then 75 mg/day if ≥60 kg, or 50 mg/day if <60 kg	PO	**PYR-SDZ**[c] or **PYR-CLD**[c] PYR-AVQ[c,d] PYR-AZT[c–e]	**First line (AI)** **Alternative to PYR-SDZ (AI)** BII CII	PYR/ SDZ + CLD [68]
Sulfadiazine (SDZ)	Intermediate (13 h)	2 × 3 g/day if ≥60 kg or 2 × 2 g/day if ≥60 kg	IV, PO	**PYR-SDZ**	**First line (AI)**	PYR/ SDZ + CLD [68]
Sulfamethoxazole (SMX)	Intermediate (9–11 h)	2 × 25 mg/kg/day	IV, PO	SMX/TMP[c]	Second line (BI)	SMX/TMP-CLD [68]
Trimethoprim (TMP)	Intermediate (10–12 h)	2 × 5 mg/kg/day				
Azithromycin (AZT)	Long (2–4 days) Brain and ocular diffusion ++	900–1200 mg/day	PO	AZT-PYR[c,d,e]	CII	

Clindamycin (CLD)	Short (2.5 h) Tissue diffusion ++	4 × 600–900 mg/day	IV, PO, IVT	**PYR-CLD**[c]	**Alternative to PYR-SDZ (AI)**	CLD (IVT)[f]
Atovaquone (AVQ)	Long (2–3 days)	2 × 750–1500 mg/day (with food)	PO	AVQ + PYR[c,d] or AVQ-SDZ[d]	BII	

PO, per os; IV, intravenous; IVT, intravitreal

A: Both strong evidence for efficacy and substantial clinical benefit support recommendation for use. Should always be offered

B: Moderate evidence for efficacy – or strong evidence for efficacy but only limited clinical benefit – supports recommendation for use. Should generally be offered

*C: Evidence for efficacy is insufficient to support a recommendation for or against use. Or evidence for efficacy might not outweigh adverse consequences (*e.g.*, drug toxicity, drug interactions) or cost of the treatment or alternative approaches. Optional*

I-III: Quality of evidence supporting the recommendation. I: At least one properly designed randomized, controlled trial. II: At least one well-designed clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one center), or from multiple time-series studies, or dramatic results from uncontrolled experiments. III: Opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees

[a]Guidelines from the European AIDS Clinical Society [64] on treatment of opportunistic infections in patients living with HIV

[b]IDSA guidelines for curative treatment of *Toxoplasma* encephalitis in HIV patients

[c]Adding folinic acid 10–15 mg/day (po) to prevent myelotoxicity associated with PYR therapy or TMP/SMX

[d]Very limited clinical experience with these therapies, should be used only in the absence of alternatives [62]

[e]In ocular toxoplasmosis good efficacy of AZT combined with PYR; AZT can be used at lower dose (250–500 mg/day) for a better tolerance [62]

[f]Efficacy of intravitreal CLD associated with steroids in ocular toxoplasmosis [62]

In addition, as immune restoration is part of treatment success, clinical improvement in transplant patients can require reduction or discontinuing immunosuppressive therapy, which should be discussed, depending on the type of graft and the risk of rejection or GVHD [60, 81].

The monitoring of treatment efficacy is based on clinical examination, keeping in mind that radiologic improvement can be delayed without suggesting treatment failure, as shown in *Toxoplasma* encephalitis in HIV patients. In patients having a positive *Toxoplasma* blood PCR at diagnosis, the treatment efficacy can be followed through iterative PCRs, which are expected to turn negative in case of successful therapy.

Curative treatment must be followed by secondary prophylaxis/maintenance therapy (see below).

Prevention of Toxoplasmosis in Transplant Patients

Prevention of toxoplasmosis relies on multiple approaches, including serologic screening of donors and recipients, patient education, chemoprophylaxis, and diagnostic follow-up of patients after transplantation.

Pretransplant Serologic Screening for Toxoplasmosis

Serologic screening allows clinicians to target patients most at risk for toxoplasmosis: (i) seropositive HSCT patients, (ii) seronegative SOT patients, and (iii) seropositive heart recipients (Table 1). Systematic donor/recipient *Toxoplasma* serologic screening is not uniformly adopted in all countries, either because the risk of toxoplasmosis is considered negligible or underestimated or because the universal use of TMP/SMX to prevent *Pneumocystis* pneumonia is assumed to protect against toxoplasmosis. However, whether mandatory (healthcare policy) or not, a recent European survey revealed that 24/26, 24/26, 31/35, and 25/26 transplantation centers from 11 countries screened recipients for anti-*Toxoplasma* antibodies before HSCT, liver, kidney, and heart transplantation, respectively [11]. Serologic screening of solid organ donors (heart, kidney, or liver) and HSCT donors was performed in all countries, although screening was reportedly mandatory in only seven countries (France, Greece, Italy, Romania, Slovakia, Switzerland, and Turkey) [11]. The knowledge of both recipient and donor serologic status allows starting primary chemoprophylaxis at the time of transplantation in case of toxoplasmosis mismatch, with particular attention given to heart transplant patients. Interestingly, in this study toxoplasmosis in SOT was due in equal proportions to a mismatch D+/R- or to probable foodborne infection, emphasizing the need for combining targeted primary prophylaxis through donor/recipient screening and hygienic measures.

Accurate interpretation of low-level IgG (even if detected in the gray zone) is crucial in both donor and recipient samples. It is now well established that many immune assays can detect specific IgG with titers below their positive threshold (or

in the gray zone); thus, these samples must be confirmed using a Western blot assay or a dye test [33, 82]. Indeed, misinterpreting serologic results can have serious consequences, particularly if a SOT donor or a HSCT recipient is misdiagnosed as seronegative. If a reliable confirmation method is not available, borderline IgG titers should be considered as positive. If borderline IgG titers are detected in a SOT recipient who is about to receive an organ from a Toxo+ donor, and no confirmation method is available, then it would be reasonable to manage this patient as if he were Toxo- (in other words, assume the higher-risk situation with respect to management of prophylaxis).

Serologic interpretation of IgM detection in donor or recipient serum may be also challenging. The presence of IgM may indicate recently acquired infection and should lead to measurement of IgG avidity to evaluate whether an acute infection is ongoing. In both instances, if acute infection is likely (very low IgG avidity), the (living) donor and recipient should be treated before organ/cells sampling and transplantation procedure, respectively.

Screening of cord blood samples destined to HSCT is an important issue. Cord blood can be contaminated with *Toxoplasma* tachyzoites if the fetus has been congenitally infected and thus can be a high risk for transmission to the recipient. The hallmark of *Toxoplasma* congenital infection is the detection of specific IgM in the cord blood serum, but IgM can be undetectable at delivery, depending on the trimester of maternal infection during pregnancy or on specific anti-*Toxoplasma* therapy of the mother [2, 83]. Therefore, the absence of IgM detection in cord blood cannot be a completely reliable marker to decide whether this sample is free of parasites, and it should be coupled to maternal serology performed on peripheral blood. However, it should be kept in mind that the presence of anti-*Toxoplasma* IgG without IgM doesn't rule out possible maternal infection during pregnancy (and thereby possible congenital toxoplasmosis), as maternal IgM may have disappeared by the time of delivery. For all these reasons, cord blood samples from Toxo+ women should be excluded from HSCT donation in countries where serologic follow-up from the beginning of pregnancy is not implemented [16].

Anti-*Toxoplasma* Chemoprophylaxis

For Which Patients and for How Long?

Prevention of toxoplasmosis is an object of consensus in HIV patients, and guidelines recommend the use of TMP/SMX which also protects patients from *Pneumocystis jirovecii* pneumonia [63]. Despite abundant literature calling for prophylaxis in transplant recipients, there are currently no worldwide consensus nor standardized guidelines. As the risk for *Toxoplasma* varies according to the type of graft, prophylaxis should be adapted accordingly, taking into account the serologic status of both donor and recipient. The American Society of Transplantation has recently published guidelines to prevent infection with protozoa in transplant patients and recommends donors and recipients screening for anti-*Toxoplasma* IgG to tailor chemoprophylaxis [84].

In SOT (heart, kidney, liver) patients, the major focus should be on D+/R- patients. The use of prophylaxis in orthotopic heart transplantation is standard practice in D+/R- recipients, warranting a very low incidence of toxoplasmosis despite high risk [5, 11].

Strikingly, while TMP/SMX was effective in preventing toxoplasmosis, Chehrazi-Raffle et al. reported a lower 5-year survival of D+/R- patients compared to D−/R- patients (60% vs. 87%, P = 0.04) [85]. Meanwhile, they did not find any impact of *Toxoplasma* serologic status of the recipient on the 5-year survival. These results could be explained by the low prevalence of toxoplasmosis in this series where 711/785 (91%) of recipients were seronegative. In a more balanced cohort (258 seronegative, 324 seropositive), van Hellemond et al. [86] also found no impact of *Toxoplasma* serostatus on the 4-year mortality using a multivariate analysis, although seropositivity appeared as a predictor of all-cause mortality in univariate analysis. Barge-Caballero et al. recently confirmed theses observation on a cohort of 4048 heart recipients [26]. Of note, all D+/R- patients received chemoprophylaxis. These data underline that while heart transplantation carries a high risk of organ-transmitted toxoplasmosis, it is well controlled by universal prophylaxis, provided that it is not interrupted too early. In a multicenter Spanish study reporting 22 cases, of which 18 were D+/R-, toxoplasmosis occurred at a median time of 92 days after transplantation, and none of them was diagnosed while on chemoprophylaxis, suggesting that the prophylaxis course was too short (when utilized, they used 3–6 months of prophylaxis) [6]. Therefore, in D+/R- heart TP, a minimum duration of 12 months is recommended, or even lifelong in high-risk patients, as recommended in the new guidelines from the American Society of Transplantation [84]. Similar observations can be found in the literature. Sarchi et al. diagnosed three thoracic transplant patients (D+/R-) around 1 year after transplant [87].

Kidney and liver transplant should also be given careful attention in the setting of Toxo-mismatch, as lack or early interruption of targeted prophylaxis can result in rapid onset of disease and fatal outcome [88–90]. Although there are no universal guidelines, a minimum of a 6-month course is reasonable, as the onset of *Toxoplasma* disease occurs at a time when immunosuppression is lower, thus reducing the severity of clinical symptoms [24, 91] (Table 1).

The necessity of chemoprophylaxis to prevent reactivation in SOT patients is not widely appreciated, based on the low incidence of *Toxoplasma* reactivation. Indeed, of the 22 cases described in the study by Fernandez-Sabé [6], only 2 cases occurred in seropositive patients prior to transplantation, highlighting that reactivation is uncommon if anti-*Pneumocystis* prophylaxis with TMP/SMX is usual practice, as it was described in this chapter. In the era of widespread use of TMP/SMX in this setting, some authors have even questioned the interest of *Toxoplasma* serologic screening of donors and recipients [92], but blind TMP/SMX prophylaxis for 3–6 months doesn't protect efficiently D+/R- heart transplant patients, as said above.

Furthermore, *Toxoplasma* reactivation should not be neglected if anti-*Pneumocystis* prophylaxis relies on aerosolized pentamidine or if TMP/SMX is withdrawn, as toxoplasmosis can occur very shortly thereafter [13, 51, 93–96]. Again the optimal duration is debatable, but several reactivation infections were

reported after 3 months of transplantation without prophylaxis, thus supporting the use of 3–6 months of prophylaxis in SOT seropositive patients. However, a recent systematic review suggests that reactivation of *Toxoplasma* disease is uncommon unless associated with recent rejection [24]. Therefore, the authors propose targeting prophylaxis to seropositive recipients with recent rejection or unusual immunosuppression for 3–6 months (Table 1).

In HSCT seropositive patients, the risk of *Toxoplasma* reactivation is generally well appreciated but diversely taken into account among countries, often according to the seroprevalence in the general population. D−/R+ allo-HSCT patients are the population at highest risk, but given the potential severity of disease, prophylaxis is justified in all patients. TMP/SMX prophylaxis tends to be the rule in Europe, aiming at providing protection against both *Toxoplasma* and *Pneumocystis* [11]. This universal practice is indeed efficacious, as Conrad et al. [18] showed in a case-control study that the absence of effective anti-*Toxoplasma* prophylaxis was an important risk factor (odds ratio 11.95; 95% CI 3.04–46.88; $p < 0.001$). However, there are two significant drawbacks associated with TMP/SMX, in that its myelosuppressive effect often leads to a delayed start of prophylaxis in allo-HSCT patients and that breakthrough infections have been described, possibly associated with suboptimal dosages or regimens, noncompliance, or inadequate oral absorption due to gastrointestinal intolerance [15]. The decision to postpone prophylaxis onset until HSCT engraftment (usually around 1 month after the transplant) is difficult to handle, as a fair proportion of cases occur within the first month (30% of patients in the series of Conrad et al.). In the systematic review of Gajurel et al. [15], 11% and 32% of patients developed *Toxoplasma* disease and *Toxoplasma* infection, respectively, before day 30. Hence, prophylaxis should ideally be initiated upon transplantation whenever feasible. Patients for whom prophylaxis is delayed should benefit from a close follow-up with blood PCR (see below).

Despite frequent concern about myelotoxicity, Fontanet et al. [97] found no effect of TMP/SMX on the speed of myeloid and platelet engraftment when comparing patients who underwent continued prophylaxis with those had interrupted prophylaxis from the day of stem cell infusion until day 30.

Prophylaxis Protocols

In a systematic review and meta-analysis, Gajurel et al. [15] showed that in allo-HSCT, TMP/SMX prophylaxis was more efficacious in preventing reactivation toxoplasmosis when given at a dose of 160 mg/800 mg at least three times a week. Indeed, 57% of breakthrough infections have been observed when given two times a week even at higher dosages. The preferred duration should be of 6 months or more and guided by CD4+ T cell monitoring [15]. Overall, this protocol is the most frequently applied in Europe [11, 98].

In SOT, the same regimen is commonly used (160 mg/800 mg × 3/week) for heart recipient, but 80/400 mg daily is another efficient regimen widely applied for kidney or liver recipients to reduce hepatic and renal toxicity [11, 24] and allow for ease of daily medication dosing. Prophylaxis with a regimen of dapsone 50 mg daily, plus pyrimethamine 50 mg weekly, plus folinic acid 10 mg weekly can be considered

in sulfa-allergic patients after checking for glucose-6-phosphate dehydrogenase deficiency [84].

Following a reactivation episode, secondary prophylaxis is required for at least 6–12 months, but data on the duration and drug regimen are scarce. Trimethoprim-sulfamethoxazole [5] or pyrimethamine-sulfadiazine (25 mg/2 g daily or 50 mg/2 g three times a week) [26] have been proposed for maintenance therapy, as used in HIV-infected patients [99, 100].

Patient Counseling and Hygienic Measures

Primary prophylaxis aims to prevent orally acquired *Toxoplasma* infection. Hygienic recommendations should be provided to any seronegative transplant recipient, in order to prevent primary infection, as usually done in counseling of pregnant women [101]. As presented in Table 3, these measures require caution with at-risk foods, e.g., meat, shellfish, and vegetables/fruits, which are consumed under- or uncooked, drinking water, and to respect hand hygiene after manipulating raw meat, gardening, or any contact with soils or cats.

Additionally, any transplant recipient who would travel to South America should be aware of the risk of acquiring more virulent *Toxoplasma* strains, even if he is already immune (R+) [102, 103].

Table 3 Hygienic counseling for prevention of *Toxoplasma* infection

Prevention of foodborne infection	Prevention of cat-related infection	Prevention of environmental transmission
Peel or wash vegetables, fruits, and herbs carefully if eaten raw Drink bottled water Cook meat thoroughly, at safe temperatures (63–74 °C or 145–165 °F) [107]; avoid microwave cooking or freeze meat for 5 days at −20 °C if consumed undercooked Carefully wash hands, dishes, and kitchen tools with soapy water after contact with raw meat or unwashed vegetables/fruits Avoid raw or undercooked seafood (oysters, mussels, etc.) Avoid consumption of crudités outside home Do not drink unpasteurized goat's milk	Wash hands after contact with cat Frequently change the litter box (oocysts become infective 2–5 days after shedding in cat's feces), rinse the box with hot water (>60 °C), and wear gloves and mask to manipulate the litter Feed the cat with dried or canned marketed food	Wear gloves for gardening Carefully wash hands and nails after outside activities in contact with soil or sand that might be contaminated with cat feces Avoid accidental water ingestion when practicing aquatic activities in fresh water

Diagnostic Follow-Up After Transplantation

Serologic follow-up of D+/R- SOT patients was reported to be performed by 40–50% of European centers, depending of the type of graft [11]. Serologic follow-up (on a 2-year basis or more) should be part of the systematic workup implemented in D+/R- SOT patients to document possible seroconversion after prophylaxis ends. Such findings should further prompt questioning of the patients for mild or unnoticed symptoms, clinical examination, and/or imaging to evaluate the clinical impact (Fig. 4).

Monitoring using PCR on the blood has also been proposed to detect early *Toxoplasma* infection in heart D+/R- recipients in the first weeks following transplant and is implemented in some centers [3]. In this setting, Dhakal et al. proposed that SOT patients with positive peripheral blood for *Toxoplasma* DNA be given prophylaxis if not yet implemented or be treated [24]. La Hoz et al. recommend careful monitoring of patients with discontinued prophylaxis and PCR testing [84].

A follow-up by PCR on peripheral blood performed on a weekly or twice-monthly basis has been proposed in allo-HSCT patients in the early weeks/months

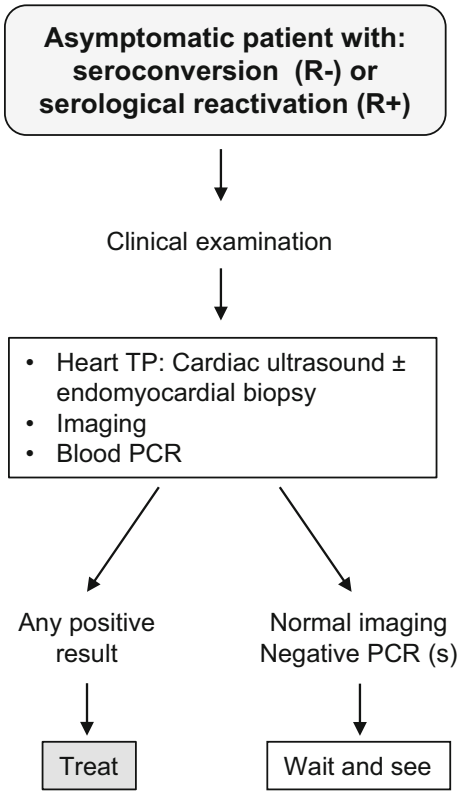


Fig. 4 Management of *Toxoplasma*-seropositive recipients with serologic reactivation or *Toxoplasma*-seronegative recipients at transplantation with seroconversion during follow-up

following transplantation [10, 59, 104], to guide rapid preemptive treatment. However, this practice is not universally applied, mainly because of concern about cost versus benefit or because of systematic TMP/SMX prophylaxis. Some studies have provided arguments in favor of PCR monitoring to improve survival [3, 9, 34, 105, 106]. In most studies, treatment of patients with circulating *Toxoplasma* DNA avoids full-blown infection. In a French study, centers with routine PCR monitoring had better survival rates than centers with no PCR monitoring [3]. Although no randomized study was designed to demonstrate the proficiency of such follow-up, we recommend that patients with delayed TMP/SMX prophylaxis initiation or under other prophylaxis protocols due to intolerance may benefit from PCR monitoring. Serologic follow-up of allo-HSCT patients is of little interest as antibody synthesis is severely impaired for several months and transfusions often complicate the interpretation of results.

Conclusion

Prevention of toxoplasmosis in transplant patients is a crucial but difficult task and relies on a multidisciplinary approach, gathering pretransplant serologic data, patient background, type of graft and chemoprophylaxis in the short term, and clinical and diagnostic follow-up in the long term. As any misdiagnosis can have serious consequences, the accurate interpretation of diagnostic results is of utmost importance to target patients at high risk who should receive chemoprophylaxis or may benefit from immediate curative treatment.

Key Points

- Toxoplasmosis is efficiently preventable by universal pretransplant donor and recipient *Toxoplasma* serology screening and targeted chemoprophylaxis with TMP/SMX.
- D+/R- SOT recipients and D−/R+ HSCT patients are the most at-risk patients for acquired and reactivation toxoplasmosis, respectively, and must be given TMP/SMX.
- *Toxoplasma*-seropositive heart and other SOT patients should be given TMP/SMX for a minimum of 6–12 months, and of 3 months, respectively.
- *Toxoplasma*-seronegative transplant patients should be advised on how to avoid infection.
- The preferred regimen for HSCT patients should be 160 mg/800 mg TMP/SMX × 3/week.
- In patients at high risk with signs and symptoms of disease, or in whom this is otherwise a major concern for disease, curative treatment should be started promptly awaiting first diagnostic results.

References

1. Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ. 2013;91(7):501–8.
2. Robert-Gangneux F, Dardé M-L. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012;25(2):264–96.
3. Robert-Gangneux F, Sterkers Y, Yera H, Accoceberry I, Menotti J, Cassaing S, et al. Molecular diagnosis of toxoplasmosis in Immunocompromised patients: a 3-year Multicenter retrospective study. J Clin Microbiol. 2015;53(5):1677–84.
4. Roux A, Canet E, Valade S, Gangneux-Robert F, Hamane S, Lafabrie A, et al. *Pneumocystis jirovecii* pneumonia in patients with or without AIDS. France Emerg Infect Dis. 2014;20(9):1490–7.
5. Derouin F, Pelloux H. ESCMID study group on clinical parasitology. Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infect. 2008;14(12):1089–101.
6. Fernàndez-Sabé N, Cervera C, Fariñas MC, Bodro M, Muñoz P, Gurguí M, et al. Risk factors, clinical features, and outcomes of toxoplasmosis in solid-organ transplant recipients: a matched case-control study. Clin Infect Dis. 2012;54(3):355–61.
7. Desoubeaux G, Cabanne É, Franck-Martel C, Gombert M, Gyan E, Lissandre S, et al. Pulmonary toxoplasmosis in immunocompromised patients with interstitial pneumonia: a single-centre prospective study assessing PCR-based diagnosis. J Clin Pathol. 2016;69(8):726–30.
8. Sumi M, Aosai F, Norose K, Takeda W, Kirihara T, Sato K, et al. Acute exacerbation of *Toxoplasma gondii* infection after hematopoietic stem cell transplantation: five case reports among 279 recipients. Int J Hematol. 2013;98(2):214–22.
9. Martino R, Bretagne S, Einsele H, Maertens J, Ullmann AJ, Parody R, et al. Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. Clin Infect Dis. 2005;40(1):67–78.
10. Meers S, Lagrou K, Theunissen K, Dierickx D, Delforge M, Devos T, et al. Myeloablative conditioning predisposes patients for *Toxoplasma gondii* reactivation after allogeneic stem cell transplantation. Clin Infect Dis. 2010;50(8):1127–34.
11. Robert-Gangneux F, Meroni V, Dupont D, Botterel F, Garcia JMA, Brenier-Pinchart M-P, et al. Toxoplasmosis in transplant recipients, Europe, 2010–2014. Emerg Infect Dis. 2018;24(8):1497–504.
12. Štajner T, Vasiljević Z, Vujić D, Marković M, Ristić G, Mićić D, et al. Atypical strain of *Toxoplasma gondii* causing fatal reactivation after hematopoietic stem cell Transplantion in a patient with an underlying immunological deficiency. J Clin Microbiol. 2013;51(8):2686–90.
13. Vaughan LB, Wenzel RP. Disseminated toxoplasmosis presenting as septic shock five weeks after renal transplantation. Transpl Infect Dis. 2013;15(1):E20–4.
14. Baliu C, Sanclemente G, Cardona M, Castel MA, Perez-Villa F, Moreno A, et al. Toxoplasmic encephalitis associated with meningitis in a heart transplant recipient. Transpl Infect Dis. 2014;16(4):631–3.
15. Gajurel K, Dhakal R, Montoya JG. *Toxoplasma* prophylaxis in haematopoietic cell transplant recipients: a review of the literature and recommendations. Curr Opin Infect Dis. 2015;28(4):283–92.
16. Robert-Gangneux F, Djurković-Djaković O. Serological and molecular screening of umbilical cord blood for toxoplasma gondii infection; a reply to Botein et al. Transpl Infect Dis. 2019;21(4):e13127.
17. Schmidt M, Sonneville R, Schnell D, Bigé N, Hamidfar R, Mongardon N, et al. Clinical features and outcomes in patients with disseminated toxoplasmosis admitted to intensive care: a Multicenter study. Clin Infect Dis. 2013;57(11):1535–41.
18. Conrad A, Le Maréchal M, Dupont D, Ducastelle-Leprêtre S, Balsat M, Labussière-Wallet H, et al. A matched case-control study of toxoplasmosis after allogeneic haematopoietic

stem cell transplantation: still a devastating complication. Clin Microbiol Infect. 2016;22(7):636–41.
19. Khalaf AM, Hashim MA, Alsharabati M, Fallon K, Cure JK, Pappas P, et al. Late-onset cerebral toxoplasmosis after allogeneic hematopoietic stem cell transplantation. Am J Case Rep. 2017;18:246–50.
20. Robin C, Leclerc M, Angebault C, Redjoul R, Beckerich F, Cabanne L, et al. Toxoplasmosis as an early complication of allogeneic hematopoietic cell transplantation. J Am Soc Blood Marrow Transplant. 2019;S1083-8791(19)30507-5.
21. Dard C, Marty P, Brenier-Pinchart M-P, Garnaud C, Fricker-Hidalgo H, Pelloux H, et al. Management of toxoplasmosis in transplant recipients: an update. Expert Rev Anti-Infect Ther. 2018;16(6):447–60.
22. Luft BJ, Naot Y, Araujo FG, Stinson EB, Remington JS. Primary and reactivated toxoplasma infection in patients with cardiac transplants. Clinical spectrum and problems in diagnosis in a defined population. Ann Intern Med. 1983;99(1):27–31.
23. Wreghitt TG, Gray JJ, Pavel P, Balfour A, Fabbri A, Sharpies LD, et al. Efficacy of pyrimethamine for the prevention of donor-acquired toxoplasma gondii infection in heart and heart-lung transplant patients. Transpl Int. 1992;5(4):197–200.
24. Dhakal R, Gajurel K, Montoya JG. Toxoplasmosis in the non-orthotopic heart transplant recipient population, how common is it? Any indication for prophylaxis? Curr Opin Organ Transplant. 2018;23(4):407–16.
25. Autier B, Dion S, Robert-Gangneux F. The liver as an organ at risk for toxoplasma transmission during transplantation: myth or reality? J Clin Pathol. 2018;71(9):763–6.
26. Barge-Caballero E, Almenar-Bonet L, Crespo-Leiro MG, Brossa-Loidi V, Rangel-Sousa D, Gómez-Bueno M, et al. Preoperative toxoplasma gondii serostatus does not affect long-term survival of cardiac transplant recipients. Analysis of the Spanish heart transplantation registry. Int J Cardiol. 2018;250:183–7.
27. Segall L, Moal M-C, Doucet L, Kergoat N, Bourbigot B. Toxoplasmosis-associated hemophagocytic syndrome in renal transplantation. Transpl Int. 2006;19(1):78–80.
28. Gatti-Mays ME, Manion M, Bowen LN, Brown GT, Danner RL, Khan O, et al. Toxoplasmosis encephalitis with immune-reconstitution inflammatory syndrome in an allogeneic stem cell transplant patient: a case report. Bone Marrow Transplant. 2016;51(12):1622–4.
29. Blanche P, Robert F, Dupouy-Camet J, Sicard D. Toxoplasmosis-associated hemophagocytic syndrome in a patient with AIDS: diagnosis by the polymerase chain reaction. Clin Infect Dis. 1994;19(5):989–90.
30. van Bilsen WPH, van den Berg CHSB, Rijnders BJA, Brinkman K, Mulder JW, Gelinck LBS, et al. Immune reconstitution inflammatory syndrome associated with toxoplasmic encephalitis in HIV-infected patients. AIDS. 2017;31(10):1415–24.
31. Duband S, Cornillon J, Tavernier E, Dumollard J-M, Guyotat D, Péoc'h M. Toxoplasmosis with hemophagocytic syndrome after bone marrow transplantation: diagnosis at autopsy. Transpl Infect Dis. 2008;10(5):372–4.
32. Gay J, Gendron N, Verney C, Joste V, Dardé M-L, Loheac C, et al. Disseminated toxoplasmosis associated with hemophagocytic syndrome after kidney transplantation: a case report and review. Transpl Infect Dis. 2019;21:e13154.
33. Douet T, Armengol C, Charpentier E, Chauvin P, Cassaing S, Iriart X, et al. Performance of seven commercial automated assays for the detection of low levels of anti-*Toxoplasma* IgG in French immunocompromised patients. Parasite. 2019;26:51.
34. Fricker-Hidalgo H, Bulabois C-E, Brenier-Pinchart M-P, Hamidfar R, Garban F, Brion J-P, et al. Diagnosis of toxoplasmosis after allogeneic stem cell transplantation: results of DNA detection and serological techniques. Clin Infect Dis. 2009;48(2):e9–15.
35. Kaiser K, Van Loon AM, Pelloux H, Ferrandiz J, Picot S, Wallace P, et al. Multicenter proficiency study for detection of *Toxoplasma gondii* in amniotic fluid by nucleic acid amplification methods. Clin Chim Acta. 2007;375(1):99–103.

36. Sterkers Y, Varlet-Marie E, Cassaing S, Brenier-Pinchart M-P, Brun S, Dalle F, et al. Multicentric comparative analytical performance study for molecular detection of low amounts of *Toxoplasma gondii* from simulated specimens. J Clin Microbiol. 2010;48(9):3216–22.
37. Roux G, Varlet-Marie E, Bastien P, Sterkers Y. French National Reference Center for toxoplasmosis network. Evolution of *Toxoplasma*-PCR methods and practices: a French national survey and proposal for technical guidelines. Int J Parasitol. 2018;48(9–10):701–7.
38. Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int J Parasitol. 2000;30(1):69–75.
39. Belaz S, Gangneux J-P, Dupretz P, Guiguen C, Robert-Gangneux F. A 10-year retrospective comparison of two target sequences, REP-529 and B1, for *Toxoplasma gondii* detection by quantitative PCR. J Clin Microbiol. 2015;53(4):1294–300.
40. Edvinsson B, Lappalainen M, Evengård B. ESCMID study Group for Toxoplasmosis. Real-time PCR targeting a 529-bp repeat element for diagnosis of toxoplasmosis. Clin Microbiol Infect. 2006;12(2):131–6.
41. Robert-Gangneux F, Belaz S. Molecular diagnosis of toxoplasmosis in immunocompromised patients. Curr Opin Infect Dis. 2016;29(4):330–9.
42. Correia CC, Melo HRL, Costa VMA. Influence of neurotoxoplasmosis characteristics on real-time PCR sensitivity among AIDS patients in Brazil. Trans R Soc Trop Med Hyg. 2010;104(1):24–8.
43. Anselmo LMP, Vilar FC, Lima JE, Yamamoto AY, Bollela VR, Takayanagui OM. Usefulness and limitations of polymerase chain reaction in the etiologic diagnosis of neurotoxoplasmosis in immunocompromised patients. J Neurol Sci. 2014;346(1–2):231–4.
44. Petersen E, Edvinsson B, Lundgren B, Benfield T, Evengård B. Diagnosis of pulmonary infection with *Toxoplasma gondii* in immunocompromised HIV-positive patients by real-time PCR. Eur J Clin Microbiol Infect Dis. 2006;25(6):401.
45. Bourdin C, Busse A, Kouamou E, Touafek F, Bodaghi B, Le Hoang P, et al. PCR-based detection of Toxoplasma gondii DNA in blood and ocular samples for diagnosis of ocular toxoplasmosis. J Clin Microbiol. 2014;52(11):3987–91.
46. De Groot-Mijnes JDF, Rothova A, Van Loon AM, Schuller M, Ten Dam-Van Loon NH, De Boer JH, et al. Polymerase chain reaction and Goldmann-Witmer coefficient analysis are complimentary for the diagnosis of infectious uveitis. Am J Ophthalmol. 2006;141(2):313–8.
47. Errera M-H, Goldschmidt P, Batellier L, Degorge S, Héron E, Laroche L, et al. Real-time polymerase chain reaction and intraocular antibody production for the diagnosis of viral versus toxoplasmic infectious posterior uveitis. Graefes Arch Clin Exp Ophthalmol. 2011;249(12):1837–46.
48. Scheepers MA, Lecuona KA, Rogers G, Bunce C, Corcoran C, Michaelides M. The value of routine polymerase chain reaction analysis of intraocular fluid specimens in the diagnosis of infectious posterior uveitis. ScientificWorldJournal. 2013;2013:545149.
49. Fardeau C, Romand S, Rao NA, Cassoux N, Bettembourg O, Thulliez P, et al. Diagnosis of toxoplasmic retinochoroiditis with atypical clinical features. Am J Ophthalmol. 2002;134(2):196–203.
50. Talabani H, Asseraf M, Yera H, Delair E, Ancelle T, Thulliez P, et al. Contributions of immunoblotting, real-time PCR, and the Goldmann-Witmer coefficient to diagnosis of atypical toxoplasmic retinochoroiditis. J Clin Microbiol. 2009;47(7):2131–5.
51. Patrat-Delon S, Gangneux J-P, Lavoué S, Lelong B, Guiguen C, Le Tulzo Y, et al. Correlation of parasite load determined by quantitative PCR to clinical outcome in a heart transplant patient with disseminated toxoplasmosis. J Clin Microbiol. 2010;48(7):2541–5.
52. Menotti J, Vilela G, Romand S, Garin YJ-F, Ades L, Gluckman E, et al. Comparison of PCR-enzyme-linked immunosorbent assay and real-time PCR assay for diagnosis of an unusual case of cerebral toxoplasmosis in a stem cell transplant recipient. J Clin Microbiol. 2003;41(11):5313–6.

53. Rodríguez Carnero P, Hernández Mateo P, Martín-Garre S, García Pérez Á, del Campo L. Unexpected hosts: imaging parasitic diseases. Insights Imaging. 2017;8(1):101–25.
54. Sakamoto N, Maeda T, Mikita K, Kato Y, Yanagisawa N, Suganuma A, et al. Clinical presentation and diagnosis of toxoplasmic encephalitis in Japan. Parasitol Int. 2014;63(5):701–4.
55. Weenink JJ, Weenink AG, Geerlings SE, van Gool T, Bemelman FJ. Severe cerebral *toxoplasma* infection cannot be excluded by a normal CT scan. Neth J Med. 2009;67(4):3.
56. Mueller-Mang C, Mang TG, Kalhs P, Thurnher MM. Imaging characteristics of toxoplasmosis encephalitis after bone marrow transplantation: report of two cases and review of the literature. Neuroradiology. 2006;48(2):84–9.
57. Sumi M, Norose K, Hikosaka K, Kaiume H, Takeda W, Kirihara T, et al. Clinical characteristics and computed tomography findings of pulmonary toxoplasmosis after hematopoietic stem cell transplantation. Int J Hematol. 2016;104(6):729–40.
58. Lavinsky D, Romano A, Muccioli C, Belfort R. Imaging in ocular toxoplasmosis. Int Ophthalmol Clin. 2012;52(4):131–43.
59. Martino R, Maertens J, Bretagne S, Rovira M, Deconinck E, Ullmann AJ, et al. Toxoplasmosis after hematopoietic stem cell transplantation. Clin Infect Dis. 2000;31(5):1188–95.
60. Campbell AL, Goldberg CL, Magid MS, Gondolesi G, Rumbo C, Herold BC. First case of toxoplasmosis following small bowel transplantation and systematic review of tissue-invasive toxoplasmosis following noncardiac solid organ transplantation. Transplantation. 2006;81(3):408–17.
61. Konstantinovic N, Guegan H, Stäjner T, Belaz S, Robert-Gangneux F. Treatment of toxoplasmosis: current options and future perspectives. Food Waterborne Parasitol. 2019;15:e00036.
62. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoya JG. Treatment of toxoplasmosis: historical perspective, animal models, and current clinical practice. Clin Microbiol Rev. 2018;31(4):e00057-17.
63. Kaplan JE, Benson C, Holmes KK, Brooks JT, Pau A, Masur H, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep. 2009;58(RR-4):1–207; quiz CE1-4.
64. European AIDS Clinical Society (EACS). EACS guidelines 2018 [Internet]. http://www.eacsociety.org/files/2018_guidelines-9.1-english.pdf
65. Dannemann B, McCutchan JA, Israelski D, Antoniskis D, Leport C, Luft B, et al. Treatment of toxoplasmic encephalitis in patients with AIDS. A randomized trial comparing pyrimethamine plus clindamycin to pyrimethamine plus sulfadiazine. The California Collaborative Treatment Group. Ann Intern Med. 1992;116(1):33–43.
66. Katlama C, De Wit S, O'Doherty E, Van Glabeke M, Clumeck N. Pyrimethamine-clindamycin vs. pyrimethamine-sulfadiazine as acute and long-term therapy for toxoplasmic encephalitis in patients with AIDS. Clin Infect Dis. 1996;22(2):268–75.
67. Torre D, Casari S, Speranza F, Donisi A, Gregis G, Poggio A, et al. Randomized trial of trimethoprim-sulfamethoxazole versus pyrimethamine-sulfadiazine for therapy of toxoplasmic encephalitis in patients with AIDS. Italian Collaborative Study Group. Antimicrob Agents Chemother. 1998;42(6):1346–9.
68. Mele A, Paterson PJ, Prentice HG, Leoni P, Kibbler CC. Toxoplasmosis in bone marrow transplantation: a report of two cases and systematic review of the literature. Bone Marrow Transplant. 2002;29(8):691–8.
69. Chirgwin K, Hafner R, Leport C, Remington J, Andersen J, Bosler EM, et al. Randomized phase II trial of atovaquone with pyrimethamine or sulfadiazine for treatment of toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome: ACTG 237/ANRS 039 Study. AIDS Clinical Trials Group 237/Agence Nationale de Recherche sur le SIDA, Essai 039. Clin Infect Dis. 2002;34(9):1243–50.
70. Saba J, Morlat P, Raffi F, Hazebroucq V, Joly V, Leport C, et al. Pyrimethamine plus azithromycin for treatment of acute toxoplasmic encephalitis in patients with AIDS. Eur J Clin Microbiol Infect Dis. 1993;12(11):853–6.

71. Jacobson JM, Hafner R, Remington J, Farthing C, Holden-Wiltse J, Bosler EM, et al. Dose-escalation, phase I/II study of azithromycin and pyrimethamine for the treatment of toxoplasmic encephalitis in AIDS. AIDS. 2001;15(5):583–9.
72. Gallant J. Get rich quick with old generic drugs! The Pyrimethamine pricing scandal. Open Forum Infect Dis. 2015;2(4):1–2.
73. Goswami RP, Goswami RP, Rahman M, Ray Y, Tripathi SK. Alternative treatment approach to cerebral toxoplasmosis in HIV/AIDS: experience from a resource-poor setting. Int J STD AIDS. 2015;26(12):864–9.
74. Arens J, Barnes K, Crowley N, Maartens G. Treating AIDS-associated cerebral toxoplasmosis – pyrimethamine plus sulfadiazine compared with cotrimoxazole, and outcome with adjunctive glucocorticoids. S Afr Med J. 2007;97(10):956–8.
75. Holland GN, Lewis KG. An update on current practices in the management of ocular toxoplasmosis. Am J Ophthalmol. 2002;134(1):102–14.
76. Jasper S, Vedula SS, John SS, Horo S, Sepah YJ, Nguyen QD. Corticosteroids as adjuvant therapy for ocular toxoplasmosis. Cochrane Database Syst Rev. 2017;2017(1):CD007417.
77. Butler NJ, Furtado JM, Winthrop KL, Smith JR. Ocular toxoplasmosis II: clinical features, pathology and management. Clin Exp Ophthalmol. 2013;41(1):95–108.
78. Ozgonul C, Besirli CG. Recent developments in the diagnosis and treatment of ocular toxoplasmosis. Ophthalmic Res. 2017;57(1):1–12.
79. Rothova A, Meenken C, Buitenhuis HJ, Brinkman CJ, Baarsma GS, Boen-Tan TN, et al. Therapy for ocular toxoplasmosis. Am J Ophthalmol. 1993;115(4):517–23.
80. Bosch-Driessen LH, Verbraak FD, Suttorp-Schulten MSA, van Ruyven RLJ, Klok AM, Hoyng CB, et al. A prospective, randomized trial of pyrimethamine and azithromycin vs pyrimethamine and sulfadiazine for the treatment of ocular toxoplasmosis. Am J Ophthalmol. 2002;134(1):34–40.
81. Chung H, Kim J-G, Choi S-H, Lee SY, Yoon YH. Bilateral *Toxoplasma* Retinochoroiditis simulating Cytomegalovirus retinitis in an allogeneic bone marrow transplant patient. Korean J Ophthalmol. 2008;22(3):197–200.
82. Mouri O, Kendjo E, Touafek F, Fekkar A, Konte O, Imbert S, et al. The impact of lowering the cut-off value on the sensitivity of the Platelia Elisa IgG (Bio-Rad) test for toxoplasmosis diagnosis. Parasite. 2015;22:22.
83. Olariu TR, Press C, Talucod J, Olson K, Montoya JG. Congenital toxoplasmosis in the United States: clinical and serologic findings in infants born to mothers treated during pregnancy. Parasite. 2019;26:13.
84. La Hoz RM, Morris MI, Infectious Diseases Community of Practice of the American Society of Transplantation. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, Babesia, Acanthamoeba, Balamuthia, and Naegleria in solid organ transplant recipients-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13546.
85. Chehrazi-Raffle A, Luu M, Yu Z, Liou F, Kittleson M, Hamilton M, et al. Toxoplasma gondii serology and outcomes after heart transplantation: contention in the literature. Transplant Proc. 2015;47(6):1949–53.
86. van Hellemond JJ, van Domburg RT, Caliskan K, Birim O, Balk AH. Toxoplasma gondii serostatus is not associated with impaired long-term survival after heart transplantation. Transplantation. 2013;96(12):1052–8.
87. Sarchi E, Genco F, Di Matteo A, Castiglioni B, Minoli L, Meroni V. Surveillance of *Toxoplasma gondii* infection in recipients of thoracic solid organ transplants. New Microbiol. 2007;30(3):299–302.
88. Miltgen G, Paillot J, Vanlemmens C, Di Martino V, Heyd B, Millon L, et al. Fatal acute respiratory distress by *Toxoplasma gondii* in a toxoplasma seronegative liver transplant recipient. New Microbiol. 2016;39(2):160–2.
89. Vaessen N, Verweij JJ, Spijkerman IJB, van Hoek B, van Lieshout L. Fatal disseminated toxoplasmosis after liver transplantation: improved and early diagnosis by PCR. Neth J Med. 2007;65(6):222–3.

90. Rogers NM, Peh C-A, Faull R, Pannell M, Cooper J, Russ GR. Transmission of toxoplasmosis in two renal allograft recipients receiving an organ from the same donor. Transpl Infect Dis. 2008;10(1):71–4.
91. Martina M-N, Cervera C, Esforzado N, Linares L, Torregrosa V, Sanclemente G, et al. Toxoplasma gondii primary infection in renal transplant recipients. Two case reports and literature review. Transpl Int. 2011;24(1):e6–12.
92. Gourishankar S, Doucette K, Fenton J, Purych D, Kowalewska-Grochowska K, Preiksaitis J. The use of donor and recipient screening for toxoplasma in the era of universal trimethoprim sulfamethoxazole prophylaxis. Transplantation. 2008;85(7):980–5.
93. Wendum D, Carbonell N, Svrcek M, Chazouilléres O, Fléjou J-F. Fatal disseminated toxoplasmosis in a toxoplasma seropositive liver transplant recipient. J Clin Pathol. 2002;55(8):637.
94. Barcán LA, Dallurzo ML, Clara LO, Valledor A, Macías S, Zorkin E, et al. Toxoplasma gondii pneumonia in liver transplantation: survival after a severe case of reactivation. Transpl Infect Dis. 2002;4(2):93–6.
95. Botterel F, Ichai P, Feray C, Bouree P, Saliba F, Raspa RT, et al. Disseminated toxoplasmosis, resulting from infection of allograft, after Orthotopic liver transplantation: usefulness of quantitative PCR. J Clin Microbiol. 2002;40(5):1648–50.
96. Manz T, Grotz W, Orszagh M, Volk B, Kirste G, Neumann HP. A patient with neurological deficits and seizures after renal transplantation. Nephrol Dial Transplant. 2001;16(3):631–3.
97. Fontanet A, Chalandon Y, Roosnek E, Mohty B, Passweg JR. Cotrimoxazole myelotoxicity in hematopoietic SCT recipients: time for reappraisal. Bone Marrow Transplant. 2001;46(9): 1272–3.
98. Baden LR, Katz JT, Franck L, Tsang S, Hall M, Rubin RH, et al. Successful toxoplasmosis prophylaxis after orthotopic cardiac transplantation with trimethoprim-sulfamethoxazole. Transplantation. 2003;75(3):339–43.
99. Béraud G, Pierre-François S, Foltzer A, Abel S, Liautaud B, Smadja D, et al. Cotrimoxazole for treatment of cerebral toxoplasmosis: an observational cohort study during 1994–2006. Am J Trop Med Hyg. 2009;80(4):583–7.
100. Podzamczer D, Miró JM, Ferrer E, Gatell JM, Ramón JM, Ribera E, et al. Thrice-weekly sulfadiazine-pyrimethamine for maintenance therapy of toxoplasmic encephalitis in HIV-infected patients. Spanish Toxoplasmosis Study Group. Eur J Clin Microbiol Infect Dis. 2000;19(2):89–95.
101. Robert-Gangneux F. It is not only the cat that did it: how to prevent and treat congenital toxoplasmosis. J Infect. 2014;68:S125–33.
102. Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, et al. Severe acquired toxoplasmosis in Immunocompetent Adult Patients in French Guiana. J Clin Microbiol. 2002;40(11):4037–44.
103. Pena HFJ, Gennari SM, Dubey JP, Su C. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. Int J Parasitol. 2008;38(5):561–9.
104. Bretagne S, Costa J-M, Foulet F, Jabot-Lestang L, Baud-Camus F, Cordonnier C. Prospective study of toxoplasma reactivation by polymerase chain reaction in allogeneic stem-cell transplant recipients. Transpl Infect Dis. 2000;2(3):127–32.
105. Cavattoni I, Ayuk F, Zander AR, Zabelina T, Bacher A, Cayroglu E, et al. Diagnosis of *Toxoplasma gondii* infection after allogeneic stem cell transplant can be difficult and requires intensive scrutiny. Leuk Lymphoma. 2010;51(8):1530–5.
106. Busemann C, Ribback S, Zimmermann K, Sailer V, Kiefer T, Schmidt CA, et al. Toxoplasmosis after allogeneic stem cell transplantation – a single Centre experience. Ann Hematol. 2012;91(7):1081–9.
107. CDC-Centers for Disease Control and Prevention. CDC – Toxoplasmosis – Prevention & Control [Internet]. 2018. Disponible sur: https://www.cdc.gov/parasites/toxoplasmosis/prevent.html

Strongyloides: Emerging Challenges and Prevention

53

Ricardo M. La Hoz and Alfredo G. Puing

Contents

Introduction 1281
Epidemiology 1282
Clinical Impact on Transplant 1282
Diagnosis Including Clinical Presentation and Available/Emerging Diagnostics 1283
Management Including Emerging Treatment Modalities and Options for Prophylaxis/Prevention 1285
Conclusions 1288
Key Points 1288
References 1289

Introduction

Strongyloidiasis is an infection caused by the intestinal nematode or roundworm *Strongyloides stercoralis.* This parasite can complete its life cycle in the environment and human host. It also has an auto-infective cycle in the human host that results in persistent infections that can last up to four decades, a feature unique among nematodes [1]. The immune response of the host regulates the extent of autoinfection, while the severity of the disease correlates with the worm burden. The primary reservoir of the parasite is soil contaminated with human feces that harbor *Strongyloides* larvae. The infection cycle begins when human skin is breached by filariform larvae. They penetrate the intact skin and migrate via the venous

R. M. La Hoz (✉)
Division of Infectious Diseases and Geographic Medicine, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA
e-mail: Ricardo.LaHoz@UTSouthwestern.edu

A. G. Puing
Division of Infectious Diseases, Department of Medicine, City of Hope National Medical Center, Duarte, CA, USA
e-mail: apuing@coh.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_52

circulatory system to the lungs where they penetrate the alveolar space, ascend to the pharynx, and are swallowed. The larvae finally arrive in the duodenal mucosa where they molt twice to become adult female worms. Adult females reproduce asexually by parthenogenesis in the human intestine, laying eggs that become rhabditiform larvae. This stage can be excreted in the stool, thus completing the parasite's life cycle, or transform to filariform larvae while still in the intestines. In the auto-infective cycle, the filariform larvae penetrate the intestinal mucosa or perianal skin where they again gain access to the venous circulatory system. The molting of rhabditiform to filariform larvae is accelerated in the milieu of immunosuppression, allowing a potentially massive number of larvae to proliferate in the intestine. As a result, solid organ and hematopoietic stem cell transplant recipients are at risk for complicated and even life-threatening infections with *Strongyloides stercoralis*.

Epidemiology

*Strongyloide*s is a neglected global public health problem. It was initially estimated that it infects approximately 100 million people worldwide [2]. Accounting for the inaccuracies due to diagnostic techniques and underreporting, a prevalence of 370 million persons is more accurate [3]. The parasite is endemic throughout the tropics and subtropics. A country-specific Bayesian meta-analysis determined that Africa, Asia, and Latin America are disproportionately affected [4]. Societies where fecal contamination of the soil is rare have a low prevalence. Studies in the United States (USA) estimate a prevalence among immigrants of 4–46% depending on the population studied [4–6]. In the USA, *Strongyloides* continues to be endemic to the Appalachian region [7–9]. Solid organ and hematopoietic stem cell transplant (HSCT) programs must consider the epidemiology of this parasite when developing screening protocols for the evaluation of transplant candidates and solid organ transplant (SOT) donors.

Clinical Impact on Transplant

Strongyloidiasis can be a devastating disease in transplant recipients. The mortality rate approaches 50% in hyper-infection syndrome and 70% in disseminated infection [10–12]. A retrospective analysis at MD Anderson between 1971 and 2003 estimated an infection frequency of 1.0 per 10,000 new cancer diagnoses [13]. In this study, hyper-infection syndromes were more frequently observed among HSCT recipients. The reported mortality of HSCT recipients with *Strongyloides* hyper-infection syndrome is about 80% [14, 15]. A retrospective study from Stanford University Medical Center reviewed 185 autopsies of HSCT recipients; 35 patients (19%) had a total of 41 missed infections. Parasitic infections represented 5% of the missed infections. Half of those were due to *Strongyloide*s [16]. The Ad Hoc Disease Transmission Advisory Committee (DTAC) of the Organ Procurement and Transplantation Network (OPTN)

reviewed *Strongyloides* potential donor-derived transmission events from 2008 to 2017 [17]. Organs procured from 15 donors, involved in a *Strongyloides* proven or probable donor derived event, were transplanted to 43 recipients; 19 (44%) developed strongyloidiasis. Five of the 19 recipients (26%) died at 45 days follow-up, with all deaths attributed to *Strongyloides*. These studies should encourage SOT and HSCT programs to be vigilant about ensuring appropriate screening and treatment of transplant candidates and organ donors prior to transplantation.

Diagnosis Including Clinical Presentation and Available/Emerging Diagnostics

Strongyloidiasis in solid organ and hematopoietic stem cell transplant recipients could be the result of de novo acquisition, re-infection, or reactivation of chronic intestinal infection [18–21]. In SOT recipients, it could also be a consequence of donor-derived infection [22]. Reactivation and donor-derived infection occur in the initial months after transplantation when immunosuppression is highest [20, 21]. Case series describe a median time to presentation in HSCT recipients of 21 days (range, 2–64) and for donor-derived strongyloidiasis in SOT recipients of 13 weeks (range, 7–33) [20, 22]. Clinical syndromes include acute infection, chronic infection, hyper-infection syndrome, and disseminated disease. Risk factors for hyper-infection syndrome and disseminated diseases have been linked to the immune status of the host and are mainly related to immunosuppressive medications. Human T-lymphotropic virus type I (HTLV-1) co-infection is also a risk factor for progression to hyper-infection syndrome and disseminated disease [23]. In the hyper-infection syndrome, there is an accelerated proliferation of larvae in the duodenum as a consequence of an increased rate of molting of rhabditiform to filariform larvae during the auto-infective cycle. Larvae migrate through the bowel wall and then travel through the venous system to the lungs and back to the small bowel. In this syndrome, larvae are restricted to the pulmonary and gastrointestinal system. Larvae cause symptoms as they migrate through the host and include fever, dyspnea, wheezing, hemoptysis, acute respiratory distress syndrome, anorexia, nausea, vomiting, abdominal pain, diarrhea, ileus, obstruction, and gastrointestinal bleeding. Common complications of the hyper-infection syndrome include septic shock (50%) and bacteremia (40%) caused by translocation of gut flora from bowel damage by migrating larvae. Disseminated disease is characterized by the spread of the larvae to other organs [10–12, 18]. Meningitis, peritonitis, pancreatitis, cholecystitis, liver abscess, and other complications have been described.

There is a lack of a reference standard for the diagnosis of strongyloidiasis [24]. Although eosinophilia is reported in patients with *Strongyloides* infection, it should not be used as a single criterion to screen or diagnose transplant patients or SOT donors; up to 20–30% of patients with chronic infection and 70–100% of patients with hyper-infection syndrome or disseminated disease have normal eosinophil

counts [10, 11, 25, 26]. Also, transplant recipients and deceased SOT donors often receive corticosteroids that decrease the eosinophil count [27]. The diagnosis of strongyloidiasis is usually made by serological assays or detection of rhabditiform larvae from clinical specimens. A study comparing the performance of five serological assays against a composite reference standard revealed an overall sensitivity of 75–94% and specificity of 94–100% [28]. Because other helminthic infections can cause false positives, local epidemiology is essential when considering the positive predictive value of these assays. False negatives occur in immunocompromised hosts due to the net state of immunosuppression. The reported sensitivity and specificity of serological assays in this population is 43–68% and 89–96%, respectively [29, 30]. Serological assays are mainly used in three scenarios in the field of transplantation: [1] screening transplant candidates; [2] screening deceased and living SOT donors; and [2] diagnosis of strongyloidiasis in transplant patients. Due to the low sensitivity of serological assays in the immunocompromised host, a combination of diagnostic methods is required to establish the diagnosis of strongyloidiasis in transplant recipients.

A definitive diagnosis of strongyloidiasis is achieved by identification of larvae in a clinical specimen. In uncomplicated infections, stool is the preferred sample. However, in patients with hyper-infection syndrome or disseminated disease, larvae can also be found in respiratory secretions, cerebrospinal fluid, peritoneal fluid, urine, pleural fluid, blood, and other tissue specimens. Examination of a single stool sample lacks sensitivity for the diagnosis of chronic strongyloidiasis. In patients with a low burden of infection, larvae are excreted intermittently. The sensitivity is increased to 50% by analyzing three stool samples and can approach 100% if seven serial stool samples are examined [1, 31]. The Agar Plate method is the technique with the highest sensitivity (89%) to identify larvae in the stool [32]. This method is labor intensive and requires that at least three stool samples be tested to achieve adequate performance [7]. Direct examination and formalin-ethyl acetate concentration methods are also used and have a sensitivity of 21 and 49%, respectively.

The performance of the polymerase chain reaction (PCR) to detect *Strongyloides* in stool has varied due to the different techniques used to extract DNA and neutralize inhibitors [24]. The method that obtained the best results included an extra step that involved the incubation of stool samples with glycine-SDS buffer and mechanical disruption before DNA extraction [25]. The sensitivity and specificity of PCR was 62% (42–78%) and 95% (92–97%) in a systematic review and meta-analysis [33]. Although PCR is a promising technique, it is not widely available.

Transplant patients diagnosed with strongyloidiasis should be screened for HTLV-1 by serology, as co-infection is associated with a high risk of relapse and disseminated disease. HTLV-1 downregulates the T-helper type 2 (Th2) immune response (decreasing interleukin-5 production and total immunoglobulin E levels), therefore impairing eosinophil recruitment and helminthic-killing activity [23, 34, 35]. Due to the increased risk of relapse in patients with HTLV-1 co-infection, some experts recommend intermittent treatment during periods of augmentation of immunosuppression [21].

Management Including Emerging Treatment Modalities and Options for Prophylaxis/Prevention

Ivermectin is the preferred anti-parasitic to treat strongyloidiasis (Table 1) and is available in several formulations, including oral, rectal, and subcutaneous [36–39]. The efficacy of ivermectin is restricted to those parasites found in the intestinal stages [40, 41]. A meta-analysis assessing the efficacy and safety of ivermectin versus albendazole for the treatment of chronic strongyloidiasis concluded that more people were cured with ivermectin [36]. The studied doses of ivermectin for the treatment of chronic strongyloidiasis were 200 mcg/kg by mouth daily for 1 or 2 days [37–39]. A multicenter open-label randomized controlled trial compared a single dose of ivermectin 200 mcg/kg to four doses of ivermectin 200 mcg/kg (given on days 1, 2, 15, and 16) for the treatment strongyloidiasis. The primary endpoint (proportion of participants with clearance of infection at 12 months) occurred in 86% of the single-dose group and 85% of the four-dose group ($p = 0.75$). Adverse events were mild albeit more common in the multi-dose group [42]. Immunosuppressed patients were excluded from this study. Therefore, some experts still recommend ivermectin 200 mcg/kg by mouth for 2 days followed by another series 2 weeks later (the duration of one autoinfection cycle) for the treatment of transplant patients [37, 38, 43]. This recommendation balances the devastating effects that *Strongyloides* may have in transplant patients and the tolerability of ivermectin. Immigrants from endemic areas for filarial diseases such as *Loa loa* (West and Central Africa, within the past 3–5 years) should be screened with a blood smear to exclude microfilaremia as the administration of ivermectin in this setting has been associated with an increased risk of encephalopathy [44]. In patients co-infected with filarial parasites, the Mazzotti reaction may occur within 7 days of administration of ivermectin. It is characterized by fever, urticaria, lymphadenopathy, myalgias, tachycardia, and hypotension [45]. Albendazole has a primary cure rate of only 38–68%, relegating it to second-line therapy for chronic strongyloidiasis [36, 46–48].

There is a paucity of information to guide treatment of hyper-infection syndrome and disseminated disease. Recommended interventions include a combination of anti-parasitic therapy, broad-spectrum antimicrobials for Gram-negative bacterial

Table 1 Management of *Strongyloides stercoralis* infection

		Solid organ transplant	Hematopoietic stem cell transplant
Treatment	Chronic strongyloidiasis	200 mcg/kg by mouth for 2 days and repeated 2 weeks later	
	Hyper-infection syndrome	Ivermectin 200 mcg/kg by mouth daily for at least 2 weeks after the last positive stool sample. Ivermectin 200 mcg/kg subcutaneous is recommended for those unable to tolerate oral therapy or with severe ileus. Broad-spectrum antimicrobials for gram-negative sepsis Supportive care Reduction of immunosuppression if feasible	
	Disseminated disease		

sepsis, supportive care during the critical phase of illness, and, if possible, reduction of immunosuppression. If the patient can tolerate oral therapy, ivermectin 200 mcg/kg by mouth daily is recommended for at least 2 weeks after the last positive stool sample to cover a complete autoinfection cycle [21]. Some experts advocate for combination therapy with albendazole until there is evidence of clinical improvement [43]. The optimal treatment route for patients unable to tolerate oral therapy due to ileus, intestinal obstruction, or obtundation is uncertain. Options in this scenario include rectal or subcutaneous ivermectin (200 mcg/kg/daily) [49, 50]. Case reports describe subtherapeutic levels of ivermectin with rectal administration; thus, subcutaneous administration is favored [21, 51, 52]. In the USA, use of the veterinary subcutaneous formulation of ivermectin for treatment of human patients requires an emergency investigational new drug (eIND) approval from the Food and Drug Administration (FDA) Division of Anti-Infective Agents (301-796-1400) [53].

Criteria to define cure have not been established [54]. Because larval stool output may be intermittent, stool examination does not have the necessary sensitivity to determine cure [24, 54]. Eosinophilia, when present, is often slow to resolve. In a case series from a non-endemic area, eosinophilia resolved by 6 months in the majority of patients [26]. Transplant patients on prednisone may not have any eosinophilia. A retrospective single-center study demonstrated that serology titers progressively wane after therapy; a decrease of at least half of the initial optical density (ELISA or LIPS) or reduction of at least two titers (IFAT) after 3 months was suggested as criteria to define clinical cure [55]. Unfortunately, commercial laboratories do not routinely report quantitative results for serology, and the using serology as a test of cure has not been validated in transplant patients. A prospective single-center study from a non-endemic area, which limits confounding from re-infection, assessed the long-term response to ivermectin in 48 patients by PCR. Only 21 patients (67% immunocompromised) adhered to the follow-up (1–4 years). All of them had a positive PCR at follow-up, and 14 (67%) had microbiologic evidence of recurrence by either the agar plate and or direct stool analysis. Three of the seven patients with a positive PCR and negative parasitological studies had a clinical presentation suspicious for recurrence of strongyloidiasis [56]. Although the performance of PCR as a test of cure and to predict the risk of recurrence is unclear, the study emphasizes the need to establish long-term follow-up for transplant candidates and recipients diagnosed with strongyloidiasis to monitor for recurrence.

Patients should be educated to wear closed footwear in endemic areas to reduce the risk of de novo acquisition or re-infection. One of the following strategies should be implemented to decrease the risk of post-transplant reactivation in SOT and HSCT recipients (Table 2). In hyper-endemic areas with limited resources, a proposed approach is to treat all transplant candidates [57]. In endemic areas with available diagnostic tools, the alternative is to implement universal screening. In non-endemic areas, a targeted screening approach based on epidemiological risk factors for strongyloidiasis has been successfully used [58]. Based on the epidemiology of *Strongyloides*, proposed risk factors include patients who were born or lived in tropical or subtropical regions, candidates with unexpected eosinophilia, travel to an endemic area, and patients who were born or lived in American Appalachia. Two

Table 2 Prevention of *Strongyloides stercoralis* infection

		Solid organ transplant	Hematopoietic stem cell transplant
Pre-transplant screening indications[a,b]	Donor	Screening is recommended	Not routinely performed
	Recipient	Screening is recommended	Screening is recommended
Transplant candidate/recipient education		Avoid walking barefoot in endemic areas	

[a]Universal or targeted screening strategy depends on the local epidemiology
[b]Proposed risk factors in the targeted strategy include patients who were born or lived in tropical or subtropical regions, unexplained eosinophilia, travel to an endemic area, and patients who were born or lived in the American Appalachia

approaches have been used to screen transplant patients with epidemiological risk factors for strongyloidiasis: combined serological and microbiologic assays versus serology alone [58, 59]. The former is labor intensive but may overcome the risk of a false-negative serology in immunocompromised transplant candidates.

To minimize the risk of donor-derived strongyloidiasis among SOT recipients, living and deceased SOT donors should be screened for *Strongyloides* (Table 2). The decision as to whether to implement a universal or targeted screening approach should depend on local epidemiology. A universal strategy is favored in endemic areas, whereas a targeted screening strategy is favored in non-endemic areas. Serology is the favored screening test for both living and deceased SOT donors [22, 59, 60]. Testing deceased donors to reduce the incidence of donor-derived strongyloidiasis has proven to be an effective strategy [22]. From 2009 to 2013, the Centers for Disease Control and Prevention investigated seven clusters of donor-derived strongyloidiasis, in which donor screening had not been performed before transplantation. Six of the seven donors involved had epidemiological risk factors for strongyloidiasis. Eleven of the 20 recipients (55%) developed strongyloidiasis, of which 2 (18%) died from complications of the disease. In contrast, LiveOnNY (the organ procurement organization serving the southwest of New York State – formerly New York Organ Donor Network) started a targeted screening strategy in 2010, in which procurement was done without delay due to pending tests results. Once available, results were promptly communicated to transplant centers, and recipients from positive donors were treated. With this approach, there were no reported cases of donor-derived strongyloidiasis during the study period [22]. A 10-year analysis from the OPTN DTAC committee described an increase in the proportion of reports initiated due to a positive result on a deceased donor screening *Strongyloides* test and a decrease in the proportion of cases adjudicated as a proven and probable transmission during the study period. This trend occurred during a time frame of improved donor screening and efficient recipient treatment [17]. Despite this data, a survey in 2016 of all 52 Organ Procurement Organizations in the USA revealed that only 6 (10%) of them were screening deceased donors for *Strongyloides* [61].

Living donors, SOT, and HSCT candidates testing positive for *Strongyloides* should be treated before organ procurement or transplantation, respectively, using

the recommended regimen for chronic strongyloidiasis. We also recommend treating transplant candidates with epidemiological risk factors requiring urgent transplantation in whom testing is not feasible. SOT patients receiving organs from deceased donors testing positive for *Strongyloides* should be treated as soon as the donor screening results become available with ivermectin following the recommendations for chronic strongyloidiasis. Additionally, during the investigation of a potential *Strongyloides* donor-derived event, all recipients should be treated empirically; delaying therapy until the investigation is concluded is highly discouraged [21, 22].

Conclusions

Strongyloides is endemic throughout the tropics and subtropics. Due to immigration patterns, patients born in endemic areas now have access to transplantation or may become organ donors in developed countries. Medical advances in endemic areas are also making transplantation more accessible in these regions. To prevent the consequences of the severe manifestations of strongyloidiasis, solid organ and hematopoietic stem cell transplant programs need to be mindful of the epidemiology of this parasite during the evaluation of transplant candidates and donors in the case of solid organ transplantation.

Key Points

- Due to the low sensitivity of serological assays in the immunocompromised host, a combination of diagnostic methods is required to establish the diagnosis of strongyloidiasis in transplant recipients.
- In patients with hyper-infection syndrome or disseminated disease, to establish a diagnosis, numerous specimens should be tested, including stool, lower respiratory samples, and any other affected body fluids and tissues.
- Screen those transplant candidates and recipients diagnosed with strongyloidiasis for HTLV-1 by serology, as this affects subsequent management.
- Oral ivermectin is the anti-parasitic of choice to treat uncomplicated strongyloidiasis.
- Educate transplant candidates and recipients about the use of closed footwear when in endemic areas so as to minimize the risk of de novo infection or re-infection.
- Screen SOT and HSCT candidates with epidemiological risk factors for *Strongyloides* with serology to minimize the risk of reactivation. Treat those that test positive with ivermectin. Transplant candidates testing positive should not be declined for transplantation based on this criterion alone.
- Screen living SOT donors with epidemiological risk factors for *Strongyloides* with serology to minimize the risk of a donor-derived event. Organs from living

donors testing positive can be procured after the donor is treated with oral ivermectin and adequate consent obtained from the recipient.

- Screen deceased SOT donors with epidemiological risk factors for *Strongyloides* with serology. Organ procurement from donors being screened for *Strongyloides* should not be delayed awaiting results. Transplant centers should be notified of a positive donor test and the results promptly reported. SOT recipients receiving organs from deceased donors should be treated with oral ivermectin without delay. SOT deceased donors undergoing screening for *Strongyloides* or testing positive should not be excluded from donation based on this criterion alone.
- Transplant centers notified of a potential donor-derived event with *Strongyloides* should immediately treat their patients empirically with oral ivermectin. Delaying treatment awaiting the outcome of the investigation is strongly discouraged.

References

1. Pelletier LL Jr, Gabre-Kidan T. Chronic strongyloidiasis in Vietnam veterans. Am J Med. 1985;78(1):139–40.
2. Genta RM. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. Rev Infect Dis. 1989;11(5):755–67.
3. Bisoffi Z, Buonfrate D, Montresor A, Requena-Mendez A, Munoz J, Krolewiecki AJ, et al. *Strongyloides stercoralis*: a plea for action. PLoS Negl Trop Dis. 2013;7(5):e2214.
4. Schar F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, et al. *Strongyloides stercoralis*: global distribution and risk factors. PLoS Negl Trop Dis. 2013;7(7):e2288.
5. Ostera G, Blum J. Strongyloidiasis: risk and healthcare access for Latin American immigrants living in the United States. Curr Trop Med Rep. 2016;3:1–3.
6. Posey DL, Blackburn BG, Weinberg M, Flagg EW, Ortega L, Wilson M, et al. High prevalence and presumptive treatment of schistosomiasis and strongyloidiasis among African refugees. Clin Infect Dis. 2007;45(10):1310–5.
7. Siddiqui AA, Berk SL. Diagnosis of *Strongyloides stercoralis* infection. Clin Infect Dis. 2001;33(7):1040–7.
8. Starr MC, Montgomery SP. Soil-transmitted helminthiasis in the United States: a systematic review – 1940–2010. Am J Trop Med Hyg. 2011;85(4):680–4.
9. Russell ES, Gray EB, Marshall RE, Davis S, Beaudoin A, Handali S, et al. Prevalence of *Strongyloides stercoralis* antibodies among a rural Appalachian population – Kentucky, 2013. Am J Trop Med Hyg. 2014;91(5):1000–1.
10. Geri G, Rabbat A, Mayaux J, Zafrani L, Chalumeau-Lemoine L, Guidet B, et al. *Strongyloides stercoralis* hyperinfection syndrome: a case series and a review of the literature. Infection. 2015;43(6):691–8.
11. Lam CS, Tong MK, Chan KM, Siu YP. Disseminated strongyloidiasis: a retrospective study of clinical course and outcome. Eur J Clin Microbiol Infect Dis. 2006;25(1):14–8.
12. Igra-Siegman Y, Kapila R, Sen P, Kaminski ZC, Louria DB. Syndrome of hyperinfection with *Strongyloides stercoralis*. Rev Infect Dis. 1981;3(3):397–407.
13. Safdar A, Malathum K, Rodriguez SJ, Husni R, Rolston KV. Strongyloidiasis in patients at a comprehensive cancer center in the United States. Cancer. 2004;100(7):1531–6.
14. Wirk B, Wingard JR. *Strongyloides stercoralis* hyperinfection in hematopoietic stem cell transplantation. Transpl Infect Dis. 2009;11(2):143–8.
15. Marcos LA, Terashima A, Canales M, Gotuzzo E. Update on strongyloidiasis in the immunocompromised host. Curr Infect Dis Rep. 2011;13(1):35–46.

16. Multani A, Allard LS, Wangjam T, Sica RA, Epstein DJ, Rezvani AR, et al. Missed diagnosis and misdiagnosis of infectious diseases in hematopoietic cell transplant recipients: an autopsy study. Blood Adv. 2019;3(22):3602–12.
17. La Hoz RM, Vece G, Danziger-Isakov L, Florescu D, Malinis M, Lilly K, et al., editors. Donor Derived Strongyloidiasis, a Preventable Event. American Transplant Congress; 2019.
18. Roxby AC, Gottlieb GS, Limaye AP. Strongyloidiasis in transplant patients. Clin Infect Dis. 2009;49(9):1411–23.
19. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.
20. Iori AP, Ferretti A, Gentile G, Gabrielli S, Perrone S, Barberi W, et al. *Strongyloides stercoralis* infection in allogeneic stem cell transplant: a case report and review of the literature. Transpl Infect Dis. 2014;16(4):625–30.
21. La Hoz RM, Morris MI. Practice ASTIDCo. Intestinal Parasites including Cryptosporidium, Cyclospora, Giardia, & Microsporidia, *Entamoeba histolytica*, Strongyloides, Schistosomiasis, & Echinococcus: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;e13618
22. Abanyie FA, Gray EB, Delli Carpini KW, Yanofsky A, McAuliffe I, Rana M, et al. Donor-derived *Strongyloides stercoralis* infection in solid organ transplant recipients in the United States, 2009–2013. Am J Transplant. 2015;15(5):1369–75.
23. Gotuzzo E, Terashima A, Alvarez H, Tello R, Infante R, Watts DM, et al. *Strongyloides stercoralis* hyperinfection associated with human T cell lymphotropic virus type-1 infection in Peru. Am J Trop Med Hyg. 1999;60(1):146–9.
24. Buonfrate D, Formenti F, Perandin F, Bisoffi Z. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. Clin Microbiol Infect. 2015;21(6):543–52.
25. Repetto SA, Ruybal P, Solana ME, Lopez C, Berini CA, Alba Soto CD, et al. Comparison between PCR and larvae visualization methods for diagnosis of *Strongyloides stercoralis* out of endemic area: a proposed algorithm. Acta Trop. 2016;157:169–77.
26. Loutfy MR, Wilson M, Keystone JS, Kain KC. Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. Am J Trop Med Hyg. 2002;66(6):749–52.
27. Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. J Allergy Clin Immunol. 1994;94(6 Pt 2):1202–13.
28. Bisoffi Z, Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, et al. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. PLoS Negl Trop Dis. 2014;8(1):e2640.
29. Luvira V, Trakulhun K, Mungthin M, Naaglor T, Chantawat N, Pakdee W, et al. Comparative diagnosis of Strongyloidiasis in immunocompromised patients. Am J Trop Med Hyg. 2016;95(2):401–4.
30. Schaffel R, Nucci M, Carvalho E, Braga M, Almeida L, Portugal R, et al. The value of an immunoenzymatic test (enzyme-linked immunosorbent assay) for the diagnosis of strongyloidiasis in patients immunosuppressed by hematologic malignancies. Am J Trop Med Hyg. 2001;65(4):346–50.
31. Nielsen PB, Mojon M. Improved diagnosis of *Strongyloides stercoralis* by seven consecutive stool specimens. Zentralbl Bakteriol Mikrobiol Hyg A. 1987;263(4):616–8.
32. Campo Polanco L, Gutierrez LA, Cardona AJ. Diagnosis of *Strongyloides Stercoralis* infection: meta-analysis on evaluation of conventional parasitological methods (1980–2013). Rev Esp Salud Publica. 2014;88(5):581–600.
33. Buonfrate D, Requena-Mendez A, Angheben A, Cinquini M, Cruciani M, Fittipaldo A, et al. Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection-A systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(2): e0006229.
34. Terashima A, Alvarez H, Tello R, Infante R, Freedman DO, Gotuzzo E. Treatment failure in intestinal strongyloidiasis: an indicator of HTLV-I infection. Int J Infect Dis. 2002;6(1):28–30.

35. Porto AF, Neva FA, Bittencourt H, Lisboa W, Thompson R, Alcantara L, et al. HTLV-1 decreases Th2 type of immune response in patients with strongyloidiasis. Parasite Immunol. 2001;23(9):503–7.
36. Henriquez-Camacho C, Gotuzzo E, Echevarria J, White AC Jr, Terashima A, Samalvides F, et al. Ivermectin versus albendazole or thiabendazole for *Strongyloides stercoralis* infection. Cochrane Database Syst Rev. 2016;1:CD007745.
37. Igual-Adell R, Oltra-Alcaraz C, Soler-Company E, Sanchez-Sanchez P, Matogo-Oyana J, Rodriguez-Calabuig D. Efficacy and safety of ivermectin and thiabendazole in the treatment of strongyloidiasis. Expert Opin Pharmacother. 2004;5(12):2615–9.
38. Suputtamongkol Y, Premasathian N, Bhumimuang K, Waywa D, Nilganuwong S, Karuphong E, et al. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. PLoS Negl Trop Dis. 2011;5(5):e1044.
39. Gann PH, Neva FA, Gam AA. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. J Infect Dis. 1994;169(5):1076–9.
40. Amato Neto V, Carignani FL, Matsubara L, Braz LM. [The treatment of rats experimentally infected with Strongyloides venezuelensis by orally administered ivermectin]. Rev Soc Bras Med Trop. 1997;30(6):481–4.
41. Grove DI. The effects of 22, 23-dihydroavermectin B1 on Strongyloides ratti and S. stercoralis infections in mice. Ann Trop Med Parasitol. 1983;77(4):405–10.
42. Buonfrate D, Salas-Coronas J, Munoz J, Maruri BT, Rodari P, Castelli F, et al. Multiple-dose versus single-dose ivermectin for *Strongyloides stercoralis* infection (strong treat 1 to 4): a multicentre, open-label, phase 3, randomised controlled superiority trial. Lancet Infect Dis. 2019;19(11):1181–90.
43. Greaves D, Coggle S, Pollard C, Aliyu SH, Moore EM. *Strongyloides stercoralis* infection. BMJ. 2013;347:f4610.
44. Gardon J, Gardon-Wendel N, Demanga N, Kamgno J, Chippaux JP, Boussinesq M. Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for Loa loa infection. Lancet. 1997;350(9070):18–22.
45. Ito T. Mazzotti reaction with eosinophilia after undergoing oral ivermectin for scabies. J Dermatol. 2013;40(9):776–7.
46. Datry A, Hilmarsdottir I, Mayorga-Sagastume R, Lyagoubi M, Gaxotte P, Biligui S, et al. Treatment of *Strongyloides stercoralis* infection with ivermectin compared with albendazole: results of an open study of 60 cases. Trans R Soc Trop Med Hyg. 1994;88(3):344–5.
47. Marti H, Haji HJ, Savioli L, Chwaya HM, Mgeni AF, Ameir JS, et al. A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of Strongyloides stercoralis and other soil-transmitted helminth infections in children. Am J Trop Med Hyg. 1996;55(5):477–81.
48. Suputtamongkol Y, Kungpanichkul N, Silpasakorn S, Beeching NJ. Efficacy and safety of a single-dose veterinary preparation of ivermectin versus 7-day high-dose albendazole for chronic strongyloidiasis. Int J Antimicrob Agents. 2008;31(1):46–9.
49. Barrett J, Broderick C, Soulsby H, Wade P, Newsholme W. Subcutaneous ivermectin use in the treatment of severe *Strongyloides stercoralis* infection: two case reports and a discussion of the literature. J Antimicrob Chemother. 2016;71(1):220–5.
50. Tarr PE, Miele PS, Peregoy KS, Smith MA, Neva FA, Lucey DR. Case report: rectal adminstration of ivermectin to a patient with Strongyloides hyperinfection syndrome. Am J Trop Med Hyg. 2003;68(4):453–5.
51. Bogoch II, Khan K, Abrams H, Nott C, Leung E, Fleckenstein L, et al. Failure of ivermectin per rectum to achieve clinically meaningful serum levels in two cases of Strongyloides hyperinfection. Am J Trop Med Hyg. 2015;93(1):94–6.
52. Grein JD, Mathisen GE, Donovan S, Fleckenstein L. Serum ivermectin levels after enteral and subcutaneous administration for Strongyloides hyperinfection: a case report. Scand J Infect Dis. 2010;42(3):234–6.
53. Administration FaD. Emergency IND Timeline [Website]. 2017. Available from: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm597130.htm

54. Requena-Mendez A, Chiodini P, Bisoffi Z, Buonfrate D, Gotuzzo E, Munoz J. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. PLoS Negl Trop Dis. 2013;7(1):e2002.
55. Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, Albonico M, et al. Accuracy of five serologic tests for the follow up of *Strongyloides stercoralis* infection. PLoS Negl Trop Dis. 2015;9(2):e0003491.
56. Repetto SA, Ruybal P, Batalla E, Lopez C, Fridman V, Sierra M, et al. Strongyloidiasis outside endemic areas: long-term parasitological and clinical follow-up after ivermectin treatment. Clin Infect Dis. 2018;66:1558.
57. Clemente WT, Pierrotti LC, Abdala E, Morris MI, Azevedo LS, Lopez-Velez R, et al. Recommendations for Management of Endemic Diseases and Travel Medicine in solid-organ transplant recipients and donors: Latin America. Transplantation. 2018;102(2):193–208.
58. Fitzpatrick MA, Caicedo JC, Stosor V, Ison MG. Expanded infectious diseases screening program for Hispanic transplant candidates. Transpl Infect Dis. 2010;12(4):336–41.
59. Requena-Mendez A, Buonfrate D, Gomez-Junyent J, Zammarchi L, Bisoffi Z, Munoz J. Evidence-based guidelines for screening and Management of Strongyloidiasis in non-endemic countries. Am J Trop Med Hyg. 2017;97(3):645–52.
60. Levi ME, Kumar D, Green M, Ison MG, Kaul D, Michaels MG, et al. Considerations for screening live kidney donors for endemic infections: a viewpoint on the UNOS policy. Am J Transplant. 2014;14(5):1003–11.
61. Abanyie FA, Valice E, Delli Carpini KW, Gray EB, McAuliffe I, Chin-Hong PV, et al. Organ donor screening practices for *Strongyloides stercoralis* infection among US organ procurement organizations. Transpl Infect Dis. 2018;20:e12865.

Chagas Disease: Coming to a Transplanted Patient Near You

54

Lígia Camera Pierrotti and Karim Yaqub Ibrahim

Contents

Introduction 1294
Epidemiology 1294
Modes of Transmission 1295
The Burden of Disease 1297
Natural History and Pathogenesis 1299
Diagnosis 1306
Methods of Laboratory Diagnosis 1306
Acute ChD Diagnosis 1308
Chronic ChD Diagnosis 1310
Reactivation of ChD Diagnosis 1310
ChD in the Transplant Scenario 1311
Acute ChD Transmitted Through the Graft from Infected Donors 1312
Transfusion-Transmitted ChD Related to the Transplant Procedure 1313
ChD Reactivation Among Chronic *T. cruzi*-Infected Recipients 1314
De Novo Infection 1317
Pretransplant Risk Assessment and Management of Donors and Recipients 1320
Pretransplant Risk Assessment 1320
Acceptability Criteria of a Positive Donor 1320
Pretransplant Treatment of a Positive Donor 1321
Pretransplant Treatment of a Positive Recipient 1321
Posttransplant Management of the Recipients 1322
Posttransplant Surveillance of Negative Recipient from Positive Donor or Positive Recipient 1322
Posttransplant Prophylaxis for Seronegative and Seropositive Recipients 1323
Posttransplant Preemptive Therapy 1324
ChD Treatment 1324
Nifurtimox 1325
Benznidazole 1327
Immunosuppressive Management 1327

L. C. Pierrotti (✉) · K. Y. Ibrahim
Infectious Disease Clinical Division, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil
e-mail: pierrot@usp.br; karim.yaqub@hc.fm.usp.br

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_53

Monitoring After Treatment and Criteria of Cure 1327
Secondary Prophylaxis 1328
Prevention 1328
Conclusion 1329
Key Points 1329
References 1330

Introduction

Chagas disease (ChD), also known as American trypanosomiasis, is a zoonotic protozoan infection caused by the flagellate parasite *Trypanosoma cruzi* (*T. cruzi*). ChD was first described by the Brazilian sanitary physician Carlos Justiniano Chagas in 1909, more than a century ago. ChD remains an important public health problem classified as a neglected disease by the World Health Organization (WHO). There are an estimated seven to eight million infected people in the world, mostly in Latin American countries where it is endemic [1].

ChD disease has two successive phases, acute and chronic. Acute infection is mostly characterized by asymptomatic high parasitemia that is progressively controlled after a few months without treatment. Untreated infection can progress to a chronic form of ChD characterized by low parasitemia, which may remain asymptomatic for life or progress to cause irreversible cardiac, gastrointestinal, or peripheral nervous system disease. Immunocompromised individuals can also present with reactivation episodes of chronic ChD characterized by high parasitemia and clinical manifestations similar to the acute disease. Transplant physicians in endemic and non-endemic countries should be aware of the risk of *T. cruzi* transmission from donors to recipients as well as the risk of ChD reactivation in previously infected patients receiving immunosuppression posttransplant.

This chapter aims to raise awareness among transplant physicians regarding the risk and clinical management of *T. cruzi* donor-to-recipient transmission and post-transplant ChD reactivation in recipients from both endemic and non-endemic countries by discussing the global burden of ChD and its consequences in the field of transplantation. Prompt diagnosis and timely treatment of ChD in transplant patients is associated with good outcome, whereas lack of initial clinical suspicion resulting in a late diagnosis has been associated with fatal outcomes.

Epidemiology

According to estimates by WHO, more than 70 million people living in Latin American endemic countries are at risk for acquiring ChD; 7–8 million people are infected worldwide; and over 10,000 people die annually from clinical complications of ChD [1, 2]. Global estimates of infected people and the status of vector transmission are presented in Fig. 1. ChD is geographically limited primarily to Central and Southern American countries and Mexico because of the distribution

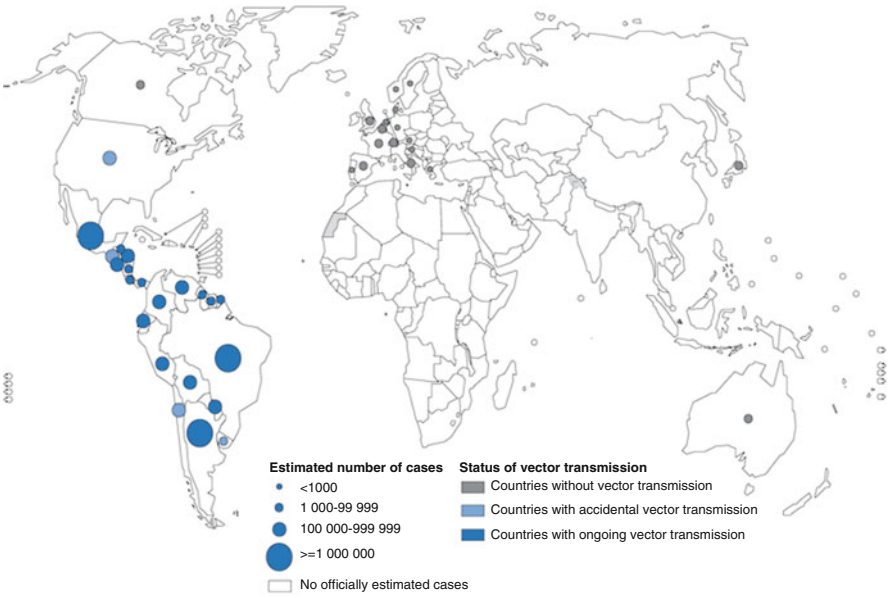


Fig. 1 Distribution of cases of *Trypanosoma cruzi* infection, based on official estimates and status of vector transmission, worldwide, 2006–2009. (Source: Reprinted from WHO [2], Copyright (2010))

of insect vectors responsible for disease transmission. However, since the 1990s, ChD has become a global health problem spreading outside Latin America, a phenomena related to increased international travel and migration flows from endemic to non-endemic areas including Europe, the USA, Canada, and Japan [3].

Modes of Transmission

ChD is transmitted to humans primarily through feces from triatomine insects containing *T. cruzi* parasites. Alternative modes of transmission consist of oral transmission by contaminated beverage and food, vertical transmission (mother to child), transmission via blood transfusion, and tissue and organ transplantation [4].

Vector transmission of ChD is limited to the Americas, involving more than 150 triatomine species (Hemiptera, Family Reduviidae, subfamily Triatominae) known as blood-sucking bugs or "kissing bugs" [5]. The most common triatomine species for *T. cruzi* transmission belong to the genera *Triatoma, Rhodnius*, and *Panstrongylus* [6]. The vector defecates after sucking blood, and the parasites are transmitted to humans through infected feces in contact with disrupted skin as a consequence of the vector bite or other skin lesions or through direct contact of feces with mucous membranes of the eyes or mouth. This zoonosis has involved sylvan or domestic mammals and nonmammal animals (marsupials, Chiroperas,

Rodentia, Edentata, native primates) serving as reservoirs hosts and several domestic and native species of *Triatoma.* Domestic (intra- and peridomicile) triatomine bugs typically live in cracks and holes in walls and roofs of poor housing, in rural and peri-urban areas. This condition is present from the Southern USA and Northern Mexico all the way to Argentina and Chile in the south [7].

Thanks to the intergovernmental Southern Cone Initiative coordinated by the Pan American Health Organization (PAHO)/WHO implemented to eliminate domestic triatomines in 1991, interruption of vector transmission of *T. cruzi* has been achieved in several Southern Cone and Central American countries. PAHO/WHO certifications of virtual or effective interruptions of domestic *T. infestans* populations were issued to Uruguay (1997 and 2012), Chile (1999), Brazil (2006), eastern Paraguay (2008), some parts of Argentina (2001, 2004, 2011, 2012), and southern Peru; and certifications of interruption domestic *R. prolixus* populations were issued to Southern Mexico (2009), Guatemala (2008), Honduras (2010), Nicaragua (2010), El Salvador (2010), and Costa Rica (2011) [8, 9]. Consequently, the number of new cases of infections in Latin America was drastically reduced. On the other hand, *T. cruzi* transmission by ubiquitous native vector populations in natural ecotopes in the Americas continues to occur; however, there is no current evidence of continuous or sustained domestic transmission [10].

An additional aim of the Southern Cone Initiative was the reduction or elimination of transmission via blood transfusions [10]. The reported prevalence of *T. cruzi* infection among blood donors from 1980 to 1989 varied from 1.3% to 51.5%, and the infectivity rate from one unit of infected whole blood was estimated to be around 12–25% [11]. In most Latin American countries, screening for *T. cruzi* infection in blood and organ donors is now compulsory [8]. As a consequence, the prevalence of infected blood samples in the Southern Cone countries has decreased. However, the implementation of serological screening as a control strategy for ChD remains a challenge in many endemic Latin American countries such as Bolivia where approximately 50% of the blood banks do not screen samples [8]. Moreover, there are many differences in the serological procedures and rules regulating the practices of blood screening for ChD.

Therefore, the risk of transfusion-transmitted ChD can present great variability. The residual risk of infection in endemic countries with fully implemented screening strategies is estimated at 1:200,000 units [12]. Transfusion-transmitted ChD has been also reported in non-endemic areas. A systematic review of reported cases in North America and Spain identified a total of seven cases [13]. In the USA, the Food and Drug Administration (FDA) recommends that blood donors should be tested at least one time for antibodies to *T. cruzi* using a licensed test; donors who test negative are qualified to return for repeat donation without further testing. This was implemented after it was realized that donor screening questions were not effective in identifying those at risk for transmission of ChD in non-endemic countries.

Aside from vector- and transfusion-transmitted ChD, transmissions from chronically infected pregnant women to the fetus and through solid-organ transplantation (SOT) or hematopoietic stem cell transplantation (HSCT) are additional modes of *T. cruzi* infection [3], occurring in both endemic and non-endemic countries.

T. cruzi can also be transmitted orally through beverages and food contaminated by infected vector's feces or urine, infected triatomines, or secretions from the anal glands of infected marsupials [6]. Oral transmission of *T. cruzi* has emerged as one of the major forms of ChD transmission in regions such as the Amazon basin and has been responsible for several outbreaks in endemic countries, usually affecting several people simultaneously. It has been related to the consumption of contaminated unpasteurized products such as *açaí* and other fruit juices and sugarcane [14].

The Burden of Disease

Although differences in the estimates of infected individuals in the Americas have been observed reflecting different methods employed to diagnose *T. cruzi* infection, the reported numbers are consistently high, and ChD remains one of the biggest public health problems in the world. Nevertheless, due to several governmental initiatives promoting advances in vector control and quality assurance of blood transfusion in many Latin American countries, the epidemiological measures of ChD in Latin America reported by WHO in 2015 showed a significant reduction compared to the previously reported data [1] (Table 1). The prevalence of *T. cruzi* infection has decreased significantly from an estimate of 30 million in the 1990s to about 7–8 million in the 2010s [7]. The estimated prevalence of *T. cruzi*-infected people in Latin America varies from 0.7% in Mexico to 15% in Bolivia [15] (Table 2). In endemic areas, according to recent estimates based on 2010 data, there are 5,742,167 infected people living in 21 Latin American countries, of which the majority are living in Argentina (1,505,235), Brazil (1,156,821), Mexico (876,458), and Bolivia (607,186) [1].

In the non-endemic areas, ChD is detected among the migrant population from endemic areas. Epidemiological data regarding the burden of ChD in non-endemic areas are limited and mostly acquired by indirect estimates based on the number of migrants from Latin American endemic countries living in non-endemic areas or information from screened blood donations [16–19]. Therefore, the real burden of ChD in non-endemic countries is uncertain, given the underestimation of the number

Table 1 Prevalence and incidence of vector-borne transmission of Chagas disease in 21 endemic countries in Latin America, in the years 1990, 2000, 2006, and 2010

	Estimated numbers			
Parameters	1990	2000	2006	2010
Total population at risk	100,000,000	40,000,000	28,000,000	70,199,360
Number of infected individuals	30,000,000	18,000,000	15,000,000	5,742,167
New cases per year acquired by vector transmission	700,000	200,000	41,2000	29,925

Note: The estimated number of total population of risk is based on official PAHO/WHO data. The higher number of estimated population at risk reported in 2010 compared with previous estimated rates was due to the different criteria used
Source: Adapted from Dias et al. [34]

of migrants and the lack of a surveillance system for ChD in these countries [20, 21]. The estimated prevalence of *T. cruzi* infection in immigrants from endemic areas ranges from 2% in the USA to 5.2% in Spain [15] (Table 2).

The total estimate of ChD cases living in Europe is between 68,318 and 123,078 with most of them living in Spain, Italy, and the UK [20]. The pooled prevalence of chronic ChD among Latin American immigrants living in Europe was 4.2%, ranging from 2.2% to 6.7% [19]. Immigrants from Bolivia had the highest prevalence (18.1%), followed by Paraguay (5.5%), Nicaragua (4.6%), Honduras (4.2%), El Salvador (3.7%), Argentina (2.2%), Mexico (1.5%), and Chile (1.0%) [19]. Prevalence among immigrants from other countries was less than 1% [17–19].

In the USA, from 2007 to 2013, the American Association of Blood Banks reported 1,908 confirmed cases of *T. cruzi* infection through screening of blood donors, and the Centers for Disease Control and Prevention (CDC) approved a total of 422 benznidazole or nifurtimox treatments for ChD during the same period [21]. More recent data estimates 238,091 cases of *T. cruzi* infection are in the USA as of 2012 [22]. Comparing the 2005 and 2012 data, the number of infected

Table 2 Estimated prevalence of *T. cruzi*-infected people living in endemic countries and of the *T. cruzi*-infected immigrants from endemic countries living in non-endemic countries

	Estimated prevalence (year)
T. cruzi-infected people living in endemic countries	
Argentina	8.2% (1990)
Bolivia	15.4% (1990)
Brazil	1.3% (1995)
Chile	2.8% (1990)
Colombia	3.9% (1990)
Costa Rica	4.3% (1990)
Ecuador	1.2% (1990)
El Salvador	6.1% (1990)
Honduras	5.8% (1990)
Guatemala	7.9% (1990)
Mexico	0.7% (1990)
Nicaragua	1.7% (1990)
Panama	9.0% (1990)
Paraguay	9.3% (1990)
Peru	3.0% (1990)
Uruguay	1.2% (1990)
Venezuela	4.0% (1990)
T. cruzi-infected immigrants from endemic countries living in non-endemic countries	
Australia	3.8% (2006)
Canada	3.5% (2006)
Europe (15 countries excluding Spain)	2.9% (1999–2005)
Spain	5.2% (2008)
USA	2.0% (2007)

Source: Adapted from Schmunis and Yadon [15]

immigrants decreased about 20% in the USA. This decrease, however, is probably not accurate, reflecting further the decrease of Latin Americans immigrants observed in the US Census surveys conducted after 2005. In addition, those authors have estimated an additional 109,000 cases of ChD among undocumented immigrants in 2012. The states with higher prevalence of immigrants with ChD (over 10,000 cases) were California, Texas, Florida, and New York, reflecting the distribution of Latin American immigrants in the USA [22].

Based on a systematic review, the risk factors for ChD among immigrants have included older age, living in a rural area, living in a mud/adobe house, having heard about ChD, knowing someone with ChD, having lived in a place with a thatched roof, and having a mother or grandmother born in an endemic country bitten by a triatomine [23].

Natural History and Pathogenesis

The life cycle of the parasite is shown in Fig. 2. *T. cruzi* has two distinct forms in infected humans: flagellate form in the peripheral blood and biological fluids (trypomastigote) and nonflagellated form in the tissues (amastigote) [24].

During a blood meal from an infected host, the triatomine vector ingests trypomastigotes which undergo transformation to epimastigotes (Fig. 2). After 3–4 weeks, infective metacyclic trypomastigotes develop in the triatomine's hindgut and are eliminated in the feces during subsequent blood meals, infecting other individuals [25, 26]. The trypomastigotes invade host cells and transform into intracellular amastigotes, which then multiply by binary fission and transform into blood form trypomastigotes. Afterwards, these trypomastigotes disseminate by infecting adjacent cells, via bloodstream or lymphatic system. Any nucleated mammalian cell can be infected, but *T. cruzi* parasites have a tropism toward cardiovascular, reticuloendothelial, nervous, and muscular systems [26].

ChD is characterized by three well-defined phases of infection or disease: acute, chronic, and reactivation phase (Fig. 3). Acute infection is mostly asymptomatic with high parasitemia. In immunocompetent individuals, infection is usually controlled after a few months even without treatment with trypanocidal drugs, and untreated patients will develop lifelong chronic infection, characterized by low-level parasitemia. The majority of people remain asymptomatic, while infection persists in the indeterminate form, and approximately 30–40% of the patients will progress to irreversible cardiac, gastrointestinal, and/or, more rarely, peripheral nervous system diseases [25, 27]. If untreated, immunosuppressed individuals with chronic infection are at risk of reactivation of ChD, characterized by episodes of high parasitemia and development of clinical and laboratory manifestations.

Vast regional diversity of the course and severity of chronic ChD probably reflects the complex interactions between the genetic variability among *T. cruzi* strains, host immunogenetics, and eco-epidemiological characteristics [28]. *T. cruzi* parasites show high genetic diversity and are classified into six discrete typing units (DTUs), named TcI to TcVI [29]. As an example, TcI has been

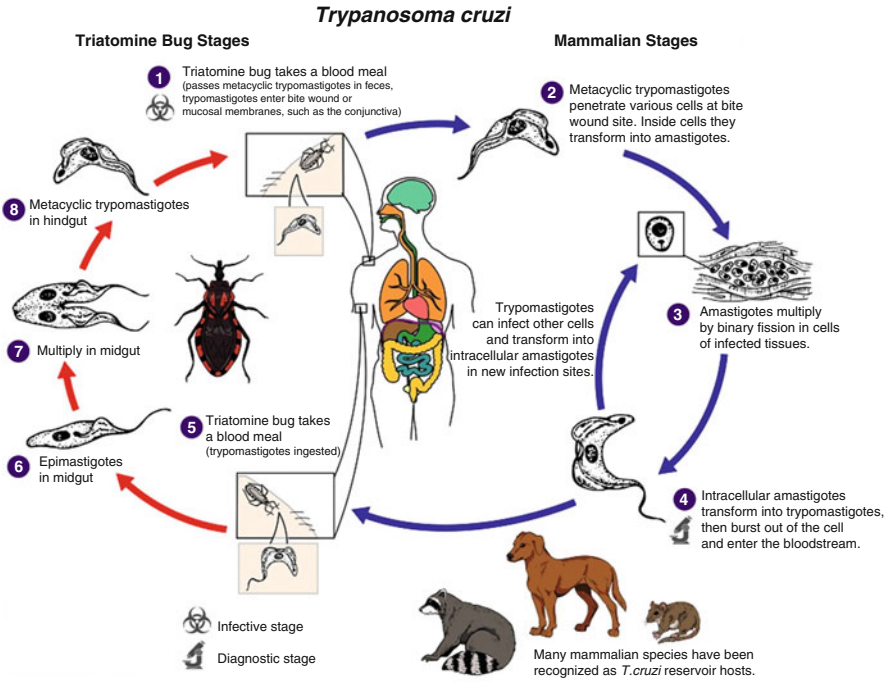


Fig. 2 Life cycle of *Trypanosoma cruzi*. (Source: Parasitic diseases in humans transmitted by vectors [24])

correlated with higher parasitemia and incidence of cardiomyopathy compared to the other *T. cruzi* DTUs [30].

After a variable incubation period, ranging from 7 to 15 days in the case of vector transmission and from 30 to 420 days in the case of transfusion- or transplant-associated transmission, the infected patient develops acute infection characterized by high parasitemia. In immunocompetent individuals, most of the patients are asymptomatic, and infection is usually controlled after a few months even without treatment with trypanocidal drugs. During the acute phase, about 50% of cases are asymptomatic or oligosymptomatic [31, 32]. When present, the main clinical manifestations are Romaña's sign characterized by unilateral painless edema of the eyelids (Fig. 4) and present in about 50% of the patients or inoculation chagoma in the skin characterized by edema at the infection site and present in about 25% of the patients. Other symptoms are fever, malaise, headache, anorexia, myalgia, lymphadenopathy, hepatomegaly, splenomegaly, rash, and diarrhea [27, 33]. Severe disease characterized by acute myocarditis, pericardial effusion, or meningoencephalitis has been reported in less than 5% of patients. The clinical manifestations of acute ChD are similar regardless of whether transmission was by vector, transfusion, HSCT, or SOT, except for the absence of an inoculation chagoma and a longer incubation period in transfusion- or transplant-associated transmission [4, 34].

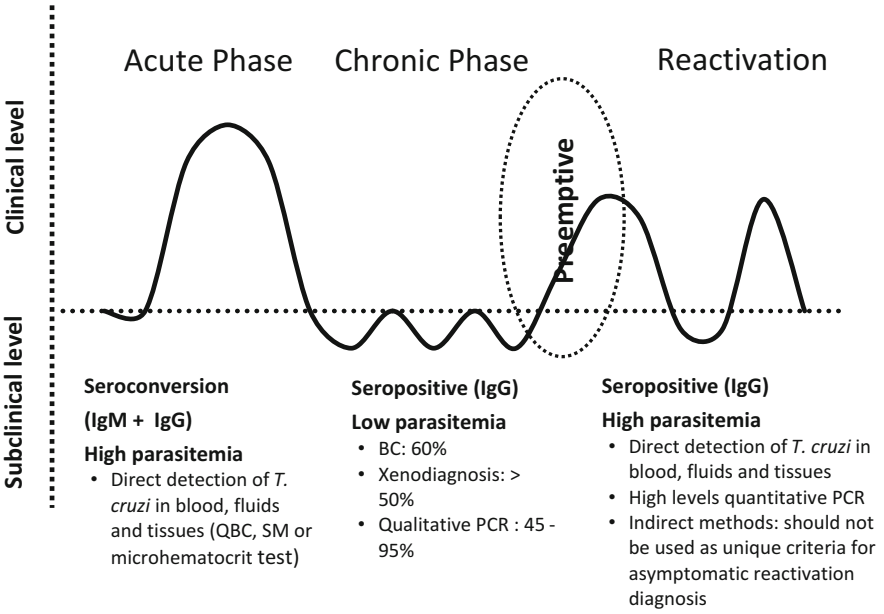


Fig. 3 Natural history of Chagas disease and the diagnostic characteristic in different phases of illness. Ig, immunoglobulin; QBC, quantitative buffy coat; SM, Strout method; BC, blood culture; PCR, polymerase chain reaction

Following parasite dissemination to the lymphatic system and bloodstream, the host's cell-mediated immunity controls parasite replication with a decrease in parasitemia in 1–2 months. Spontaneous resolution of the symptoms then occurs, and infection progresses to the chronic phase without treatment [4]. In this phase, the parasitemia is low and intermittent, and the parasites remain mostly in muscle (cardiac, smooth, and skeletal) and ganglion cells where they undergo further cycles of intracellular multiplication [4].

Approximately 60–70% of patients with chronic infection will evolve to an indeterminate clinical form of ChD characterized by positive serological tests, absence of clinical manifestations, normal electrocardiogram (ECG), and normal radiological examination of the chest [4, 34]. The remaining individuals will develop a determinate form with organ involvement 10–30 years after acute infection, evolving to symptomatic clinical forms related to the involvement of the heart, digestive system, and/or nervous system [27].

Heart involvement is the most serious and frequent form of the chronic ChD occurring in about 20–30% of chronically infected cases [35], and ChD remains the major cause of end-stage cardiomyopathy in endemic regions associated with high mortality [36]. Cardiac disease results from low-intensity continuous focal immune-mediated myocardial injury triggered by persistent *T. cruzi* myocardial infection, therefore resulting in progressive myocardial dysfunction and fibrosis and cardiomegaly (Fig. 5a, b). In addition, autonomic nervous system innervation and

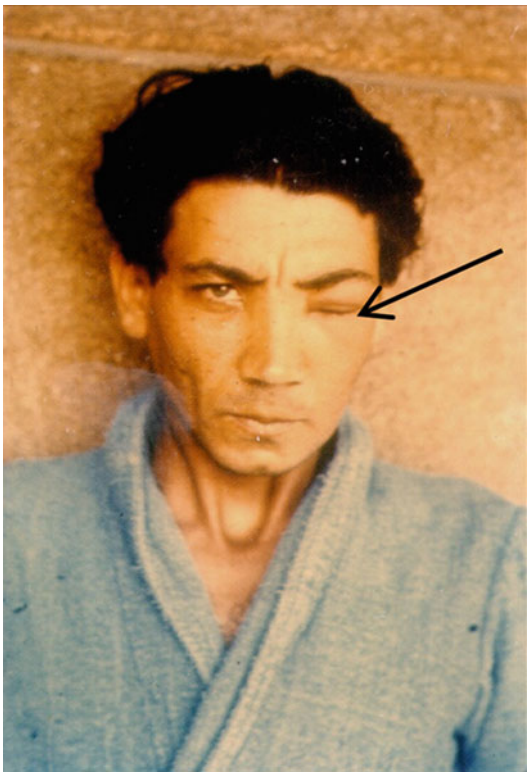


Fig. 4 Acute Chagas disease showing unilateral bipapebral edema in the local of insect bite – Romaña sign (arrow). (Courtesy: Prof. Mário Shiroma, São Paulo, SP – Brazil)

coronary microcirculation disturbances may amplify the pathogenesis of chronic Chagas cardiomyopathy (CCC) [35]. CCC is more frequent among individuals suffering over 30 years of infection and slowly evolves to arrhythmias, congestive heart failure, and thromboembolism [37, 38]. At first, cardiac involvement can be oligosymptomatic with arrhythmias detected only by the ECG or by 24-h Holter monitoring; CCC is essentially an arrhythmogenic disease where any type of atrial and ventricular arrhythmia as well as a wide variety of abnormalities of the conduction system may occur. The most common ECG abnormalities are sinus bradycardia, first-degree atrioventricular block, isolated ventricular extrasystoles, or typical findings such as second-degree and third-degree atrioventricular blocks and right bundle branch block [35, 37]. Congestive heart failure secondary to ventricular dysfunction progresses from mild symptoms of fatigue and dyspnea to more intense symptoms of pulmonary and systemic venous congestion. Pulmonary and systemic embolisms are common manifestations in patients with congestive heart failure [35]. Anatomical changes include thinning of the ventricular walls, biventricular enlargement, apical aneurysms, and mural thrombi [4].

Clinical progression and survival rates are significantly worse in patients with CCC than in those with non-inflammatory forms of dilated cardiomyopathy. The worse prognosis of patients with CCC might be associated with the greater incidence

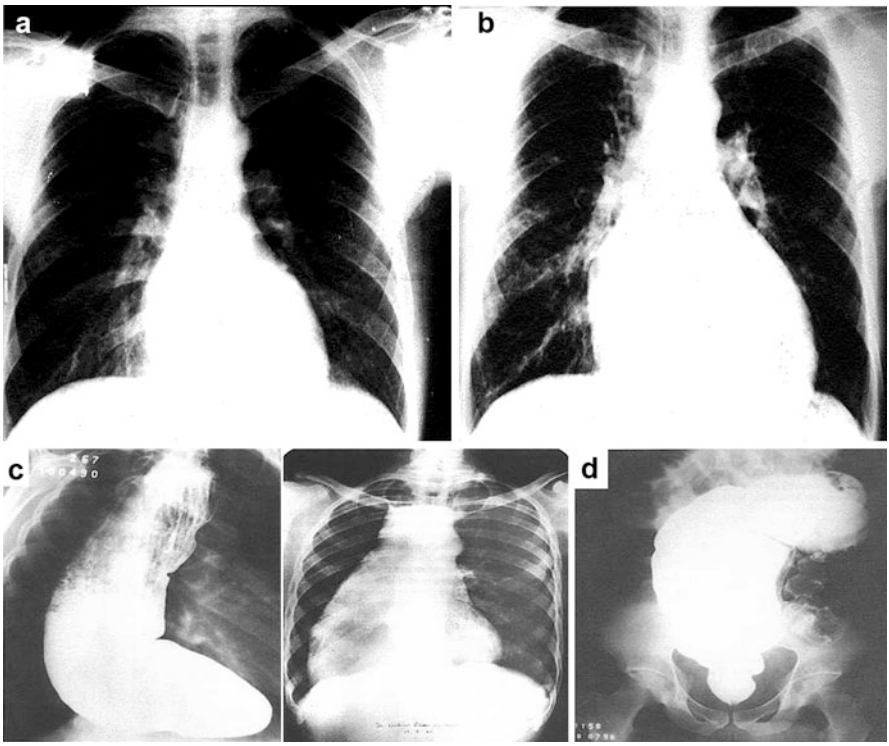


Fig. 5 Chronic ChD complications. (**a**) Patient with normal cardiac area; (**b**) Chronic Chagas cardiomyopathy characterized by the enlarged cardiac area; (**c**) contrasted X-ray of esophagus showing an enlargement of esophagus grade IV (on the right) and simple X-ray showing the shadow of esophagus on paracardiac area (on the left); (**d**) Megacolon in a patient who is 45 days without evacuation. (Courtesy: Dr. Ana Marli Christovam Sartori – Infectious Disease Department – Faculdade de Medicina da Universidade de São Paulo – SP, Brazil)

of atrial fibrillation and apical aneurysm observed in these patients in comparison with patients with cardiomyopathy from other etiologies. The most frequent causes of death among CCC patients are sudden cardiac death, accounting for nearly two-thirds of all deaths and usually associated with ventricular tachycardia and fibrillation, followed by refractory heart failure (25–30%) and thromboembolism (10–15%) [4]. Compared to patients with non-chagasic heart failure, the 5-year mortality rate of CCC patients was worse (21.8% vs. 32.8%, $p = 0.046$) [39].

The digestive form of ChD is less common, affecting about 15–20% of chronically infected individuals, occurring mainly in Argentina, Chile, and Bolivia [4]. Gastrointestinal tract disease is caused by injury to the autonomic nervous system throughout the tract, resulting in functional abnormalities of the salivary glands, stomach, extrahepatic ducts, duodenum, and small and large intestines [34]. The functional abnormalities, however, occur mostly in the esophagus and distal colon where denervation leads to loss of motor coordination and achalasia of the

sphincters, followed by difficulty in emptying semisolid material and progressive enlargement of these organs, resulting in the characteristic megaesophagus or megacolon [4, 27].

Concerning megaesophagus (Fig. 5c), the manifestations range from asymptomatic motility disorders, mild achalasia, and esophageal reflux to severe megaesophagus and dolichomegaesophagus. Symptoms include dysphagia, odynophagia, weight loss, aspiration, cough, and regurgitation. Patients with megaesophagus may be at increased risk of esophageal cancer [4].

Megacolon and dolichomegacolon (Fig. 5d) clinical manifestations are characterized by constipation, meteorism, dyskinesia, and, less frequently, abdominal pain. Prolonged constipation can lead to fecaloma, volvulus, and bowel ischemia. The sigmoid and rectum are dilated in nearly all cases of megacolon, whereas dilatation of more proximal colonic segments is rare. Most cases of megacolon are associated with megaesophagus. An increased risk of colorectal cancer has not been found in patients with megacolon [4].

A cardiodigestive form of ChD, combination of heart disease with megaesophagus and/or megacolon, has been also reported, but the real prevalence is unknown. In most patients, the development of megaesophagus precedes the involvement of the heart and colon [4].

Peripheral nervous system involvement by ChD is characterized by a sensorimotor polyneuritis causing paresthesia and hypoesthesia [27]. This form of the disease is much less common than cardiac and digestive manifestation, and it is usually restricted to a reduction or abolition of osteo-tendinous reflex showing destruction of motor neurons and of peripheral sensory nerve fibers at the electromyography examination. The autonomic nervous system can also be involved [27].

During the chronic Chagas infection, the delicate but stable immunological balance between the immune response and the parasite replication control can be broken by immunosuppression and immune deficiency states which lead to reactivation of latent infection characterized by high parasitemia and/or clinical manifestations with the presence of the parasite in fluids and tissues. The reactivation of ChD has been reported in patients with different immunosuppressive states, such as those with human immunodeficiency virus (HIV) infection, malignancies, and systemic autoimmune diseases and those with SOT or HSCT [32, 40, 41].

The risk of reactivation is related to the intensity of immunosuppression and does not depend on the phase of chronic infection whether indeterminate or active organ involvement. Reactivation episodes are usually reported after an intensification of the immunosuppression in chronically infected patients and are characterized by high parasitemia and sometimes atypical clinical manifestations. Moreover, some clinical differences between reactivation episodes among the immunocompromised groups have been observed. Long-lasting fever, cutaneous lesions including panniculitis (Fig. 6a), and myocarditis (Fig. 6b) are common in SOT and HSCT recipients [42, 43], whereas the most common manifestations of ChD reactivation among HIV-infected individuals are meningoencephalitis and central nervous system (CNS) involvement (Fig. 6b, c) [44], conditions rarely reported in transplant recipients [45, 46].

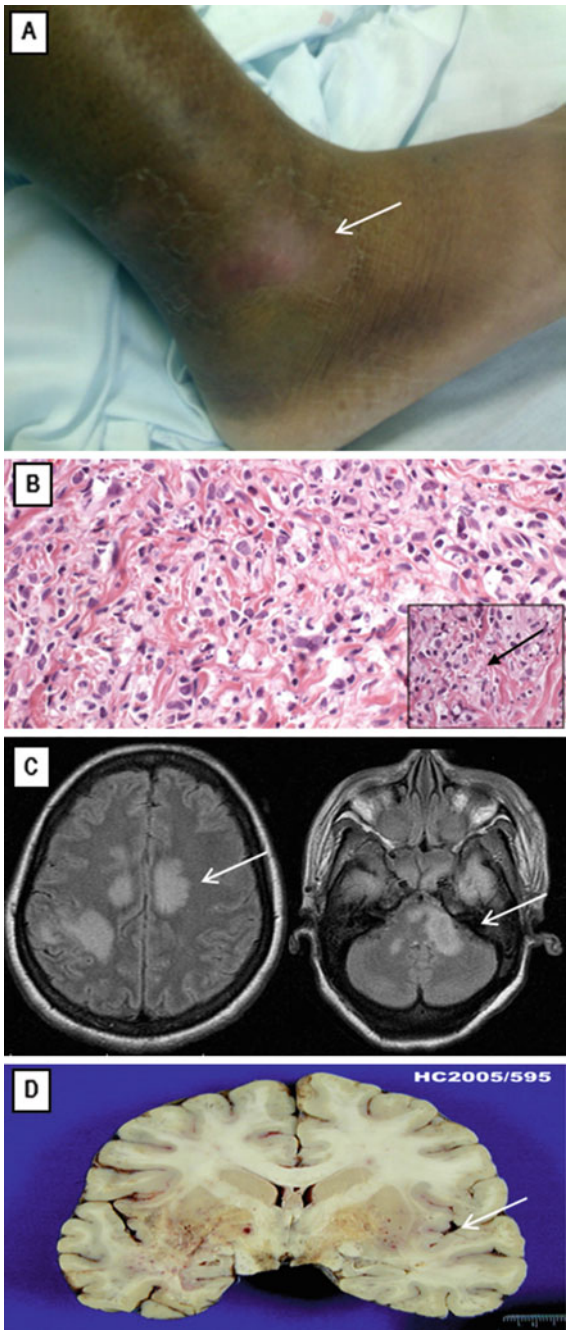


Fig. 6 Reactivation ChD manifestations. (**a**) Necrotic ulcer after nodosum erythema (arrow) in a heart transplant recipient. (**b**) Severe myocarditis with lymphocytes (H&E staining, objective magnification: 40X); myocardial fiber containing *T. cruzi* parasites (arrow). (**c**, **d**) Meningoencephalitis as clinical manifestation of reactivation of Chagas disease in an HIV-infected patient. (**c**) Computed tomography imaging showing the central nervous system involvement, including the cerebellum (arrows). (**d**) Anatomical piece showing brain lesions (arrow). (Courtesy: Dr. Karim Ibrahim Yaqub – Infectious Disease Division & Dr. Luiz A. Benvenuti – Heart Institute (InCor) – Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil)

Diagnosis

Methods of Laboratory Diagnosis

The diagnosis of ChD can be confirmed by serological and parasitological tests. Specific comments and indications of each method are summarized in Table 3.

Serological tests are based on both conventional and nonconventional methods. Conventional methods include the high-sensitivity enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IFA) methods and the high-specificity indirect hemagglutination assay (IHA) or recombinant antigen methods [47]. The pooled estimated sensitivity and specificity of 61 *T. cruzi* conventional serologic assays were 90% and 98%, respectively [48]. False-positive serology can occur by cross-reaction with *Leishmania* sp. and *T. rangeli* antibodies. Non-conventional tests, preferably recombinant antigen ELISA and Western blot with trypomastigote excretory-secretory antigen (TESA), have higher sensitivity and specificity and therefore are indicated after inconclusive results in conventional assays. Other serological methods with increased specificity include the complement-mediated lysis, latex agglutination, and radioimmunoprecipitation assay (RIPA). More recently, newer serological tests based on purified, recombinant, or synthetic peptide antigens have been used, and due to its high sensitivity, ELISA has been particularly useful to guarantee the security of blood donations [47]. Parasitologic diagnosis is based on direct and indirect tests. Direct parasitological tests involve direct examination of blood, fluid, and tissue samples for parasites, while indirect parasitological tests detect parasites by culture or xenodiagnosis.

Direct microscopic examination of the parasites based on non-concentration methods (Giemsa stain or thick smear) presents lower sensitivity compared to direct investigation methods of parasite after concentration of the clinical sample by centrifugation (Strout method, microhematocrit, and quantitative buffy coat). For these concentration methods, samples from patients are collected and centrifugated to remove the erythrocyte fraction and concentrate the parasites in the sediment. In the microhematocrit, blood samples are centrifugated in a capillary tube, and the presence of parasite is checked in the layer obtained between plasma and red blood cells (leukocyte cream). For Strout method, after the first centrifugation, the supernatant is separated and centrifugated again, and the presence of parasite is examined in the sediment obtained after the second centrifugation. Buffy coat method combined the concentration techniques with staining of parasite nucleic acids by the fluorochrome called acridine orange.

Xenodiagnosis is based on microscopic examination of the intestinal contents of triatomine nymphs 20, 30, and 60 days after they are fed with blood from a patient with suspected infection. The result is expressed as a percentage of triatomine nymphs infected by *T. cruzi* [49].

Polymerase chain reaction (PCR) assays has been increasingly used in the diagnosis of ChD, albeit with limited research in reference centers and lack of approval by Food and Drug Administration (FDA) [50]. Qualitative PCR results have not provided definitive information since the test can be negative in chronically

Table 3 Diagnostic tests according to ChD manifestation

Diagnostic test	Samples tested	Specific comments	Recommended tests		
			Acute ChD	Chronic ChD	Reactivation ChD
Direct microscopic examination of trypanosomatids	Blood fluids – CSF, cavitary fluids	Quick and easy to perform. Non-concentration methods (Giemsa stain or thick smear) present lower sensitivity compared to concentration methods (SM, microhematocrit, and QBC)	Yes	No	Yes
Xenodiagnosis	Blood	Low sensitivity if low parasitemia, highly specific; labor-intensive and time-consuming (up to 2 weeks or longer). Results can be semiquantitative indicating the percentage of nymphs per assay, useful for monitoring risk of ChD reactivation	No	Yes	No Predictive of ChD reactivation
Blood culture	Blood	Low sensitivity if low parasitemia, highly specific; time-consuming (up to 120 days to get positive results)	No	Yes	No
Polymerase chain reaction	Blood, CSF	High sensitivity and specificity. Not commercially available, performed by only few research/ reference centers, need standardization protocols. Results can be quantitative (IU/ml), useful for monitoring risk of ChD reactivation and monitor therapy response	Yes	Yes, in special situations to confirm inconclusive serological results	Yes Predictive of ChD reactivation Monitor therapy response

(continued)

Table 3 (continued)

Diagnostic test	Samples tested	Specific comments	Recommended tests		
			Acute ChD	Chronic ChD	Reactivation ChD
Serological testing	Blood	Conventional tests – ELISA, IFA, IHA have less than 100% sensitivity and specificity; diagnosis based on two different tests performed in parallel	Possibly useful to confirm the diagnosis in difficulty diagnosis cases	Yes	No

CSF, cerebrospinal fluid; SM, Strout method; BC, QBC, quantitative buffy coat; ELISA, enzyme-linked immunosorbent assay; IFA, indirect immunofluorescence assay; IHA, indirect hemagglutination assay

infected individuals. Furthermore, positive results are not able to distinguish between acute and reactivation from chronic infection. On the other hand, quantitative PCR (qPCR) tests have demonstrated good accuracy for *T. cruzi* quantification in peripheral blood samples [51, 52]. Parasite load quantification is particularly useful both for monitoring therapeutic response [53] and immunocompromised patients at risk of reactivation [54–56] (see the discussion below). PCR tests are also important for molecular typing of *T. cruzi* and can identify the lineage and genetic diversity of the parasites.

Sensitivity and specificity of noncommercial PCR tests are highly variable due to several technical factors, such as the sample volume, conservation conditions, methods used to extract DNA whether commercial kits or in-house methods, cycling protocols, and the parasite sequences and primers selected [57]. In addition, there is a possible effect of *T. cruzi* DTUs and their respective parasite burden among infected individuals [30]. Therefore, there is an urgent need for PCR tests to follow a standardized protocol. Due to the lack of standardized PCR tests, and the genetic variability of *T. cruzi* strains, currently an isolated positive result cannot be considered accurate enough to confirm or discard cases of acute or chronic ChD, and a combination of two or more PCR assays with different target genes is recommended in order to increase the accuracy [58].

Acute ChD Diagnosis

In the acute phase of infection, parasitemia is high in the peripheral blood, and parasitological examination is the most appropriate. Simultaneous performance of different modalities of direct parasitological examination of the peripheral blood (with or without centrifugation) using microscopy (with or without staining) is recommended [34] (Fig. 7a–c). Fresh examination for parasites is quicker and easier to perform, and it is the preferred diagnostic method in patients with up to 30 days of

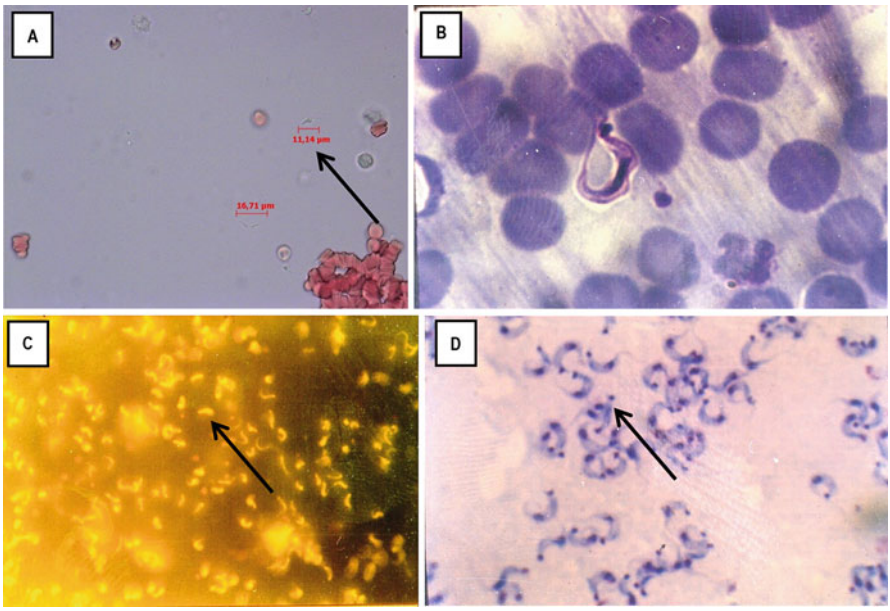


Fig. 7 (**a**–**c**) Parasitemia detection by direct methods in blood samples from a patient with acute Chagas disease. (**a**) Direct visualization of trypomastigote form of *T. cruzi* on peripheral blood smear. (**b**) Trypomastigote form of *T. cruzi* visualized in a Giemsa-stained peripheral blood smear. (**c**) Trypomastigote form of *T. cruzi* detected by quantitative buffy coat (QBC). (**d**) Trypomastigota form of *T. cruzi* visualized in a cerebrospinal fluid. (Courtesy: Vera Lúcia Teieira de Freitas and Laboratório de Investigação Médica – Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil)

fever. Concentrated methods have a higher sensitivity compared to non-concentrated ones, and they perform better in symptomatic patients with more than 30 days of evolution due to the expected decline in parasitemia. Blood samples should be examined within 24 h due to the risk of parasite lysis that may reduce sensitivity. Whenever the results of fresh examination and concentration methods are negative in the initial sample, additional samples must be tested until ChD suspicion is discarded, symptoms are resolved, or a different diagnosis is confirmed [34].

Parasites have also been observed directly in cerebrospinal fluid (CSF) when neurological signs were present (Fig. 7d) or in biopsied tissues such as the skin, heart, CNS, or any other involved organ when signs of reactivation were present [25, 27].

Indirect parasitological testing like xenodiagnosis and blood culture is time-consuming and laborious, thus are not indicated for the diagnosis of acute infection since treatment must be promptly started. However, positive results are useful to confirm acute ChD given the high specificity of these tests.

Serological tests are complementary and useful in acute ChD diagnosis and should always be performed. When repeated direct examinations are negative as commonly occurs during the late acute infection phase, serological tests are crucial to confirm suspected ChD [34]. Serological diagnosis of acute ChD is based on the

presence of anti-*T. cruzi* IgM antibodies by indirect IFA or the presence anti-*T. cruzi* IgG antibodies by conventional tests where an increase in IgG concentration of at least two IgG titers after a minimum interval of 21 days in preferably paired samples is diagnostic or seroconversion by any of the methods.

Additionally, in cases where a strong clinical and epidemiological suspicion of acute ChD exists, but direct parasitological examination is negative, the diagnosis of *T. cruzi* infection can be confirmed by molecular testing using PCR.

Chronic ChD Diagnosis

To confirm the diagnosis of chronic infection, it is necessary to detect the anti-*T. cruzi* IgG antibodies by two conventional methods with distinct antigenic preparations. Preferably, a high-sensitivity ELISA or IFA test should be performed simultaneously with a high specificity test like IHA or recombinant antigens [47].

Two conventional serological tests provide an accurate diagnosis in more than 95% of samples. Use of three serological tests in parallel can reduced indeterminate results to less than 2% [47]. Samples with indeterminate results (i.e., one negative and one positive result) should be retested. Repeatedly indeterminate results should be further tested by nonconventional tests, preferably using recombinant antigen ELISA and TESA-Western blot.

Chronic phase of ChD is characterized by low parasite burdens and negative results of direct parasitological methods. Low level of parasitemia can be detected intermittently by indirect parasitological methods with highly variable positivity rates, ranging from 25% to 94% for blood cultures [59].

Detection of *T. cruzi* DNA by PCR assays in chronically infected individuals shows a high variability, and therefore a negative result is not sufficiently accurate to rule out a diagnosis of chronic ChD. In a comparative study of different diagnosis methods for ChD, the rates of PCR detection among chronically infected patients with positive, negative, and inconclusive serologies were 83.5%, 47.6%, and 46.2%, respectively [59].

In conclusion, considering the intermittent parasitemia, negative indirect parasitological methods and PCR cannot rule out the diagnosis of chronic ChD. When positive, however, they confirm chronic ChD which is useful particularly in cases where serologic tests present inconclusive results.

Reactivation of ChD Diagnosis

ChD reactivation is defined as increased parasitemia regardless of clinical symptoms. Detection of *T. cruzi* by direct examination is the gold standard for diagnosis of ChD reactivation. In a symptomatic individual, ChD reactivation is confirmed by direct detection of trypomastigotes in the peripheral blood and fluids or intracellular amastigotes in biopsy specimens, including the skin, myocardium, bone marrow, and brain. Once the parasitemia increases, the parasite can be detected by direct methods or even incidentally through the manual differential leucocyte count analysis by visualizing the parasites on blood smear [34].

Asymptomatic reactivation of ChD is characterized by finding the parasite in direct microscopic examination of blood. This condition was first reported in an HIV-infected patient in Brazil [60]. However, direct examination is not usually positive during the early phase of reactivation, thus delaying the diagnosis. Detection of the parasite in blood and tissues by PCR methods is able to confirm the diagnosis of reactivation days to weeks earlier compared to conventional direct methods [55, 61–63]. Studies have reported that parasite DNA is present in blood around 30 days before clinical onset of clinical reactivation [54, 61, 62]. On the other hand, negative parasitemia in patients with clinical suspicion of reactivation does not exclude this diagnosis, and direct parasitological tests must be repeated [49].

Asymptomatic ChD reactivation presenting with subclinical parasitemia, which can only be detected by indirect parasitological methods such as blood culture and xenodiagnosis or by qPCR, must be distinct from the chronic stage of ChD. In chronically infected individuals, positive blood culture or qPCR results do not necessarily imply reactivation, since asymptomatic low-level parasitemia may occur in chronic disease. Therefore, association of quantitative method is recommended to see the increase of parasitemia. Real-time qPCR needs validation and definition of cutoff values to distinguish between reactivation and increased parasitemia in chronic ChD immunocompromised patients [64].

The distinction between chronic parasitemia and reactivation is particularly difficult among immunocompromised patients as these individuals have higher levels of chronic parasitemia compared to immunocompetent individuals. A Brazilian study in patients with chronic *T. cruzi* infection compared parasitemia detected by qualitative and quantitative methods among non-HIV-infected individuals ($n = 57$), coinfected *T. cruzi*-HIV individuals without ChD reactivation ($n = 29$), and coinfected *T. cruzi*-HIV individuals with ChD reactivation ($n = 5$) [64]. The authors showed increased parasitemia across these groups measured by qualitative and qPCR. Qualitative PCR was positive in 50.9%, 89.7%, and 100% individuals, and median parasitemia by real-time qPCR was 0, 1.57, and 1428.90 parasites/ml, respectively.

Semiquantitative xenodiagnosis that indicates the percentage of positive nymphs per assay may predict the occurrence of ChD reactivation. Episodes of reactivation among coinfected HIV-positive patients have been observed in 50% of patients who show >20% positive nymphs per assay in a follow-up period of 5 years [49].

Serology is not useful in the diagnosis of ChD reactivation. Blood culture and nonquantitative xenodiagnoses are time-consuming and do not confirm the diagnosis of ChD reactivation.

ChD in the Transplant Scenario

ChD in transplant recipients may occur in one of the following ways: (i) through the graft from infected donors; (ii) through blood transfusion during the transplant procedure; (iii) by reactivation of a latent infection in chronically infected recipients; and (iv) de novo acquisition posttransplant.

The most experience regarding ChD in the transplant scenario is derived from cardiac [65] and kidney transplantation [42, 66]. There are a few reported cases among liver and lung transplant recipients and among HSCT recipients.

Acute ChD Transmitted Through the Graft from Infected Donors

Acute ChD may occur in *T. cruzi* seronegative recipients of organs or bone marrow from a seropositive donor (D+/R−). The risk of transmission from infected donors to non-infected recipients is probably the same in endemic and non-endemic countries. The lack of systematic screening of donor and recipients in most non-endemic areas, and the fact that affected donors are frequently unaware of their status, probably makes the frequency of unexpected *T. cruzi* transmission higher in non-endemic compared to endemic countries.

Acute ChD Through Solid-Organ Transplantation

There are several reported cases of accidental donor-derived *T. cruzi* infection in SOT recipients in endemic and non-endemic countries [66–72]. Accidental transmission is a challenge especially in non-endemic countries, where systematic serological ChD screening is lacking [66, 73, 74]. Based on a survey study reported in 2011, only 19% of all US organ procurement organizations performed either universal or targeted donor screening for *T. cruzi* infection [50].

The *T. cruzi* transmission rates using kidneys and livers from chronically infected donors range from 10% to 22%, with variable findings across different series, as shown in Table 4. The period between transplantation and detection of infection is

Table 4 Donor-derived *T. cruzi* infection from seropositive donors to seronegative recipients[a]

Organ	Rate of transmission	Prophylaxis	Parasitemia or molecular screening after transplant	Preemptive therapy (number of patients)	Country	Ref
Kidney	3/15	No	Yes	1	Argentina	[42]
Kidney	0/9	Yes	No	–	Brazil	[92]
Kidney	2/15	No	Yes	2	United States	[66]
Kidney	0/6	No	Yes	0	Argentina	[46]
Liver	0/2	Yes	Yes	0	Spain	[77]
Liver	2/9	No	Yes	2	Argentina	[75]
Liver	0/6	Yes	Yes	0	Brazil	[117]
Liver	2/10	No	Yes	1	United States	[66]
Liver	0/4	No	Yes	0	Argentina	[94]
Heart	3/4	No	Yes	1	United States	[66]

[a]Each series include at least two patients

variable, ranging from 27 to 203 days [42, 66, 75]. Clinical presentation includes fever, malaise, anorexia, hepatosplenomegaly, acute myocarditis, and pancytopenia [40, 41]; fulminant myocarditis is also reported [66].

Experience with lung transplantation from infected donors is limited to a few case reports; transmission of ChD was reported in three out of four negative lung transplant recipients [63, 66, 76, 77]. Time from lung transplantation to detection of infection ranged from 10 to 61 weeks [63, 66, 76]. The estimated transmission rates from the reported cases, however, are likely lower than the true figures, considering possible publication bias. The rates of ChD transmission after heart transplantation are much higher (75% or more), and reports showed that recipients experience poor outcomes with high mortality rates [66, 78]. There are no reported cases with intestine transplantation.

Acute ChD Through Hematopoietic Stem Cell Transplantation

There is only one case report of ChD transmission in a patient following HSCT in literature. The patient was a *T. cruzi* seronegative marine from Spain who underwent HSCT from a *T. cruzi* seropositive donor originally from Latin America [79]. Ninety-four days after HSCT, the patient was readmitted to the hospital with severe weakness, fever, edema of the face and legs, and hyporeflexia. Trypomastigotes were identified in the blood and probably in the CSF. Despite of treatment with nifurtimox and initial clinical response, the patient developed a bacterial infection with sepsis and died. During this clinical course, the patient received many blood products from potentially infected donors; however, further studies failed to show evidence of positive serologic tests for the HSCT donor or any of the blood donors.

Transfusion-Transmitted ChD Related to the Transplant Procedure

Transmission of ChD by blood transfusion has been recognized since 1952, and the risk is related to disease prevalence in the population as well as systematic blood donor screening [80]. All blood components from infected donors have the potential to transmit *T. cruzi*, but transmission risk has been reported to be higher for platelet transfusion because trypomastigotes are predominantly separated into the platelet fraction during centrifugation [4, 12]. *T. cruzi* can survive in blood components at 4 °C up to 18 days and when stored at room temperature up to 250 days [12]. In addition, platelet leukoreduction or irradiation may reduce but do not eliminate the risk of *T. cruzi* transmission [13]. Finally, although there is evidence of viable parasites in cryopreserved components, there is no clear evidence of transfusion-transmitted ChD through frozen-thawed cellular products [12]. Blood derivatives, such as human albumin solution, clotting factor concentrates, and immunoglobulins, are not infective [12]. Based on a systematic review of transfusion-transmitted *T. cruzi* cases in North America and Spain, the infectivity risk is estimated to be 0–1.5% for red blood cells units, 5.6–25.7% for platelets, and 0–3.7% for frozen plasma or cryoprecipitate units [13]. In all reported cases,

platelets (from both whole blood and apheresis) or whole blood were implicated [13].

In most endemic countries, systematic screening of blood donors for ChD is mandatory. In non-endemic countries, the approach has either been based on the deferral from donations of people at risk of ChD or testing those at risk and accepting them for blood donation if specific laboratory test results are negative [40]. The second approach has been the chosen strategy in countries where there is a substantial Latin American population, such as the USA, Spain, France [81], and more recently Japan [82].

Individuals requiring blood transfusion during the transplantation period are at additional risk of *T. cruzi* acquisition from positive blood donors. Nevertheless, infection can be transmitted to recipients by either transfusion or transplant, and in some cases, the real source of infection cannot be identified.

Fever is the most common symptom, occurring in 80–100% of individuals with transfusion-transmitted ChD, and may be the only symptom. Lymphadenopathy and splenomegaly are also frequent findings [34]. Transplant clinicians should be aware that the incubation period is usually longer than that observed in vector-borne transmission, ranging from 30 to 120 days, although much shorter period of 8 days has been reported [34]. Therefore, ChD must be included in the differential diagnosis in transplant recipients presenting fever of unknown origin, especially if they received a transfusion.

There is no reported case of proved transfusion-transmitted *T. cruzi* infection among SOT recipients. An old report from Chile in 1989 identified ten patients with acute ChD where blood transfusion was suspected as the mechanism of infection in these cases [83].

A fatal case of disseminated *T. cruzi* infection with myocarditis and encephalitis was reported in a 25-year-old male patient with high-risk acute myeloid leukemia who received an unrelated cord blood transplant [84]. The patient developed persistent fever 11 days after transplantation. Trypamastigotes of *T. cruzi* were detected on peripheral blood and bone marrow smears 35 days posttransplantation. Treatment for acute ChD with benznidazole was initiated immediately, but the patient died 2 days later. An infected blood donor was identified retrospectively.

ChD Reactivation Among Chronic *T. cruzi*-Infected Recipients

A possible explanation for ChD reactivation in transplant recipients is that adrenal glands and skeletal muscle may be the reservoirs of *T. cruzi* and the parasites are released into the bloodstream during posttransplant immunosuppression. Parasitemia can thus cause fever and unspecific symptoms, or parasites can invade tissues causing different manifestations, such as myocarditis, encephalitis, and panniculitis [85].

In general, the risk of reactivation in chronically infected recipients is directly related to the intensity of immunosuppression, rather than a specific immunosuppressive protocol; therefore, the risk is higher in the first year after transplantation

and also with subsequent intensifications of immunosuppression (i.e., treatment of rejection). Nevertheless, late reactivation has been reported, and clinicians should be aware of this possibility. Gomez-P et al. reported two fatal reactivations of *T. cruzi* occurring 6 years after kidney and 7 years after heart transplants which were only diagnosed at autopsy [86].

ChD reactivation may remain completely asymptomatic, detected only by direct parasitological testing or qPCR positive results; if symptoms occur, the most common are cutaneous lesions, myocarditis, encephalitis, and other nonspecific manifestations [40, 87]. Reactivation with cutaneous manifestations has been described following kidney, heart, and liver transplantation and HSCT, with similar clinical presentation seen in other immunocompromised patients [88] (Fig. 8a–g).

ChD Reactivation in Non-heart Solid-Organ Transplantation

ChD reactivation among chronically infected recipients after kidney and liver transplantation varies from 10% to 35% [42, 43, 89–93]. The two largest case series of positive recipients were reported by Riarte et al. [42] and Balderramo et al. [94]. Riarte et al. reported 5 (22%) cases of ChD reactivation among 23 chronically infected kidney transplant recipients in Argentina [42]. The patients did not receive prophylaxis and were monitored by direct parasitological and serological tests; reactivations were detected 37 days to 29 months posttransplant. All but one patient were successfully treated before developing symptoms; one patient with asymptomatic parasitemia recovered spontaneously without treatment. Balderramo et al. reported three (33%) asymptomatic cases of ChD reactivation among nine chronically infected liver transplant recipients [94]. Similarly, the patients did not receive prophylaxis and were monitored for *T. cruzi* parasitemia by real-time qPCR. All patients were successfully treated with preemptive therapy based on positive PCR results detected at days 15, 62, and 152 posttransplantation. There are a few case reports of subsequent ChD reactivation after acute infection acquired through the transplant [42, 66].

ChD Reactivation in Heart SOT

ChD cardiomyopathy represents the third leading cause of heart transplantation in Latin America [95], and heart transplant is currently the treatment of choice for chagasic patients with end-stage heart failure. This condition is associated with a worse outcome compared to other causes of heart failure, and it is an independent predictor of waitlist mortality [85]. In a Brazilian cohort, the 100-day waitlist mortality among chagasic patients was 30% compared to 16% for non-chagasic patients with heart failure ($p = 0.002$), despite no difference in the rates of transplantation [96].

Patients with CCC should be screened for megaesophagus before heart transplantation as the presence of severe esophageal dilatation may be a relative contraindication for heart transplant; surgical correction should be considered for eligible patients [85].

After heart transplantation for CCC, a high proportion of patient present with ChD reactivation, with rates of reactivation ranging from 27% to 50% and even

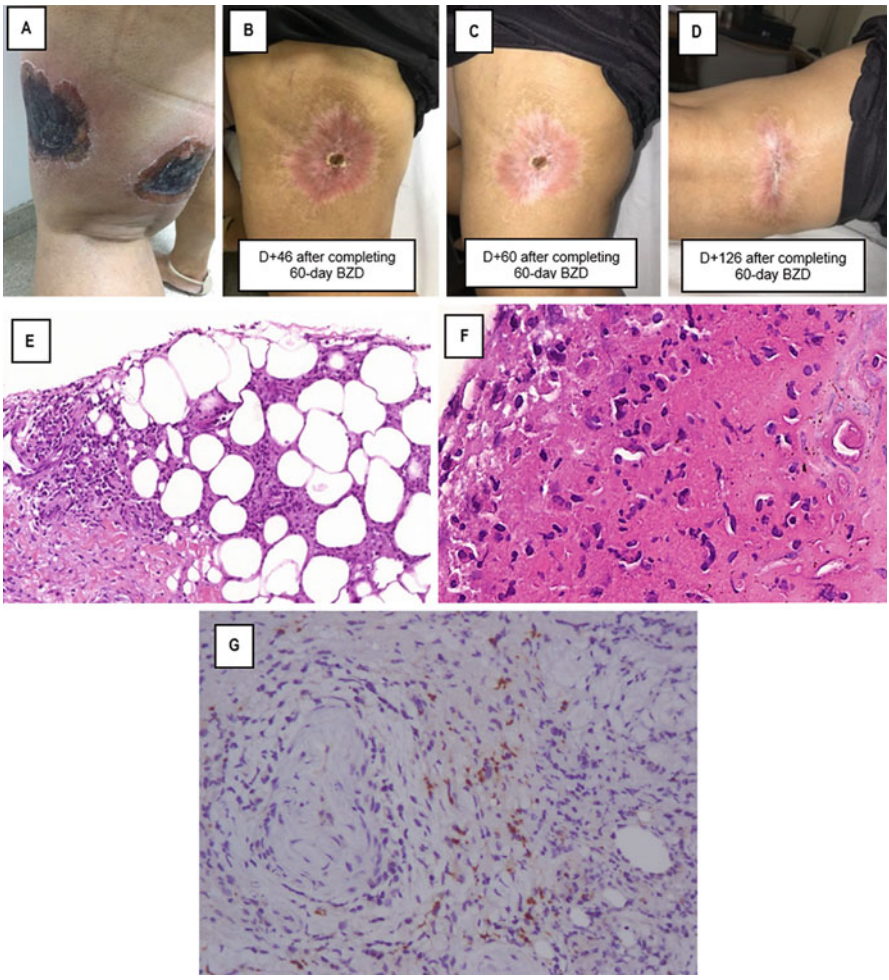


Fig. 8 Chronic chagasic 49-year-old female patient 2 months after kidney transplant. (**a**) Two left thigh eroded crusted lesions with about 4 weeks of evolution. (**b**–**d**) Clinical evolution of cutaneous lesions after completing 60-day BZD and surgical debridement. (**e**, **f**) Subcutaneous tissue showing panniculitis, pandermic macrophage inflammatory infiltrate with amastigote forms visualized on an H&E stain (x20 and x400); 10D. (**g**) Positive *T. cruzi*-immunohistochemistry (x400). (Courtesy: Lígia Camera Pierrotti – Infectious Disease Division, Hospital das Clínicas – Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil. Maria Irma Seixas Duarte – Pathology Department – Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil)

reported to be as high as 90% [55, 56, 61, 97–101]. These patients frequently present with several episodes of reactivation, ranging from one to eight episodes per patient [98], despite previous correct use of trypanocidal drugs. Nevertheless, reactivation of ChD does not impact the heart transplant outcome. Graft and patient survival rates of chagasic patients are usually similar to those without ChD [102]. The reported mortality rates associated with reactivation ChD are low, at about 0.9% [101].

The most frequent clinical manifestation of ChD among heart transplant recipients is myocarditis given the tropism of the parasite for cardiomyocytes, thus increasing the risk of graft dysfunction [101]. The patients present with symptoms of heart allograft failure similar to acute rejection, including high-degree atrioventricular block and cardiogenic shock in severe cases [103].

Among heart transplant recipients, risk factors associated with higher reactivation rates are rejection, neoplasms, and use of mycophenolate mofetil [99]. Although *T. cruzi* parasites persist in the myocardium of the native heart, and an explanted heart at transplant is usually associated with high-grade myocarditis among chronically infected patients, however, the parasite persistence and parasite load in the myocardium of the native heart explanted are not associated with the risk of ChD reactivation posttransplant [104].

ChD Reactivation in Hematopoietic Stem Cell Transplantation

Immunosuppression secondary to HSCT and treatment for graft versus host disease (GvHD) is usually a trigger for the reactivation of ChD among seropositive patients [43, 105–109]. Table 5 summarizes published case reports and series in the literature.

Altclas J et al. described a cohort of 1,328 HSCT transplant patients (441 allogeneic and 887 autologous HSCT) from 1989 to 2002 from 4 health centers in Argentina [107]. The incidence of ChD in this study was 1.9% (25 *T. cruzi*-infected patients – 12 underwent autologous and 13 allogeneic HSCT). All 25 patients were in the indeterminate phase of ChD. Positive recipients were monitored by Strout and serological methods performed weekly during the first 2 months, every 15 days during the following month, and monthly thereafter. This follow-up took place in the first 2 months after autologous HSCT and throughout the immunosuppressive period after allogeneic HSCT; with any clinical suspicion of reactivation, the protocol returned to a weekly frequency until the etiological cause was diagnosed. During the follow-up period, 6 out of 25 (24%) patients presented with reactivation of ChD characterized by high parasitemia detected by positive Strout method (2 autologous and 4 allogeneic HSCT recipients). One patient presented several skin lesions with *T. cruzi* amastigote forms in tissue.

Dictar et al. followed 28 allogeneic and 206 autologous HSCT recipients from 1989 until 1996; the incidence of ChD was 2% (5 of 234 patients) [105]. Out of five patients, only one presented with reactivation of ChD (20%) during a 7-year period of follow-up, characterized by an asymptomatic positive blood. However, in this study, positive blood culture was considered the only diagnostic criteria for ChD reactivation diagnosis. Given that intermittent and low-level parasitemia is characteristic of chronically infected individuals, a positive blood culture might not mean reactivation; hence, the case reported by Dictar et al. is considered inconclusive. The other four chagasic patients presented no clinical or laboratory evidence of reactivation.

De Novo Infection

After transplantation, de novo acquisition can occur from vector exposure or blood transfusions. Vector exposure represents a risk for patients who reside or travel to

Table 5 Chagas disease reactivation among chronically infected HSCT patients

Reference	Country of origin	Hematological condition	Protocol surveillance	Reactivation rates	Clinical manifestation	Reactivation diagnosis	Treatment	Outcome
Dictar et al. [105]	Argentina	02 NHL/Auto HSCT 01 MM/Auto HSCT 01 CML/Allo HSCT 01 AML/Allo HSCT	Yes. SM, BC, serology – up to 60 days for Auto HSCT and during period of IS for Allo HSCT	1/5 HSCT (20%) 1/3 Auto HSCT	No	Positive BC[a] at 4 days pre-HSCT	Preemptive BZD 5–8 mg/kg/day for 30 days	Survival
Altclas et al. [106]	Argentina	CML/Allo HSCT	Yes. SM and BC weekly during the first 2 months, every 15 days during the third month, and monthly while under IS	Case report	No	Positive SM	BZD 400 mg/day	Survival
Altclas et al. [107]	Argentina	13 Auto HSCT 12 Allo HSCT	Yes. Weekly by the SM and serology weekly during the first 2 months, every 15 days during the third month, and then monthly controls from then on	6/25 HSCT (24%) 4/13 Auto HSCT 2/12 Allo HSCT	1 panniculitis with *T. cruzi* amastigotes in the tissue	5 Positive SM[b] 1 Positive BC	BZD 5 mg/kg/day for 30–60 days	Survival

Riganti et al. [43]	Argentine	CML/Allo HSCT	No	Case report	Multiple panniculitis, fever and asthenia	*T. cruzi* amastigotes on skin biopsy; positive SM	Nifurtimox 600 mg/day	Death
Angheben et al. [108]	Italy	ALL/Allo HSCT	No	Case report	Fever, pancytopenia, elevated LDH and CRP and hepatomegaly	Trypomastigotes of *T. cruzi* on peripheral blood smear; increased q PCR; negative BC and QBC	BZD 10 mg/kg/day	Death
Guiang et al. [109]	El Salvador	MM/Auto HSCT	No	Case report	No	Negative BC and QBC; positive PCR[b]	BZD 5 mg/kg/day for 60 days	Death

NHL, non-Hodgkin lymphoma; Auto HSCT, autologous hematopoietic stem cell transplant; MM, multiple myeloma; CML, chronic myeloid leukemia; Allo HSCT, allogeneic hematopoietic stem cell transplant; IS, immunosuppression; AML, acute myeloid leukemia; SM, Strout method; BC, blood culture; BZD, benzonidazole; PCR, polymerase chain reaction; qPCR, quantitative PCR; QBC, quantitative buffy coat

[a]BC detects low parasitemia and it can be also positive in chronic chagasic patients; positive BC has not been considered a criterion of ChD reactivation

[b]PCR has high sensitivity, so it can also be positive in chronic chagasic patient; positive qualitative PCR has not been considered a criterion of ChD reactivation

endemic regions; these individuals should be counseled to avoid vector exposure through bites or through inadvertent oral intake of contaminated food [87]. If an exposure risk occurs, the transplant patient should be screened for acute infection through direct parasitological examination of blood and other clinical specimens when indicated. Alternatively, serological tests and qPCR should be performed in cases where direct methods examination is negative. If infection is detected, this individual should be treated for acute infection and should be monitored thereafter as a *T. cruzi* chronically infected transplant patient is at risk of reactivation.

Pretransplant Risk Assessment and Management of Donors and Recipients

Pretransplant Risk Assessment

Due to risk of donor-transmitted infection, screening tests to detect chagasic patients should be performed in all potential donors and candidates at risk for this infection. In most endemic areas, pretransplant screening of donors and candidates is mandatory [40, 87]. In non-endemic areas, screening should be considered for those at risk, including individuals who were born in endemic areas or to mothers from endemic areas, individuals who lived for a prolonged time in endemic areas, and those who received unscreened blood in the past [110–113].

Screening is based on commercially available serological tests to detect chronic infection and should follow the current international recommendation for chronic ChD diagnosis. For clinical use, a serological diagnosis is confirmed by at least two positive results with different conventional serological assays using lysates of the epimastigote form of the parasite grown in lipid culture [114]. However, a single highly sensitive test such as ELISA with recombinant antigens is acceptable to certify the suitability of blood donors [47].

In some cases, the impaired humoral immunity eventually present in donors and candidates for transplant may hamper the detection of antibodies by conventional serologic tests [59].

Acceptability Criteria of a Positive Donor

Solid-Organ Transplantation

Based on cumulative successful experience of transplantation using *T. cruzi*-infected donors and considering the shortage of transplantable organs, most of the programs and guidelines have expanded donor criteria to include the use of kidneys and livers, and probably lungs, from chronically infected donors to negative recipients [87, 93, 113, 115]. However, the decision to risk possible infection in seronegative recipients receiving organs from infected donors needs to strike a balance between

the benefits of transplantation and the risk acceptance of the possibility of recipients getting the infection; an informed consent letter by the medial team needs to be acknowledged by the recipients. The decision should take into account the availability of monitoring exams and treatment if infection occurs.

Given that the heart and intestines are potentially chronically infected with a high burden of latent *T. cruzi*, these organs from known infected donors should not be accepted.

Although rare, organs from donors with acute infection should be temporarily deferred, while those from donors who died of acute disease should be not accepted.

Hematopoietic Stem Cell Transplantation

The American Society of Hematology has also published recommendations focusing on prior testing of transplant candidates and on the fact that although a positive serology is not a contraindication for recipients, seropositive HSCT donors are not accepted [115, 116]. In exceptional circumstances, however, when no seronegative donor is available, then a seropositive donor may be considered, and the recipients from this donor should be followed appropriately [107].

Pretransplant Treatment of a Positive Donor

Infected living donors should receive antitrypanosomal treatment for 30–60 days, and donation should take place as soon as possible after completing the treatment [34, 93]. If the transplant procedure needs to take place before completing treatment, the donors should be treated for at least 14 days before the donation [34]. Although the antitrypanosomal treatment response among chronically infected patients is considered minimal, the rationale for this recommendation is to decrease parasitemia and consequently the risk of parasite transmission.

Pretransplant Treatment of a Positive Recipient

Pretransplant treatment of chronically infected recipients is not indicated, given the lack of efficacy of antitrypanosomal drugs on quiescent infections and the risk of drug toxicity. In addition, a recent study in liver transplant recipients with ChD has suggested that pretransplant treatment of parasitemic patients could postpone the reactivation episode to later after transplant [94]. Among two out of nine seropositive candidates with positive parasitemia who received pretransplant treatment, one evolved with relapse of parasitemia at 60 days posttransplant requiring retreatment. In contrast, two out of seven seropositive candidates with negative parasitemia and no pretransplant treatment presented early ChD reactivation (<15 days) posttransplant. The authors suggested that patients with pretransplant *T. cruzi* parasitemia may have a late ChD reactivation and, therefore, they need to have a longer monitoring period.

Posttransplant Management of the Recipients

Posttransplant Surveillance of Negative Recipient from Positive Donor or Positive Recipient

Posttransplant monitoring of seronegative recipients of organs from positive donors or seropositive recipients is mandatory to provide prompt diagnosis of acute or reactivation ChD and timely treatment before the development of clinically significant disease. In both groups of patients, the posttransplant surveillance should be based on both clinical and laboratory monitoring.

Although the current protocols and guidelines may differ a little between the timelines proposed, all of them have highly recommended close monitoring of the patient for the first 3–6 months, until at least 2 years posttransplant; they recommend resuming monitoring if immunosuppression is increased [34, 40, 41, 111, 115]. Laboratory surveillance must be done weekly or every other week for the first months and then monthly until at least 6 months and intensified monitoring if immunosuppression is increased. Surveillance should be based on direct parasitological tests in blood (Strout method, buffy coat) and/or molecular methods. *T. cruzi* DNA analysis by PCR has been proposed as the method of choice, since it is able to detect low parasitemia earlier in reactivation and permit prompt preemptive therapy before development of clinical symptoms, which is more often observed with high parasitemia.

For heart transplant recipients, in addition to the above, parasite monitoring should include routine immunohistochemistry on all endomyocardial biopsies to search for intracellular parasites regardless of indication for biopsy [99].

In Tables 6 and 7, we present a proposal for monitoring SOT and HSCT recipients. Moreover, we recommend additional testing in the setting of intensified immunosuppression, unexplained febrile illness, or episodes of suspected graft rejection when immunosuppression may be intensified.

Table 6 Monitoring protocol for negative recipient from *T. cruzi*-infected donor or for positive recipient post SOT

Time (days)	Clinical evaluation	QBC/ Strout	Blood smear	IgM ELISA[a]	Quantitative PCR
0				X	
15	X	X	X	X	X
30	X	X	X	X	X
60	X	X	X	X	X
90	X	X	X	X	X
180	X	X	X		X
270	X	X	X		X
360	X	X	X		X

QBC, quantitative buffy coat; PCR, polymerase chain reaction
[a]Only for negative recipient and positive donor

Table 7 Monitoring protocol for negative recipient from *T. cruzi*-infected donor or for positive recipient post HSCT

Time (days)	Clinical evaluation	QBC/ Strout	Blood smear	IgM ELISA[a]	Quantitative PCR
−7		X	X		X
0		X	X		X
7	X	X	X		X
15	X	X	X	X	X
30	X	X	X	X	X
60	X	X	X	X	X
90	X	X	X	X	X
180	X	X	X	X	X
270	X	X	X		X
360	X	X	X		X
540	X	X	X		X
720	X	X	X		X

QBC, quantitative buffy coat; PCR, polymerase chain reaction
[a]Only for negative recipient and positive donor

In transplant recipients with clinical manifestations consistent with acute or reactivation ChD, such as persistent fever for more than 7 days, with at least one or more of the following findings, pancytopenia, panniculitis, meningoencephalitis, or myocarditis, clinicians must consider a diagnosis of ChD if the donor or the recipient is from endemic areas. Histological examination of affected organs can show *T. cruzi* amastigotes. In these cases, serological testing on stored pretransplant serum can confirm the diagnosis of chronic ChD in donor and/or recipients. During the posttransplant period, serological results should be interpreted with caution. Among seronegative individuals under immunosuppression, seroconversion after acute infection may be delayed or never occur. On the other hand, negative seroconversion in chronic ChD patients on immunosuppression during the posttransplant period has been reported. Therefore, in both scenarios, false-negative serological results should be suspected when there is a high clinical probability of ChD [42].

Transplant recipients who become infected through donor-derived *T. cruzi* infection should be treated for acute infection and then managed as chronically infected ChD patients. Since there is no criteria for cure, treated patients remain at risk for further reactivation while on immunosuppression regardless of effective antiparasitic therapy.

Posttransplant Prophylaxis for Seronegative and Seropositive Recipients

Posttransplant Prophylaxis for Seronegative Recipients of Organs from Positive Donors

Data supporting the benefits of prophylaxis for seronegative recipients of organs from infected donors are not uniform. Two published Brazilian studies have shown that the use

of benznidazole prophylaxis 14–60 days posttransplant prevented donor-derived infection in seronegative kidney [92] and liver [117] recipients. However, both studies were carried out on a small number of patients (nine kidney and six liver transplant recipients), and laboratory surveillance to detect *T. cruzi* transmission was not systematically performed according to the current protocol. In fact, parasitemia surveillance was not mentioned in the study of kidney transplant recipients [92]. Other authors have reported successful transplantation of organs from positive donors to negative recipients, without parasite transmission even in the absence of prophylaxis [72, 75].

Therefore, considering the lack of prospective studies supporting posttransplant prophylaxis for seronegative recipients, potential drug toxicity, and interactions in the early posttransplant stages (see discussion in section "ChD Treatment"), in addition to the good outcomes of evidence-based surveillance and preemptive therapy, most guidelines recommend parasite monitoring and treatment if transmission occurs rather than routine prophylaxis [40, 93, 115]. The Brazilian guidelines include two possibilities: start *T. cruzi* prophylaxis for a period of 14–60 days and then proceed with serial clinical and laboratory monitoring or just monitor for *T. cruzi* without prophylaxis [34].

Posttransplant Prophylaxis for Seropositive Recipients

Current knowledge does not support universal prophylaxis for seropositive recipients to prevent ChD reactivation. This scenario has been more evident after heart transplantation where benznidazole treatment for 60 days did not prevent reactivation and treated patients experienced several episodes of reactivation in the posttransplant period [98, 99].

Posttransplant Preemptive Therapy

Preemptive posttransplant treatment for acute or reactivation ChD following laboratory evidence of ChD reactivation during monitoring is associated with good outcomes and is the best approach both for seronegative and seropositive recipients [93, 107, 111, 115].

However, chronically infected patients can have intermittent low-level parasitemia; distinguishing these individuals from those with genuine ChD reactivation based on traditional parasitological tests has been a challenge. Several studies have supported using qPCR assays to systematically monitor parasitemia after transplantation since they are highly sensitive, thus are able to provide earlier diagnosis of acute or reactivation ChD compared to parasitological methods [52, 54, 61, 62, 94].

ChD Treatment

According to current recommendations, trypanocidal treatment is indicated in the following situations: congenital transmission, infected children younger than 18 years, acute infection, laboratory accidents, and reactivation episodes in

immunocompromised individuals [95]. However, evidence supporting the benefit of treatment for chronically infected adult individuals is controversial, and the indication of trypanocidal treatment for these individuals is not at a consensus. A recent meta-analyses have concluded that there is no clear data showing the impact of treatment to avoid, stop, or revert heart pathology among chronically infected individuals [118]. Treatment of ChD beyond the transplant scenario will not be addressed in this chapter. Detailed discussion regarding this topic is available on recent publications [25, 119].

In transplant recipients, ChD treatment is strongly indicated for acute and reactivation disease. There is no data at this moment evaluating the treatment of chronic chagasic transplant patients without evidence of reactivation.

Currently, benznidazole and nifurtimox (developed more than 40 years ago) are the two specific drugs for the treatment of ChD [119]. Benznidazole is currently produced by Brazil and Argentina and largely used in Latin American countries. Nifurtimox is currently not available in many countries in Latin America, and the drug is provided for specific situations (such as suspected of resistance or serious adverse effects associated with benznidazole) through the Pan American Health Organization (PAHO)/WHO. In May 2018, benznidazole was approved by the US FDA for use in children from 2 to 12 years of age and is commercially available. Nifurtimox is not currently FDA approved and is available from the CDC Drug Service for use under an investigational protocol (Table 8).

Benznidazole is better tolerated than nifurtimox, and it has been used for the vast majority of reported cases. Alternative drugs, such as allopurinol and azole antifungals, can be used in immunocompromised patients when traditional drugs are not available, but experience with these drugs is very limited to support any recommendation [120]. Posaconazole has been used to treat chronic asymptomatic ChD, but comparative studies have showed higher treatment failure compared to benznidazole [121, 122]. There is no reported experience using this drug in transplant recipients.

In the chronic indeterminate phase, the cure rates range from 85% to 100% in children and 2% to 40% in adults. The cure rates in the acute phase are estimated to be 80–90% [25, 123].

Nifurtimox

Nifurtimox was the first developed drug against *T. cruzi*, in 1965. The drug is metabolized in the liver via cytochrome P450 and excreted preferentially by the kidneys. The mechanism of action has not been completely clarified. The drug inhibits the trypanothione reductase, a parasite-specific antioxidant defense enzyme; this deficient metabolic detoxification mechanism for oxygen is effective because *T. cruzi* is highly susceptible to oxidation and death [119].

Nifurtimox has shown more toxicity than benznidazole, and adverse effects have been reported in 43–97% of the patients, mostly characterized by anorexia, weight loss, neurological disorders (such as irritability, insomnia, disorientation, mood

Table 8 Specific available drugs to Chagas disease treatment

	Benznidazole	*Nifurtimox*
Formulation	Benznidazole (BZD; *N*-benzyl-2-nitroimidazole acetamide	Nitroheterocyclic compounds nifurtimox (NFX; 3-methyl-4-[59-nitrofurfurylideneamine] tetrahydro-4H-1,4-tiazine-1,1-dioxide)
Presentation	Tablet 100 mg	Tablet 120 mg
Adult dosing	5–7.5 mg/kg/day; recommended maximum dose 300 mg/day Up to 15 mg/kg/day in cases of meningoencephalitis	15 mg/kg/day for children and acute disease 8–10 mg/kg/day
Times per day	Two to three doses	Three to four doses
Administration	Oral	Oral
Adjust for renal function	No need	No need
Adjust for hepatic function	No need	No need
Duration of treatment	Usually 60 days For adults >60 kg, duration of treatment may be prolonged beyond the 60 days	60–90 days
Most important adverse reactions	Rash, polyneuropathy, bone marrow suppression, anorexia, nausea, vomiting	Anorexia, abdominal pain, nausea, vomiting, and weight loss
Laboratory monitoring	Baseline: complete blood cell count, hepatic enzymes, bilirubin, serum creatinine, blood urea nitrogen. While on therapy: complete blood cell count every 2–3 weeks	Baseline: complete blood cell count, hepatic enzymes, bilirubin, serum creatinine, blood urea nitrogen. While on therapy: repeat lab tests every 4–6 weeks
Drug interactions	Risk of disulfiram-like effects with concomitant use of alcohol and should be avoided Concomitant use with aspirin may increase the risk of bleeding The effect of coumarin-derived anticoagulants, such as warfarin, can be potentiated due to inhibition of its enzymatic metabolism	Alcohol increase risk of adverse effects and should be avoid Concomitant use with tacrolimus can increase tacrolimus levels
Contraindications	During pregnancy and for patients with kidney and liver failure If hypersensitivity history to the drug	

Source: Adapted from Pérez-Molina and Molina [25], de Andrade et al. [95], and Chin-Hong et al. [111]

changes, paresthesia, and peripheral neuropathy), digestive manifestation (such as nausea and vomiting), and, occasionally, fever and rash. Treatment is discontinued in up to 75% of the cases [25]. In addition, nifurtimox causes an increase in the tacrolimus levels via cytochrome P450 reductase metabolism, and strict monitoring of tacrolimus levels is recommended when both agents are used simultaneously [111].

Benznidazole

Benznidazole was developed in 1971, and it is currently the first-line treatment of ChD based on the better tolerability profile, tissue penetration, and efficacy. The drug is able to induce prompt remission of parasitemia and avoids severe complications and parasite recrudescence as observed in long-term follow-up studies [25]. The drug acts in different ways, able to induce parasite direct modification, improve phagocytosis, and increase trypanosomal death through interferon-gamma induction and inhibition of NADH-fumarate reductase [119]. Higher doses of benznidazole have been used for transplant recipients with CNS ChD involvement [46, 124].

The most frequent side effect associated with benznidazole is exanthem and urticarial dermatitis, which occur up to 30% of the patients, is not dose-dependent, and usually occurs around the 7th to 9th day of treatment. This usually responds well to antihistamines and low doses of oral corticosteroids, but if fever and lymph node enlargement occur, the drug should be promptly interrupted. Another significant side effect is polyneuropathy, which is dose-dependent and usually occurs after the 5th week of treatment. Other less frequent side effects include pain and/or tingling in the lower limbs, gastrointestinal disorders (such as nausea, vomiting, and diarrhea), and anorexia (usually much less intense that what are observed with nifurtimox). Significant leukopenia and agranulocytosis are rarely observed, but, in their presence, the treatment should be interrupted.

Immunosuppressive Management

Although there is no consensus on optimal immunosuppression during episodes of infection, the intensity of immunosuppression during acute or reactivation ChD may be evaluated and decreased wherever possible. The reported experience with heart transplantation for chagasic patients has supported the administration of lower doses of corticosteroids, adjusted cyclosporine levels (100–150 ng/ml) from the third month posttransplant, and mycophenolate-sparing regimens to reduce the risk of *T. cruzi* reactivation and rejection episodes [125]. The switch to steroid-sparing immunosuppressive regimens is not necessarily indicated.

Monitoring After Treatment and Criteria of Cure

During treatment of acute or reactivation ChD, direct parasitological tests and PCR assays should be performed at least weekly, and the treatment needs to be continued at least until two negative results are obtained [93]. Timely treatment usually has a good response with rapid decrease of parasitemia, and direct tests generally become negative after the first week of parasiticidal treatment. Afterwards, patients should be followed by clinical and laboratory methods for an indefinite period because there is no consensus for cure [95].

Although the current criteria of "definitive cure" is the persistent negative seroconversion by conventional serological tests after treatment, negative seroconversion after treatment usually takes months to several years (10–25 years) to occur, and persistently positive serology does not reveal therapeutic failure with indication of new course of therapy [25, 95, 126]. A reduction in the anti-*T. cruzi* antibody titers by conventional tests has been proposed as indicative of cure in the setting of persistently negative parasitological tests, although this criteria has not been evaluated in immunocompromised patients [95]. Therefore, treated patients remain at risk of repeated episodes of reactivation despite antitrypanosomal treatment, and parasitological tests should be periodically checked to allow early diagnosis of new episode of reactivation [32, 95]. After treatment, parasitological tests should be monitored weekly during the first 2 months, every 2 weeks through 3–6 months, and annually thereafter or at any time if increase of immunosuppression. Repeated negative parasitological assays are insufficient to ensure cure. However, positive direct parasitological tests and/or progressive increase of qPCR are unequivocal evidence of treatment failure, even in the absence of symptoms, indicating a new course of treatment.

New biomarkers including lytic antibody assays and novel serologic tests, host antigens, cytokine production, lymphocytic phenotype, and metabolic molecules are under investigation for use in clinical practice as surrogate cure [127].

Secondary Prophylaxis

Secondary prophylaxis with benznidazole at a dose of 2.5–5 mg/kg/day, orally, three times per week, is currently considered for coinfected HIV patients after standard treatment of a reactivation episode [44, 49]. There are no evidence so far, however, on the efficacy of secondary prophylaxis with benznidazole for transplant recipients, and it is not currently done for transplant recipients after antitrypanosomal treatment.

Prevention

The strategies toward ChD control have been based to promote access to diagnosis and systematic treatment for target populations, in both endemic and non-endemic countries [25]. Prevention of infection in transplant recipients is based on blood and organ donors ChD screening pre-donation [115]. There is no vaccine available for *T. cruzi* transmission prevention; however, the current knowledge about the natural history of ChD favoring the role of parasite persistence in tissues as the main driving mechanism of pathogenesis rather than the autoimmunity theory has supported the efforts to develop a vaccine [128].

Conclusion

ChD, caused by the parasite *T. cruzi*, is endemic in Latin America, and the disease has been increasingly recognized in areas where it had not traditionally occurred, including Europe and the USA. The parasite causes a serious, potentially life-threatening illness because of its ability to cause damage in host tissue. In the transplant scenario, ChD may be a consequence of acute *T. cruzi* transmission in a negative recipient from a seropositive donor or a reactivation of chronic infection in a seropositive recipient. Transmission of ChD occurs in about 10–20% of the recipients of non-heart graft and in 75% or more of the recipients of heart graft from a chronically infected donor. Reactivation of ChD occurs in about 10–35% of the chronically infected kidney and liver transplant recipients and in up to 90% of heart transplant recipients. Transplantation of the heart and intestine from a *T. cruzi*-infected donor is contraindicated; the use of other organs can be considered, and the decision to accept an organ from an infected donor is a balance between urgency of need for the organ and the acceptance of the risk of possible infection in the recipient. Recipients at risk of acute infection or reactivation should be monitored carefully with clinical and parasitological evaluation during the first 6–24 months posttransplant. Early posttransplant detection of *T. cruzi* parasitemia and treatment of acute infection and reactivation with benznidazole are associated with good outcomes in transplanted recipients.

Key Points

- Chagas disease in transplant patients involves acute infection among negative recipients receiving a graft from positive donors and reactivation episodes among positive recipients due to posttransplant immunosuppression.
- Screening should be performed in donor and recipients who were born or have resided in Chagas disease endemic areas through serological testing.
- Transplantation of the heart and intestine from a chronically *T. cruzi*-infected donor is contraindicated; the use of other organs can be considered.
- If transplantation is performed using organ from a chronically infected donor, *T. cruzi* transmission occurs in about 10–22% of kidney and liver transplant recipients and in 75% or more of heart transplant recipients from chronically infected donors.
- Reactivation of Chagas disease among *T. cruzi*-infected recipients occurs in about 10–20% of the liver and kidney and up to 90% of heart transplant recipients who are chronically infected.
- Prospective monitoring and preemptive antitrypanosomal treatment of subclinical parasitemia of the recipients at risk of transmission or reactivation rather than prophylactic treatment of candidates and recipients are indicated.
- Posttransplant monitoring for acute infection or reactivation relies on preferentially direct parasitological tests (e.g., Strout method) and qPCR of blood specimens routinely for at least 6–24 months.

- A positive result by PCR does not prove reactivation, but an increase in parasitic load measured by real-time qPCR can allow an earlier identification of reactivation parasitemia. Additional specimens and histological samples to detect parasite need to be considered in symptomatic patients.
- Treatment is based on standard courses of benznidazole or nifurtimox.
- After treatment of acute infection or reactivation episode, the patient should restart carefully monitoring with clinical and parasitological evaluation indefinite period because there is no consensus for cure. After treatment, parasitological tests should be monitored weekly during the first 2 months, every 2 weeks through 3–6 months, and annually thereafter or at any time if increase of immunosuppression.

References

1. World Health Organization (WHO). Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec. 2015 Feb 6;90(6):33–43.
2. World Health Organization (WHO). Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases. Geneva: World Health Organization, 2010 WHO/HTM/NTD/2010; 2010.
3. Schmunis GA. Epidemiology of Chagas disease in non-endemic countries: the role of international migration. Mem Inst Oswaldo Cruz [Internet]. 2007 Oct 30;102(Suppl):75–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17891282
4. Rassi A, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am [Internet]. 2012 June;26(2):275–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0891552012000116
5. Dias J, Schofield C. The evolution of Chagas disease (American trypanosomiasis) control after 90 years since Carlos Chagas discovery. Mem Inst Oswaldo Cruz [Internet]. 1999 Sept;94(Suppl 1):103–21. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02761999000700011&lng=en&tlng=en
6. Coura JR. The main sceneries of Chagas disease transmission. The vectors, blood and oral transmissions – a comprehensive review. Mem Inst Oswaldo Cruz [Internet]. 2015 May;110(3):277–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25466622
7. Moncayo A, Silveira AC. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. Mem Inst Oswaldo Cruz [Internet]. 2009 July [cited 2014 Dec 11];104(Suppl(May)):17–30. Available from: http://www.scielo.br/scielo.php?pid=S0074-02762009000900005&script=sci_arttext&tlng=ES
8. Dias JCP. Southern Cone Initiative for the elimination of domestic populations of *Triatoma infestans* and the interruption of transfusion Chagas disease: historical aspects, present situation, and perspectives. Mem Inst Oswaldo Cruz [Internet]. 2007 Aug 31;102(Suppl 1):11–8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762007000900003&lng=en&tlng=en
9. Abad-Franch F, Diotaiuti L, Gurgel-Gonçalves R, Gürtler RE. Certifying the interruption of Chagas disease transmission by native vectors: cui bono? Mem Inst Oswaldo Cruz [Internet]. 2013 Apr;108(2):251–4. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762013000200251&lng=en&tlng=en
10. Salvatella R, Irabedra P, Castellanos LG. Interruption of vector transmission by native vectors and "the art of the possible". Mem Inst Oswaldo Cruz [Internet]. 2014 Feb;109(1):122–30. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762014000100122&lng=en&tlng=en

11. Schmunis G. *Trypanosoma cruzi*, the etiologic agent of Chagas' disease: status in the blood supply in endemic and nonendemic countries. Transfusion [Internet]. 1991 July;31(6):547–57. Available from: http://doi.wiley.com/10.1046/j.1537-2995.1991.31691306255.x
12. Wendel S. Transfusion transmitted Chagas disease: is it really under control? Acta Trop [Internet]. 2010 [cited 2014 Dec 14];115(1–2):28–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20044970
13. Benjamin RJ, Stramer SL, Leiby DA, Dodd RY, Fearon M, Castro E. *Trypanosoma cruzi* infection in North America and Spain: evidence in support of transfusion transmission (CME). Transfusion [Internet]. 2012 Sept;52(9):1913–21. Available from: http://doi.wiley.com/10.1111/j.1537-2995.2011.03554.x
14. Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. Clin Infect Dis [Internet]. 2012 Mar;54(6):845–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22238161
15. Schmunis GA, Yadon ZE. Chagas disease: a Latin American health problem becoming a world health problem. Acta Trop. 2010;115(1–2):14–21.
16. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis. 2009;49(5):e52–4.
17. Rassi A, Rassi A, Marin-Neto JA. Chagas disease. Lancet. 2010;375(9723):1388–402.
18. Navarro M, Navaza B, Guionnet A, López-Vélez R. Chagas disease in Spain: need for further public health measures. Büscher P, editor. PLoS Negl Trop Dis [Internet]. 2012 Dec 27;6(12):e1962. Available from: https://dx.plos.org/10.1371/journal.pntd.0001962
19. Requena-Méndez A, Aldasoro E, de Lazzari E, Sicuri E, Brown M, Moore DAJ, et al. Prevalence of Chagas disease in Latin-American migrants living in Europe: a systematic review and meta-analysis. Rodrigues MM, editor. PLoS Negl Trop Dis [Internet]. 2015 Feb 13;9(2):e0003540. Available from: http://dx.plos.org/10.1371/journal.pntd.0003540
20. Basile L, Jansa JM, Carlier Y, Salamanca DD, Angheben A, Bartoloni A, et al. Chagas disease in European countries: the challenge of a surveillance system. Euro Surveill [Internet]. 2011 Sept 15;16(37). Available from: http://www.ncbi.nlm.nih.gov/pubmed/21944556
21. Manne-Goehler J, Reich MR, Wirtz VJ. Access to care for Chagas disease in the United States: a health systems analysis. Am J Trop Med Hyg [Internet]. 2015 July;93(1):108–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25986581
22. Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the burden of Chagas disease in the United States. Dumonteil E, editor. PLoS Negl Trop Dis [Internet]. 2016 Nov 7;10(11):e0005033. Available from: https://dx.plos.org/10.1371/journal.pntd.0005033
23. Conners EE, Vinetz JM, Weeks JR, Brouwer KC. A global systematic review of Chagas disease prevalence among migrants. Acta Trop [Internet]. 2016 Apr;156:68–78. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0001706X16300018
24. Parasitic diseases in humans transmitted by vectors – Scientific Figure on ResearchGate. [Internet]. Available from: https://www.researchgate.net/figure/Life-cycle-of-Trypanosoma-cruzi-Trypomastigotes-enter-the-host-through-the-wound-or_fig1_282655547
25. Pérez-Molina JA, Molina I. Chagas disease. Lancet [Internet]. 2018 Jan;391(10115):82–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673617316124
26. Echeverria LE, Morillo CA. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am [Internet]. 2019 Mar;33(1):119–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30712757
27. Prata A. Clinical and epidemiological aspects of Chagas disease. Lancet Infect Dis [Internet]. 2001 Sept [cited 2015 Jan 12];1(2):92–100. Available from: http://www.sciencedirect.com/science/article/pii/S1473309901000652
28. Campbell DA, Westenberger SJ, Sturm NR. The determinants of Chagas disease: connecting parasite and host genetics. Curr Mol Med [Internet]. 2004 Sept;4(6):549–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15357207
29. Zingales B, Andrade SG, Briones MRS, Campbell DA, Chiari E, Fernandes O, et al. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz [Internet]. 2009 Nov;104(7):1051–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20027478

30. Moreira OC, Ramírez JD, Velázquez E, Melo MFAD, Lima-Ferreira C, Guhl F, et al. Towards the establishment of a consensus real-time qPCR to monitor *Trypanosoma cruzi* parasitemia in patients with chronic Chagas disease cardiomyopathy: a substudy from the BENEFIT trial. Acta Trop [Internet]. 2013 Jan [cited 2014 Oct 16];125(1):23–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22982466
31. Ribeiro AL, Nunes MP, Teixeira MM, Rocha MOC. Diagnosis and management of Chagas disease and cardiomyopathy. Nat Rev Cardiol [Internet]. 2012 Oct;9(10):576–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22847166
32. Bern C. Chagas disease in the immunosuppressed host. Curr Opin Infect Dis [Internet]. 2012 Aug;25(4):450–7. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00001432-201208000-00013
33. Steverding D. The history of Chagas disease. Parasit Vectors [Internet]. 2014 July 10;7:317. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25011546
34. Dias JCP, Ramos AN Jr, Gontijo ED, Luquetti A, Shikanai-Yasuda MA, Coura JR, et al. 2nd Brazilian Consensus on Chagas disease, 2015. Rev Soc Bras Med Trop [Internet]. 2016 Dec;49:3–60. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0037-86822016000700003&lng=en&nrm=iso&tlng=en
35. Simões MV, Romano MMD, Schmidt A, Martins KSM, Marin-Neto JA. Chagas disease cardiomyopathy. Int J Cardiovasc Sci [Internet]. 2018. Available from: http://www.gnresearch.org/doi/10.5935/2359-4802.20180011
36. Freitas HFG, Chizzola PR, Paes AT, Lima ACP, Mansur AJ. Risk stratification in a Brazilian hospital-based cohort of 1220 outpatients with heart failure: role of Chagas' heart disease. Int J Cardiol [Internet]. 2005 July 10;102(2):239–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15982491
37. Rassi A, Rassi A, Little WC. Chagas' heart disease. Clin Cardiol [Internet]. 2000 Dec;23(12):883–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11129673
38. Malik LH, Singh GD, Amsterdam EA. The epidemiology, clinical manifestations, and management of Chagas heart disease. Clin Cardiol [Internet]. 2015 Sept;38(9):565–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25993972
39. Abuhab A, Trindade E, Aulicino GB, Fujii S, Bocchi EA, Bacal F. Chagas' cardiomyopathy: the economic burden of an expensive and neglected disease. Int J Cardiol [Internet]. 2013 Oct 3;168(3):2375–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23465560
40. Lattes R, Lasala MB. Chagas disease in the immunosuppressed patient. Clin Microbiol Infect [Internet]. 2014 Apr;20(4):300–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1198743X14602755
41. Kransdorf EP, Zakowski PC, Kobashigawa JA. Chagas disease in solid organ and heart transplantation. Curr Opin Infect Dis [Internet]. 2014. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25023742
42. Riarte A, Luna C, Sabatiello R, Sinagra A, Schiavelli R, De Rissio A, et al. Chagas' disease in patients with kidney transplants: 7 years of experience 1989–1996. Clin Infect Dis [Internet]. 1999 Sept [cited 2014 Dec 11];29(3):561–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10530448
43. Riganti J, Maqueda MG, Piñero MCB, Volonteri VI, Galimberti RL. Reactivation of Chagas' disease: cutaneous manifestations in two immunosuppressed patients. Int J Dermatol [Internet]. 2012 July;51(7):829–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22715827
44. Ferreira MS, Nishioka S de A, Silvestre MT, Borges AS, Nunes-Araújo FR, Rocha A. Reactivation of Chagas' disease in patients with AIDS: report of three new cases and review of the literature. Clin Infect Dis [Internet]. 1997 Dec;25(6):1397–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9431385
45. Jardim E, Takayanagui OM. Chagasic meningoencephalitis with detection of *Trypanosoma cruzi* in the cerebrospinal fluid of an immunodepressed patient. J Trop Med Hyg [Internet]. 1994 Dec;97(6):367–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7966540

46. Cicora F, Escurra V, Bibolini J, Petroni J, González I, Roberti J. Cerebral trypanosomiasis in a renal transplant recipient. Transpl Infect Dis [Internet]. 2014 Oct;16(5):813–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25040263
47. World Health Organization. WHO consultation on international biological reference preparations for Chagas diagnostic tests [Internet]. Geneva: World Health Organization; 2007 [cited 2014 Nov 17]. Available from: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:WHO+Consultation+on+International+Biological+Reference+Preparations+for+Chagas+Diagnostic+Tests#0
48. Afonso AM, Ebell MH, Tarleton RL. A systematic review of high quality diagnostic tests for Chagas disease. PLoS Negl Trop Dis [Internet]. 2012;6(11):e1881. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23145201
49. Sartori AMC, Ibrahim KY, Nunes Westphalen EV, Braz LMA, Oliveira OC, Gakiya E, et al. Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. Ann Trop Med Parasitol. 2007;101(1):31–50.
50. Schwartz BS, Paster M, Ison MG, Chin-Hong PV. Organ donor screening practices for *Trypanosoma cruzi* infection among US Organ Procurement Organizations. Am J Transplant [Internet]. 2011 Apr [cited 2014 Dec 11];11(4):848–51. Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1600-6143.2011.03436.x/full
51. Piron M, Fisa R, Casamitjana N, López-Chejade P, Puig L, Vergés M, et al. Development of a real-time PCR assay for *Trypanosoma cruzi* detection in blood samples. Acta Trop [Internet]. 2007 Sept;103(3):195–200. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17662227
52. Duffy T, Bisio M, Altcheh J, Burgos JM, Diez M, Levin MJ, et al. Accurate real-time PCR strategy for monitoring bloodstream parasitic loads in chagas disease patients. PLoS Negl Trop Dis [Internet]. 2009 Jan [cited 2014 Dec 10];3(4):e419. Available from: http://dx.plos.org/10.1371/journal.pntd.0000419.g004
53. Britto CC. Usefulness of PCR-based assays to assess drug efficacy in Chagas disease chemotherapy: value and limitations. Mem Inst Oswaldo Cruz [Internet]. 2009 July;104(Suppl):122–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19753467
54. Maldonado C, Albano S, Vettorazzi L, Salomone O, Zlocowski JC, Abiega C, et al. Using polymerase chain reaction in early diagnosis of re-activated *Trypanosoma cruzi* infection after heart transplantation. J Heart Lung Transplant [Internet]. 2004 Dec [cited 2014 Nov 21];23(12):1345–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15607662
55. Burgos JM, Diez M, Vigliano C, Bisio M, Risso M, Duffy T, et al. Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. Clin Infect Dis [Internet]. 2010 Sept 1;51(5):485–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20645859
56. Gray EB, La Hoz RM, Green JS, Vikram HR, Benedict T, Rivera H, et al. Reactivation of Chagas disease among heart transplant recipients in the United States, 2012–2016. Transpl Infect Dis [Internet]. 2018 Dec;20(6):e12996. Available from: http://doi.wiley.com/10.1111/tid.12996
57. Schijman AG, Bisio M, Orellana L, Sued M, Duffy T, Mejia Jaramillo AM, et al. International study to evaluate PCR methods for detection of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. Rodriguez A, editor. PLoS Negl Trop Dis [Internet]. 2011 Jan 11;5(1):e931. Available from: http://dx.plos.org/10.1371/journal.pntd.0000931
58. Qvarnstrom Y, Schijman AG, Veron V, Aznar C, Steurer F, da Silva AJ. Sensitive and specific detection of *Trypanosoma cruzi* DNA in clinical specimens using a multi-target real-time PCR approach. Ghedin E, editor. PLoS Negl Trop Dis [Internet]. 2012 July 3;6(7):e1689. Available from: http://dx.plos.org/10.1371/journal.pntd.0001689
59. Gomes ML, Galvao LMC, Macedo AM, Pena SDJ, Chiari E. Chagas' disease diagnosis: comparative analysis of parasitologic, molecular, and serologic methods. Am J Trop Med Hyg. 1999;60(2):205–10.

60. Sartori AMC, Neto JE, Nunes EV, Braz LMA, Caiaffa-Filho HH, Oliveira O da C, et al. *Trypanosoma cruzi* parasitemia in chronic Chagas disease: comparison between human immunodeficiency virus (HIV)-positive and HIV-negative patients. J Infect Dis [Internet]. 2002 Sept 15 [cited 2014 Dec 11];186(6):872–5. Available from: http://jid.oxfordjournals.org/content/186/6/872.short
61. Diez M, Favaloro L, Bertolotti A, Burgos JM, Vigliano C, Lastra MP, et al. Usefulness of PCR strategies for early diagnosis of Chagas' disease reactivation and treatment follow-up in heart transplantation. Am J Transplant [Internet]. 2007 June;7(6):1633–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17511688
62. Schijman AG, Vigliano C, Burgos J, Favaloro R, Perrone S, Laguens R, et al. Early diagnosis of recurrence of *Trypanosoma cruzi* infection by polymerase chain reaction after heart transplantation of a chronic Chagas' heart disease patient. J Heart Lung Transplant [Internet]. 2000 Nov [cited 2014 Dec 11];19(11):1114–7. Available from: http://www.sciencedirect.com/science/article/pii/S1053249800001686
63. Cura CI, Lattes R, Nagel C, Gimenez MJ, Blanes M, Calabuig E, et al. Early molecular diagnosis of acute Chagas disease after transplantation with organs from *Trypanosoma cruzi*-infected donors. Am J Transplant [Internet]. 2013 Dec [cited 2014 Dec 11];13(12):3253–61. Available from: http://onlinelibrary.wiley.com/doi/10.1111/ajt.12487/full
64. de Freitas VLT, da Silva SCV, Sartori AM, Bezerra RC, Westphalen EVN, Molina TD, et al. Real-time PCR in HIV/*Trypanosoma cruzi* coinfection with and without Chagas disease reactivation: association with HIV viral load and CD4 level. PLoS Negl Trop Dis [Internet]. 2011 Aug [cited 2014 Dec 11];5(8):e1277. Available from: http://www.bv.fapesp.br/pt/producao-cientifica/59461/real-time-pcr-hiv-trypanosoma/
65. Bacal F, Cp S, Pv P, Mangini S, Ai F, Ng S, et al. Transplantation for Chagas Õ disease: an overview of immunosuppression and reactivation in the last two decades. Clin Transpl. 2010;6:29–34.
66. Huprikar S, Bosserman E, Patel G, Moore A, Pinney S, Anyanwu A, et al. Donor-derived *Trypanosoma cruzi* infection in solid organ recipients in the United States, 2001–2011. Am J Transplant [Internet]. 2013 Sept [cited 2014 Dec 11];13(9):2418–25. Available from: http://onlinelibrary.wiley.com/doi/10.1111/ajt.12340/full
67. Chocair PR, Sabbaga E, Amato Neto V, Shiroma M, de Goes GM. Kidney transplantation: a new way of transmitting Chagas disease. Rev Inst Med Trop Sao Paulo [Internet]. 1981;23(6):280–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6808651
68. Ferraz AS, Figueiredo JF. Transmission of Chagas' disease through transplanted kidney: occurrence of the acute form of the disease in two recipients from the same donor. Rev Inst Med Trop Sao Paulo [Internet]. 1993;35(5):461–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8115816
69. Souza FF, Castro-E-Silva O, Marin Neto JA, Sankarankutty AK, Teixeira AC, Martinelli ALC, et al. Acute chagasic myocardiopathy after orthotopic liver transplantation with donor and recipient serologically negative for *Trypanosoma cruzi*: a case report. Transplant Proc [Internet]. 2008 Apr;40(3):875–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18455041
70. Barcán L, Luna C, Lunaó C, Clara L, Sinagra A, Valledor A, et al. Transmission of *T. cruzi* infection via liver transplantation to a nonreactive recipient for Chagas' disease. Liver Transpl [Internet]. 2005 Sept;11(9):1112–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16123968
71. Silva AE, Silva ACFL, Faleiros ACG, Guimarães CS de O, Corrêa RRM, Oliveira FA, et al. Acute Chagas' disease in postrenal transplant and treatment with benzonidazole. Ann Diagn Pathol [Internet]. 2010 June;14(3):199–203. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20471566
72. Rodriguez-Guardado A, González ML, Rodriguez M, Flores-Chavez M, Boga JA, Gascon J. *Trypanosoma cruzi* infection in a Spanish liver transplant recipient. Clin Microbiol Infect

[Internet]. 2015 July;21(7):687.e1–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25882355
73. Centers for Disease Control and Prevention (CDC). Chagas disease after organ transplantation – United States, 2001. MMWR Morb Mortal Wkly Rep [Internet]. 2002 Mar 15;51(10):210–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11922190
74. Centers for Disease Control and Prevention (CDC). Chagas disease after organ transplantation – Los Angeles, California, 2006. MMWR Morb Mortal Wkly Rep [Internet]. 2006 July 28; 55(29):798–800. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16874295
75. McCormack L, Quiñónez E, Goldaracena N, Anders M, Rodríguez V, Orozco Ganem F, et al. Liver transplantation using Chagas-infected donors in uninfected recipients: a single-center experience without prophylactic therapy. Am J Transplant [Internet]. 2012 Oct [cited 2014 Dec 11];12(10):2832–7. Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1600-6143.2012.04160.x/full
76. Corey AB, Sonetti D, Maloney JD, Montgomery SP, Rademacher BL, Taylor LJ, et al. Transmission of donor-derived *Trypanosoma cruzi* and subsequent development of Chagas disease in a lung transplant recipient. Case Rep Infect Dis [Internet]. 2017;2017:1–4. Available from: https://www.hindawi.com/journals/criid/2017/5381072/
77. Salvador F, Sánchez-Montalvá A, Sulleiro E, Berastegui C, Jauregui A, Pont T, et al. Case report: successful lung transplantation from a donor seropositive for *Trypanosoma cruzi* infection (Chagas disease) to a seronegative recipient. Am J Trop Med Hyg [Internet]. 2017 Oct;97(4):1147–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28749767
78. Casadei D. Chagas' disease and solid organ transplantation. Transplant Proc [Internet]. 2010 Nov [cited 2014 Nov 2];42(9):3354–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21094779
79. Villalba R, Fornés G, Alvarez MA, Román J, Rubio V, Fernández M, et al. Acute Chagas' disease in a recipient of a bone marrow transplant in Spain: case report. Clin Infect Dis [Internet]. 1992 Feb;14(2):594–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1554849
80. Angheben A, Boix L, Buonfrate D, Gobbi F, Bisoffi Z, Pupella S, et al. Chagas disease and transfusion medicine: a perspective from non-endemic countries. Blood Transfus [Internet]. 2015 Oct;13(4):540–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26513769
81. Castro E. Chagas' disease: lessons from routine donation testing. Transfus Med [Internet]. 2009 Feb;19(1):16–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19302451
82. Yasukawa K. Blood donor screening for *Trypanosoma cruzi* infection in Japan. Transfusion [Internet]. 2014 Mar;54(3):745–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24617589
83. Thambo S, Passalacqua W, Van Cauwelaert R, Lazcano F. Chagas' disease in patients with renal transplantation. Rev Med Chil [Internet]. 1989 Jan;117(1):18–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2518278
84. Forés R, Sanjuán I, Portero F, Ruiz E, Regidor C, López-Vélez R, et al. Chagas disease in a recipient of cord blood transplantation. Bone Marrow Transplant [Internet]. 2007 Jan; 39(2):127–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17213850
85. Benatti RD, Oliveira GH, Bacal F. Heart transplantation for Chagas cardiomyopathy. J Heart Lung Transplant [Internet]. 2017 June;36(6):597–603. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1053249817313530
86. Gomez-P CF, Mantilla-H JC, Rodriguez-Morales AJ. Fatal Chagas disease among solid-organ transplant recipients in Colombia. Open Forum Infect Dis. 2014 Jun 7;1(1):ofu032. Available from: https://doi.org/10.1093/ofid/ofu032
87. Pierrotti LC, Carvalho NB, Amorin JP, Pascual J, Kotton CN, López-Vélez R. Chagas disease recommendations for solid-organ transplant recipients and donors. Transplantation [Internet]. 2018 Feb;102:S1–7. Available from: http://insights.ovid.com/crossref?an=00007890-201802002-00001

88. Hemmige V, Tanowitz H, Sethi A. *Trypanosoma cruzi* infection: a review with emphasis on cutaneous manifestations. Int J Dermatol [Internet]. 2012 May;51(5):501–8. Available from: http://doi.wiley.com/10.1111/j.1365-4632.2011.05380.x
89. Gallerano V, Consigli J, Pereyra S, Gómez Zanni S, Danielo C, Gallerano RH, et al. Chagas' disease reactivation with skin symptoms in a patient with kidney transplant. Int J Dermatol [Internet]. 2007 June;46(6):607–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17550560
90. Nowicki MJ, Chinchilla C, Corado L, Matsuoka L, Selby R, Steurer F, et al. Prevalence of antibodies to *Trypanosoma cruzi* among solid organ donors in Southern California: a population at risk. Transplantation [Internet]. 2006 Feb 15;81(3):477–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16477238
91. Arias LF, Duque E, Ocampo C, Henao J, Zuluaga G, Varela G, et al. Detection of amastigotes of *Trypanosoma cruzi* in a kidney graft with acute dysfunction. Transplant Proc [Internet]. 2006 Apr;38(3):885–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16647498
92. Sousa A., Lobo MCS., Barbosa R., Bello V. Chagas seropositive donors in kidney transplantation. Transplant Proc [Internet]. 2004 May;36(4):868–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0041134504003859
93. Chagas' Disease Argentine Collaborative Transplant Consortium, Casadei D. Chagas' disease and solid organ transplantation. Transplant Proc [Internet]. 2010 Nov;42(9):3354–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S004113451001345X
94. Balderramo D, Bonisconti F, Alcaraz A, Giordano E, Sánchez A, Barrabino M, et al. Chagas disease and liver transplantation: experience in Argentina using real-time quantitative PCR for early detection and treatment. Transpl Infect Dis [Internet]. 2017 Dec;19(6):e12782. Available from: http://doi.wiley.com/10.1111/tid.12782
95. de Andrade JP, Marin Neto JA, de Paola AAV, Vilas-Boas F, Oliveira GMM, Bacal F, et al. I Latin American Guidelines for the diagnosis and treatment of Chagas' heart disease: executive summary. Arq Bras Cardiol [Internet]. 2011 June;96(6):434–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21789345
96. Bertolino ND, Villafanha DF, Cardinalli-Neto A, Cordeiro JA, Arcanjo MJ, Theodoropoulos TAD, et al. Prognostic impact of Chagas' disease in patients awaiting heart transplantation. J Heart Lung Transplant [Internet]. 2010 Apr;29(4):449–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20006935
97. Bacal F, Silva CP, Bocchi EA, Pires PV, Moreira LFP, Issa VS, et al. Mycophenolate mofetil increased Chagas disease reactivation in heart transplanted patients: comparison between two different protocols. Am J Transplant [Internet]. 2005 Aug;5(8):2017–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15996254
98. Fiorelli AI, Stolf NAG, Honorato R, Bocchi E, Bacal F, Uip D, et al. Later evolution after cardiac transplantation in Chagas' disease. Transplant Proc [Internet]. 2005 [cited 2014 Dec 11];37(6):2793–8. Available from: http://www.sciencedirect.com/science/article/pii/S0041134505006068
99. Campos S V, Strabelli TM V, Amato Neto V, Silva CP, Bacal F, Bocchi EA, et al. Risk factors for Chagas' disease reactivation after heart transplantation. J Heart Lung Transplant [Internet]. 2008 June [cited 2015 Jan 12];27(6):597–602. Available from: http://www.sciencedirect.com/science/article/pii/S1053249808001861
100. Godoy HL, Guerra CM, Viegas RF, Dinis RZ, Branco JN, Neto VA, et al. Infections in heart transplant recipients in Brazil: the challenge of Chagas' disease. J Heart Lung Transplant [Internet]. 2010 Mar [cited 2014 Dec 11];29(3):286–90. https://doi.org/10.1016/j.healun.2009.08.006.
101. Fiorelli AI, Santos RHB, Oliveira JL, Lourenço-Filho DD, Dias RR, Oliveira AS, et al. Heart transplantation in 107 cases of Chagas' disease. Transplant Proc [Internet]. 2011 Jan;43(1):220–4. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0041134510019615

102. Bestetti RB, Theodoropoulos TAD. Review articles. A systematic review of studies on heart transplantation for patients with end-stage Chagas' heart disease. J Card Fail [Internet]. 2009;15(3):249–55. https://doi.org/10.1016/j.cardfail.2008.10.023.
103. Kransdorf EP, Czer LSC, Luthringer DJ, Patel JK, Montgomery SP, Velleca A, et al. Heart transplantation for chagas cardiomyopathy in the United States. Am J Transplant. 2013; 13(12):3262–8.
104. Benvenuti LA, Roggério A, Nishiya AS, Campos S V., Fiorelli AI, Levi JE. *Trypanosoma cruzi* persistence in the native heart is associated with high-grade myocarditis, but not with Chagas' disease reactivation after heart transplantation. J Heart Lung Transplant [Internet]. 2014 July;33(7):698–703. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1053249814009516
105. Dictar M, Sinagra A, Verón MT, Luna C, Dengra C, De Rissio A, et al. Recipients and donors of bone marrow transplants suffering from Chagas' disease: management and preemptive therapy of parasitemia. Bone Marrow Transplant [Internet]. 1998 Feb;21(4):391–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9509974
106. Altclas J, Sinagra A, Jaimovich G, Salgueira C, Luna C, Requejo A, et al. Reactivation of chronic Chagas' disease following allogeneic bone marrow transplantation and successful pre-emptive therapy with benznidazole. Transpl Infect Dis [Internet]. 1999 June;1(2):135–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11428981
107. Altclas J, Sinagra A, Dictar M, Luna C, Verón MT, De Rissio AM, et al. Chagas disease in bone marrow transplantation: an approach to preemptive therapy. Bone Marrow Transplant [Internet]. 2005 July;36(2):123–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15908978
108. Angheben A, Giaconi E, Menconi M, Casazza G, Najajreh M, Anselmi M, et al. Reactivation of Chagas disease after a bone marrow transplant in Italy: first case report. Blood Transfus [Internet]. 2012 Oct;10(4):542–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22790268
109. Guiang KMU, Cantey P, Montgomery SP, Ailawadhi S, Qvarnstrom Y, Price T, et al. Reactivation of Chagas disease in a bone marrow transplant patient: case report and review of screening and management. Transpl Infect Dis [Internet]. 2013 Dec;15(6):E264–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24147999
110. Kotton CN, Lattes R, AST Infectious Diseases Community of Practice. Parasitic infections in solid organ transplant recipients. Am J Transplant [Internet]. 2009 Dec;9(Suppl 4):S234–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20070685
111. Chin-Hong P V, Schwartz BS, Bern C, Montgomery SP, Kontak S, Kubak B, et al. Screening and treatment of Chagas disease in organ transplant recipients in the United States: recommendations from the Chagas in transplant working group. Am J Transplant [Internet]. 2011 Apr [cited 2014 Nov 16];11(4):672–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21401868
112. Schwartz BS, Mawhorter SD. Parasitic infections in solid organ transplantation. Am J Transplant [Internet]. 2013 Mar [cited 2015 Jan 13];13(Suppl 4):280–303. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23465021
113. La Hoz RM, Morris MI. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, Babesia, Acanthamoeba, Balamuthia, and Naegleria in solid organ transplant recipients – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant [Internet]. 2019 Sept 29;33(9). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/ctr.13546
114. Otani MM, Vinelli E, Kirchhoff L V, del Pozo A, Sands A, Vercauteren G, et al. WHO comparative evaluation of serologic assays for Chagas disease. Transfusion [Internet]. 2009 June [cited 2014 Dec 23];49(6):1076–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19290995

115. Pinazo M, Miranda B, Rodríguez-Villar C, Altclas J, Brunet Serra M, García-Otero EC, et al. Recommendations for management of Chagas disease in organ and hematopoietic tissue transplantation programs in nonendemic areas. Transplant Rev (Orlando) [Internet]. 2011 July [cited 2014 Dec 11];25(3):91–101. https://doi.org/10.1016/j.trre.2010.12.002.
116. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant [Internet]. 2009 Oct;15(10):1143–238. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19747629
117. D'Albuquerque LAC, Gonzalez AM, Filho HLVN, Copstein JLM, Larrea FIS, Mansero JMP, et al. Liver transplantation from deceased donors serologically positive for Chagas disease. Am J Transplant [Internet]. 2007 Mar [cited 2014 Dec 11];7(3):680–4. Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1600-6143.2007.01663.x/full
118. Villar JC, Perez JG, Cortes OL, Riarte A, Pepper M, Marin-Neto JA, et al. Trypanocidal drugs for chronic asymptomatic *Trypanosoma cruzi* infection. In: Villar JC, editor. Cochrane database of systematic reviews [Internet]. Chichester: Wiley; 2014. p. CD003463. Available from: http://doi.wiley.com/10.1002/14651858
119. Sales Junior PA, Molina I, Fonseca Murta SM, Sánchez-Montalvá A, Salvador F, Corrêa-Oliveira R, et al. Experimental and clinical treatment of Chagas disease: a review. Am J Trop Med Hyg [Internet]. 2017 Nov 8;97(5):1289–303. Available from: http://www.ajtmh.org/content/journals/10.4269/ajtmh.16-0761
120. Almeida DR, Carvalho AC, Branco JN, Pereira AP, Correa L, Vianna P V, et al. Chagas' disease reactivation after heart transplantation: efficacy of allopurinol treatment. J Heart Lung Transplant [Internet]. 1996 Oct;15(10):988–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8913915
121. Molina I, Gómez i Prat J, Salvador F, Treviño B, Sulleiro E, Serre N, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. N Engl J Med [Internet]. 2014 May 15;370(20):1899–908. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1313122
122. Morillo CA, Waskin H, Sosa-Estani S, del Carmen Bangher M, Cuneo C, Milesi R, et al. Benznidazole and posaconazole in eliminating parasites in asymptomatic *T. Cruzi* carriers. J Am Coll Cardiol [Internet]. 2017 Feb;69(8):939–47. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0735109717301158
123. Bern C. Chagas' disease. N Engl J Med [Internet]. 2015 July 30;373(5):456–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26222561
124. Montero M, Mir M, Sulleiro E, Avalos Esquivel JL, García López E, Molina-Morant D, et al. High-dose benznidazole in a 62-year-old Bolivian kidney transplant recipient with Chagas central nervous system involvement. Int J Infect Dis [Internet]. 2019 Jan;78:103–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30391324
125. Nogueira SS, Felizardo AA, Caldas IS, Gonçalves RV, Novaes RD. Challenges of immunosuppressive and antitrypanosomal drug therapy after heart transplantation in patients with chronic Chagas disease: a systematic review of clinical recommendations. Transplant Rev [Internet]. 2018 July;32(3):157–67. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0955470X18300077
126. Machado-de-Assis GF, Silva AR, Do Bem VAL, Bahia MT, Martins-Filho OA, Dias JCP, et al. Posttherapeutic cure criteria in Chagas' disease: conventional serology followed by supplementary serological, parasitological, and molecular tests. Clin Vaccine Immunol [Internet]. 2012 Aug;19(8):1283–91. Available from: http://cvi.asm.org/lookup/doi/10.1128/CVI.00274-12

127. Requena-Méndez A, López MC, Angheben A, Izquierdo L, Ribeiro I, Pinazo M-J, et al. Evaluating Chagas disease progression and cure through blood-derived biomarkers: a systematic review. Expert Rev Anti Infect Ther [Internet]. 2013 Sept 10;11(9):957–76. Available from: http://www.tandfonline.com/doi/full/10.1586/14787210.2013.824718
128. Dumonteil E, Herrera C, Buekens P. A therapeutic preconceptional vaccine against Chagas disease: a novel indication that could reduce congenital transmission and accelerate vaccine development. PLoS Negl Trop Dis [Internet]. 2019;13(1):e0006985. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30703092

Leishmaniasis in Transplant Candidates and Recipients: Diagnosis and Management

55

Wanessa T. Clemente and Paulo H. O. Mourão

Contents

Introduction 1342
Leishmaniasis: A General Overview 1342
Visceral Leishmaniasis in Transplant Candidates and Recipients: Diagnosis and Management 1343
Disease Epidemiology 1343
Clinical Impact in Transplant (SOT and HSCT) 1344
Disease Diagnostics and Clinical Evaluation 1353
Management 1355
Conclusion 1360
Key Points 1360
Cutaneous Leishmaniasis in Transplant Candidates and Recipients: Diagnosis and Management 1360
Disease Epidemiology 1360
Clinical Impact in Transplants (SOT and HSCT) 1361
Disease Diagnostics and Clinical Evaluation 1362
Management 1365
Conclusion 1366
Key Points 1366
References 1367

W. T. Clemente (✉)
Faculty of Medicine, Departamento de Propedêutica Complementar, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil

Digestive Transplant Service, Liver Transplant Group, Instituto Alfa de Gastroenterologia do Hospital das Clínicas, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil
e-mail: wanclemente@yahoo.com.br

P. H. O. Mourão
Infection Control and Hospital Epidemiology, Laboratory Medicine, Hospital das Clínicas, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil
e-mail: phqmourao@gmail.com

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_54

Introduction

Leishmaniasis: A General Overview

Leishmania parasites can cause three different clinical presentations: localized cutaneous leishmaniasis (CL), CL with mucosal involvement (MCL), and systemic visceral leishmaniasis (VL), also called Kala-azar (Hindi for black fever) [1]. In general, the disease presentation varies depending on the species and type of host, although it is possible to have multiple combinations that may result in an atypical presentation.

More than 20 *Leishmania* species are known to be present in different endemic areas. Classically, *L. donovani* and *L. infantum* have visceral involvement; *L. tropica*, *L. aethiopica*, and *L. mexicana* complex cause CL; and *L. (Viannia) braziliensis* complex species are mainly associated with CL and MCL [2].

In a geographical context, *Leishmania* species can be divided into Old World species such as *L. major*, *L. infantum*, and *L. tropica* and New World species such as *L. amazonensis*, *L. infantum*, *L. mexicana*, *L. (V.) naiffi*, *L. (V.) braziliensis*, and *L. guyanensis*. Most Old World species commonly lead to self-limiting ulcers, whereas New World species frequently cause CL and MCL disease (which give rise to partial or total destruction of the mucous membranes of the nose, mouth, and throat) and, more rarely, diffuse and disseminated CL [1]. VL is mainly caused by *L. infantum* or *L donovani*; the parasite infects internal organs, such as the liver, spleen, and bone marrow, resulting in life-threatening diseases [3].

In the general population worldwide, the CL or MCL forms are the most prevalent manifestations of the disease comprising 75% of the total cases. It is estimated that between 600,000 and one million new cases of CL occur globally per year. Approximately 95% of CL cases occur in the Americas, the Mediterranean Basin, the Middle East, and Central Asia. On the other hand, over 90% of MCL cases are found in Bolivia, Brazil, Ethiopia, and Peru. Visceral leishmaniasis, which is considered the most serious form of the disease, is endemic in more than 80 countries and highly endemic on the Indian subcontinent and in East Africa. The global number of cases varies from 200,000 to 400,000 new cases each year, of which 90% are reported from six countries: Brazil, Ethiopia, India, Somalia, South Sudan, and Sudan. Without access to treatment, VL is fatal in more than 95% of cases within 2 years after the onset. These numbers may still be underestimated [3].

As described in several studies, contrasting with the classical presentation, some *Leishmania* species are related to more than one disease form. This occurrence points toward the finding of parasite variants linked to atypical disease manifestations and indicates genes that control disease virulence and tropism [4].

In addition, some more recently published studies have described atypical disease manifestations related to *Leishmania* variants [4–10]. In this regard, the association between *Leishmania* species and clinical outcome is an ongoing investigation, but there is evidence pointing to different parasite genotypes in relation to distinct disease forms [4].

Atypical CL presentations are sometimes difficult to diagnose and treat. A Brazilian study found that *L. braziliensis* genetic traits not commonly identified among parasites from CL are plausible risk factors for the manifestation of atypical leishmaniasis [11]. Occasionally, the precise identification of species is important mainly when the disease does not respond to drugs commonly used but readily responds to other treatment options available [11, 52].

Finally, in immunocompromised hosts, such as organ or hematopoietic stem cell transplant recipients, the number of VL cases reported is greater than that of cutaneous forms, and the reason for this difference remains unclear. Notwithstanding, species usually related to CL and MCL can provoke visceral manifestations, and conversely species usually related to VL can cause cutaneous manifestations [12].

This review of leishmaniasis in transplant candidates and recipients aims to present and discuss the most important issues concerning disease management in the transplant setting. Considering that leishmaniasis is a rare disease in the context of transplantation and that the cutaneous form has been reported even less frequently, the main focus of this manuscript will be visceral leishmaniasis.

Visceral Leishmaniasis in Transplant Candidates and Recipients: Diagnosis and Management

Disease Epidemiology

VL is caused by a protozoan from the genus *Leishmania*, which is usually transmitted to human hosts through the bite of female phlebotomine sand flies. The most important *Leishmania* species related to VL are *Leishmania donovani* and *Leishmania infantum (*syn. *L. chagasi* in Latin America), but species that cause CL (e.g., *Leishmania tropica*) can eventually visceralize, particularly in the immunocompromised host [13].

L. donovani and *L. infantum* belong to the *L. donovani* complex, and while closely related, they present some epidemiological differences. *L. donovani* shows an anthroponotic transmission mode (from human to human), while *L. infantum*, on the other hand, is zoonotic. Likewise, *L. donovani* is found only in the Old World (mainly India and East Africa) and *L. infantum* in the Mediterranean region and Latin America. In nonendemic regions, leishmaniasis cases may reflect travel and immigration patterns [3, 13] (Fig. 1).

The incidence of leishmaniasis in the general population has changed over time and is therefore difficult to estimate. According to the World Health Organization (WHO), 25,000 new cases of VL were reported in 2015, although the underreporting rate may be significant. Available data suggest that the real number of cases ranges from 0.2 to 0.4 million cases each year [2, 14].

In both solid-organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients, VL is an uncommon disease even in endemic areas. Nevertheless, transplant patients, like other immunocompromised individuals, are at an increased risk of developing disease, and presumably the frequency varies according

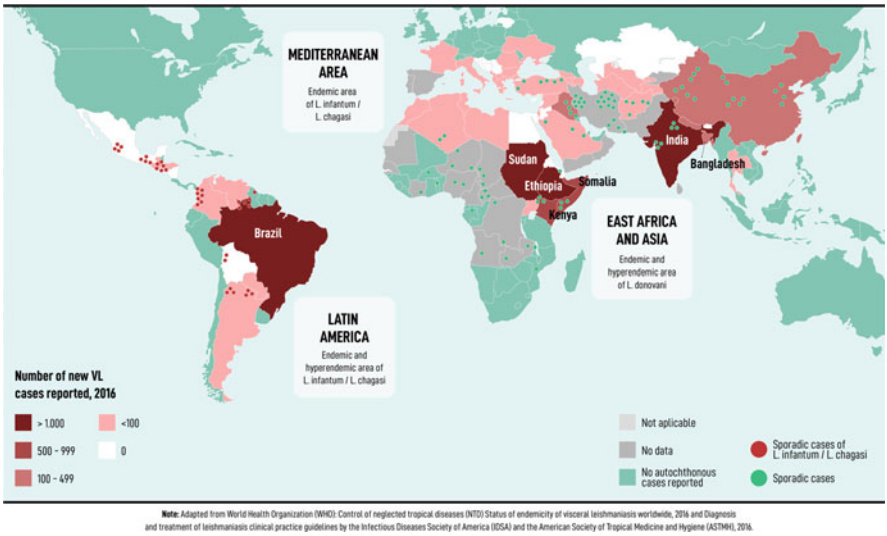


Fig. 1 Visceral leishmaniasis disease endemicity and the geographic distribution of main species

to both the prevalence of VL in the general population and the transplant type. In this scenario, VL can occur after primary infection through a sand fly bite, via transplanted organs, blood products, or consequent to reactivation of a latent infection [15, 16].

Clinical Impact in Transplant (SOT and HSCT)

Typically, VL is characterized by prolonged fever, weight loss, splenomegaly and hepatomegaly, cytopenia, hypergammaglobulinemia, and, less frequently, lymphadenopathy. In contrast, the clinical presentation in SOT recipients can be greatly altered. Fever remains the most common symptom. Unusual manifestations include post-Kala-azar dermal leishmaniasis (PKDL) and gastrointestinal tract manifestations (e.g., digestive bleeding, diarrhea) and can trigger secondary hemophagocytic lymphohistiocytosis, as presented in Table 1 [16–42] and Fig. 2. Although VL predominately affects the organs of the reticuloendothelial system, it can disseminate to other tissues, and cutaneous manifestations can occur before, during, or after VL.

In SOT recipients, the onset of symptoms may occur at any time after transplantation, although the majority of cases are diagnosed in the first 2 years post-transplant. However, this can vary depending on the transplanted organ, earlier after liver transplantation than in kidney transplant recipients, as described by Antinori et al. [43].

There are very few cases of leishmaniasis reported among HSCT recipients [44]. Thus, the limited number of cases precludes further conclusions. A recent review described a series of VL cases in both allogeneic ($N = 9$) and autologous ($N = 2$)

Table 1 Visceral leishmaniasis in transplant recipients: diagnosis, management, and outcome (2009–2018)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
36 SOT	Median: 11 months	Brazil and Spain	Fever (86%), visceromegaly (81%), and pancytopenia (47%)	Bone marrow microscopy, ~81%; Serology, ~76%	L-AmB (83.3%)	2.8% (1/36)	~26%	Clemente et al. [16]
1 KT and 1 KPT	KT: 2 years KPT: 3 years	Spain	KT: atypical presentation fever + adenopathy; no cytopenia or visceromegaly KPT: fever, pancytopenia, no visceromegaly	Lymph node biopsy and blood PCR	L-AmB (100%)	0	KPT: relapse 3 years after treatment	Sanchez et al. [17]
6 KT and 1 HT	Mean: 1.3 years	Spain	Fever: 6/7 Pancytopenia: 7/7 Splenomegaly: 7/7	Bone marrow microscopy: 7/7 Serology: 3/7	L-AmB (100%)	0	3/7	Carrasco-Antón et al. [18]
1 KT	5 years	India	Visceral and mucocutaneous nodular lesions (face and mucosa) + fever + visceromegaly + cytopenia	Bone marrow: negative Skin biopsy: + Serology: +	L-AmB	0	–	Mahesh et al. [19]
1 lung transplant	13 months	Switzerland	Persistent pancytopenia	Peripheral blood and bone marrow microscopy, whole blood PCR	L-AmB	0	–	Opota et al. [20]

(continued)

Table 1 (continued)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
1 liver transplant	3 years	Georgia	Digestive bleeding and fever; no visceromegaly or cytopenia	Cecal biopsy, bone marrow microscopy, and serology (ELISA and spot IF) Blood PCR: positive	L-AmB	0	Relapsed 1 year after treatment	Desoubeaux et al. [21]
1 liver transplant	6 months	Portugal	Fever, pancytopenia, and Splenomegaly	Serology: negative Bone marrow microscopy: positive	L-AmB	0	No relapse after 18 months of follow-up	Pereira et al. [22]
30 KT	Median: 28 months	Brazil (Piaui and Ceara states)	Fever: 70% Splenomegaly: 93% Hepatomegaly: 70%	Diagnosis method: Direct: 73% Indirect (immunological test): 27%	Amphotericin B (100%): L-AmB (93%) and deoxycholate (7%) Additionally, 23% were treated with pentavalent antimonies		Relapse (up to 6 months): 27% in patients treated with pentavalent antimonies	de Silva et al. [23]
1 HSCT	10 months	Switzerland	Pancytopenia	Bone marrow microscopy and PCR	L-AmB	–	–	Drexler and Holbro [24]
1 KT	6 years	France	Fever, asthenia, diarrhea, pancytopenia	Bone marrow microscopy, PCR, and	L-AmB poorly tolerated, treated with pentamidine Failure	0	No relapse after retreatment	Duvignaud et al. [25]

				serology: positive	after 2 months and 4 months		with L-AmB and secondary prophylaxis (pentamidine)	
1 KT	16 years	Italy	Fever, pancytopenia	Bone marrow microscopy: positive Serology: negative	L-AmB	0 (death from unrelated cause)	Relapse 5 months and 10 months after diagnosis	Pedroso et al. [26]
1 HSCT myeloma	6 years after the first HSCT	Germany	Fever, visceromegaly, pancytopenia, secondary hemophagocytic lymphohistiocytosis	Bone marrow microscopy, PCR, and serology: positive	L-AmB	0	0	Radujkovic et al. [27]
1 KT	7 years	Turkey	Fever, splenomegaly, pancytopenia, multiple skin lesions, and nasopharyngeal mucosal involvement	Bone marrow microscopy, PCR (sputum): positive Culture: negative	L-AmB	Died 4 months after treatment *A. baumannii* pneumonia	0	Yücel et al. [28]
1 liver transplant	28 months	Spain	Diarrhea, abdominal pain, visceromegaly, pancytopenia	Bone marrow microscopy and colon biopsy: positive	L-AmB	0	–	Acosta et al. [29]
1 KT	5 years	Turkey	Splenomegaly, fever, and pancytopenia	Bone marrow microscopy and culture: positive	L-AmB	0	–	Zümrütdal et al. [30]

(continued)

Table 1 (continued)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
				Serology: negative				
1 KT	8 years	Tunisia	Fever and pancytopenia	Bone marrow microscopy: positive	Pentavalent antimonial + allopurinol	0	No relapse after 1 year	Harzallah et al. [31]
1 KT	6 years	Switzerland	Fever, visceromegaly, pancytopenia, increased liver enzymes	Bone marrow microscopy, PCR, and serology: positive	L-AmB	1	Treatment failure, renal involvement, and disseminated disease	Dettwiler et al. [32]
5 KT	Mean: 18 days (5 to 32 days)	Italy	Fever: 4/5 Splenomegaly: 3/5 Renal impairment: 5/5 Cytopenia: 5/5	Bone marrow microscopy: negative ($n = 2$); not performed ($n = 3$) Serology (rK39): positive (5/5)	L-AmB	0	1/5	Veroux et al. [33]
2 KT	#1 10 years #2 7 years	Italy	#1 First episode (1996): not described; second episode (2001): nasal and colonic mucosa; third	#1 Third episode: Bone marrow microscopy: negative Peripheral blood	#1 Third episode: L-AmB + intralesional glucantime #2 L-AmB (all)	#1 Died from recurrent pneumonia and enteritis; tests for leishmaniasis	#1 2 episodes #2 4 episodes	Simon et al. [34]

			episode (2007): tongue lesion #2 First episode (2005): not described; second episode (2006): fever, purpuric skin lesions, thrombocytopenia; third episode (2007): not described; fourth episode (2008): fever, ulcers, and cytopenia; fifth episode(2010): fever and purpuric skin lesions	PCR, serology, and biopsy lesion microscopy: positive #2 Bone marrow microscopy and PCR: positive		not performed #2 Alive		
1 pancreas transplant	4 months	Spain	Fever, asthenia, diarrhea, splenomegaly, and pancytopenia	Bone marrow microscopy: positive	L-AmB	0	0	Rodriguez et al. [35]

(continued)

Table 1 (continued)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
			nasal mucosal lesion					
1 KT + series	4 years	Italy	Fever, pancytopenia, visceromegaly	Bone marrow microscopy and PCR: positive	L-AmB	0	0	Postorino et al. [36]
1 HSCT	Diagnosed at the time of the third HSCT (2014). First HSCT (2010), second (2013)	Italy	Pancytopenia	Bone marrow microscopy: positive Peripheral blood and bone marrow PCR: positive Serology: positive before and after the third HSCT	L-AmB	1	1 (2 months)	Tatarelli et al. [37]
3 HSCT	#1 15 months #2 26 months #3 5 years	Greece	#1 Fever, pancytopenia, splenomegaly #2 Fever, pancytopenia, splenomegaly, night sweats #3 Fever,	#1 Bone marrow microscopy and PCR: positive Serology: negative #2 Bone marrow microscopy: negative	L-AmB	0	2 #1 8 months #3 1 months	Komitopoulou et al. [38]

			pancytopenia, skin rush, no splenomegaly	Bone marrow PCR: positive Serology: positive #3 Bone marrow microscopy: positive Peripheral blood PCR: positive Serology: negative				
1 HSCT	5 months	Spain	Fever, rhinorrhea, odynophagia, diarrhea, splenomegaly, thrombocytopenia	Serology: positive Bone marrow microscopy and culture: positive Bone marrow PCR: negative Peripheral blood microscopy: positive	L-AmB	0	No relapse after 6 months	Bautista et al. [39]
1 HSCT	5 months	Spain (patient from Romania)	Fever, pancytopenia, no visceromegaly, ulcer on the lower lip, and oral mucosa	Bone marrow aspirate and duodenal biopsy: positive Qualitative PCR: positive	L-AmB (failure) + glucantime	1	–	Martinez-Losada et al. [40]

(continued)

Table 1 (continued)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
1 HSCT	6 months	Portugal	Thrombocytopenia	Bone marrow aspirate and PCR: positive	L-AmB	0	1 (5 months)	Brito et al. [41]
1 KT	17 months	Tunisia	Fever, pancytopenia, visceromegaly	Bone marrow microscopy and serology: positive	Glucantime® toxicity 7th day after treatment followed by L-AmB	0	0	Trabelsi et al. [42]

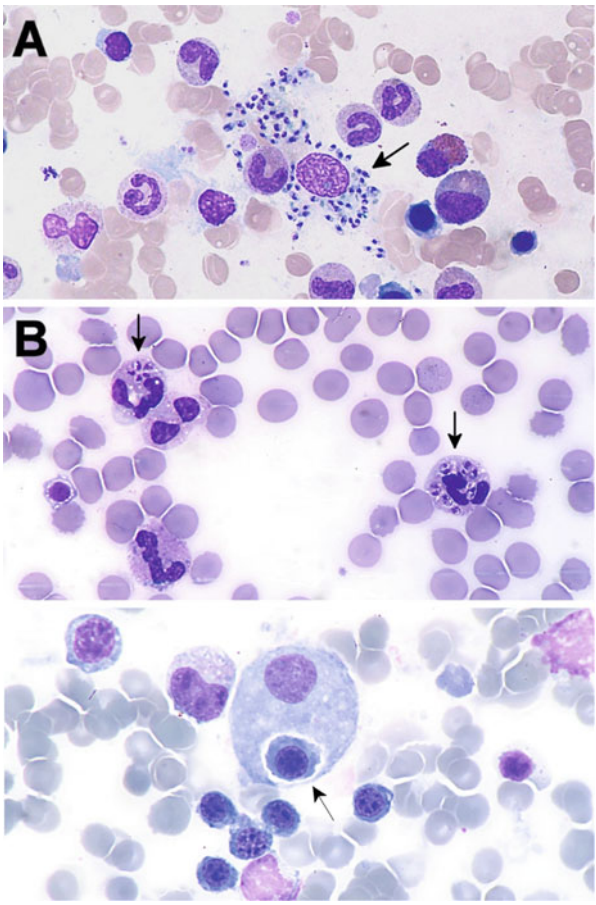


Fig. 2 Peripheral blood and bone marrow aspirate smears. (**a**) Bone marrow aspirate: numerous *Leishmania* amastigotes around the remains of a macrophage. (**b**) Peripheral blood: two neutrophils (arrows) containing phagocytized amastigotes. May-Grünwald-Giemsa stain, original magnification x 800. Hemophagocytosis: one erythroblast engulfed by macrophage (arrow) in bone marrow. Visceral leishmaniasis is one of several conditions that can trigger secondary hemophagocytic lymphohistiocytosis and should be investigate as part of the differential diagnosis in cases presenting with hemophagocytosis. May-Grünwald-Giemsa stain, original magnification x 800

HSCT recipients. The average time of disease onset was 23 weeks (0–240). In this case, relapse was seen in 50% of patients, and the overall mortality was 20% [37].

Reported VL cases in both SOT and HSCT from 2009 to 2018 are presented in Table 1.

Disease Diagnostics and Clinical Evaluation

Diagnostic confirmation is based on the demonstration of *Leishmania* parasites by microscopic examination or culture of sample tissues. A splenic aspirate has the highest sensitivity, exceeding 95%. However, there is a concern about the safety of this procedure, which may lead to life-threatening hemorrhage, particularly if performed by inexperienced personnel. Bone marrow aspirate is considered to be a safer alternative. The sensitivity ranges from 53% to 86% depending on personal expertise. Lymph node aspirates have lower sensitivity (<50%) but may be useful

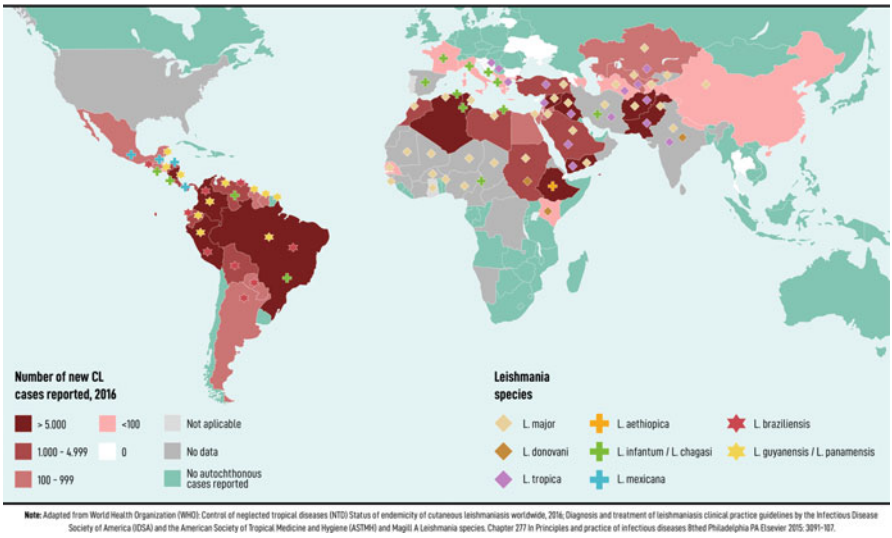
for patients with lymphadenopathy; however, the frequency of this sign varies in different geographic areas [12, 43, 45]. Peripheral blood smears have poor sensitivity (Fig. 2). In one study [46], thin smear preparations of peripheral whole blood showed sensitivity of only 1.5%. Better results were obtained with buffy coat (19.5%) or peripheral blood mononuclear cell (33.7%) smears. To date, culture isolation of splenic or bone marrow aspirates remains the reference method for parasite identification at the species level. However, culture is time-consuming, is restricted to referral research centers, and has been progressively replaced by molecular techniques [12, 45, 47].

Molecular diagnosis techniques comprise different methods, of which the PCR-based assays are the main molecular tools. Sample sites include the spleen, lymph node, bone marrow aspirates, and peripheral blood [48]. These methods present high sensitivity and specificity with both bone marrow and peripheral blood samples. According to a recent meta-analysis, whole blood PCR presents a sensitivity and specificity of 93.1% and 95.6%, respectively [49]. It is expected that conventional *Leishmania* PCR tests that use blood samples will become negative soon after the beginning of treatment and the reappearance of a positive PCR result can indicate relapse. However, asymptomatically infected individuals may have positive PCR test without any further disease development; thus, a positive PCR result does not always indicate disease [12]. In this regard, quantitative PCR may represent an important tool in early diagnosis and may indicate relapse [50].

Leishmanial antigens can be detected in serum or urine samples with a wide range of sensitivity and specificity. Methods such as the latex agglutination test (KAtex®; urine samples) present good specificity (82–100%) but low to moderate sensitivity (47–95%) [48]. The usefulness of this detection method in the transplant recipient population remains to be determined (Fig. 3).

Serological test sensitivity in SOT recipients appears to be similar to that described for immunocompetent individuals. However, serological assays in HSCT patients generally have a poor diagnostic yield, similar to that observed in HIV/AIDS patients. The most widely used serological techniques are the indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), and rapid tests, such as the immunochromatographic and direct agglutination test (DAT). Sensitivity and specificity vary according to the method, antigens used, and geographical area [12]. A systematic review with meta-analysis showed that rK39, used either in a strip or in ELISA, and the DAT are the best choices for the implementation of rapid and easy VL serodiagnosis [51]. Although rK39 is quite effective in South America and India, its use in East Africa is less satisfactory [45]. Serological limitations include the lack of agreement between methods, the inability to distinguish previous exposure from active infection, and the potential cross-reaction with other protozoa. Diagnosis based on positive serology without confirmation by direct examination should be supported by clinical correlation [12].

As suggested by van Griensven et al. [15] and recommended by a recent guideline from the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH) [52], the diagnostic strategy should be



Note: Adapted from World Health Organization (WHO): Control of neglected tropical diseases (NTD) Status of endemicity of cutaneous leishmaniasis worldwide, 2016; Diagnosis and treatment of leishmaniasis clinical practice guidelines by the Infectious Disease Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH) and Magill A Leishmania species. Chapter 277 in Principles and practice of infectious diseases 8thed Philadelphia PA Elsevier 2015: 3091-107.

Fig. 3 Cutaneous leishmaniasis disease endemicity and the geographic distribution of main species

based on a combination of methods to increase performance, including (when available) microscopy, culture, molecular, and serologic testing (Table 2) [53, 54].

Management

Risk for Donor Transmission

In light of the current knowledge, there are no precise criteria for donor screening or organ deferral [55]. Despite the high prevalence of asymptomatic infection in endemic areas, reaching up to approximately 70% depending on the test used for detection, *Leishmania* transmission through organ grafts has not been conclusively demonstrated [56–58]. A study, performed in Brazil, evaluated the risk of *Leishmania* transmission through blood transfusion. This study followed six previous seronegative patients who received blood components from seropositive donors. Two patients presented seroconversion, and one of them also had a positive PCR after the transfusion of nonleukoreduced packed red blood cells [59]. In this regard, the use of leukodepletion filters (often used with transplant patients) is considered to be an efficient method to reduce the *Leishmania* transmission hazard and may explain the relatively low rates of transfusion-transmitted leishmaniasis (TTL) [60]. According to the Pan American Health Organization (PAHO), blood donors who have had *Leishmania* infections should be permanently deferred [61]. However, compulsory screening for VL in organ donors and the deferral criteria are still not established in current international guidelines, reviews, and protocols, and there is a lack of definitive evidence that serology or molecular methods should be systematically implemented [12, 55, 62].

Table 2 Methods for visceral leishmaniasis laboratory diagnosis

Method	Comments
Direct examination	Bone marrow aspirate sensitivity varies depending on expertise of technician (53–86%) Lymph node aspirates have lower sensitivity (< 50%), and splenic aspirates have the highest sensitivity (>95%) Peripheral blood smear has poor sensitivity, varying with the preparation: peripheral whole blood shows the lowest sensitivity (1.5%) compared with those of buffy coat (19.5%) and peripheral blood mononuclear cell (33.7%) smears
Serology **Should be supported by clinical correlation**	Serological tests in SOT recipients have good performance characteristics (similar to that in immunocompetent individuals). In HSCT patients, serological assays have a poor diagnostic yield (similar to that in individuals with human immunodeficiency virus (HIV)/AIDS) Different serological techniques: indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), and rapid tests (immunochromatographic and direct agglutination test (DAT)) Serology limitations include the potential for lack of agreement between methods, the inability to distinguish previous exposure from active infection, and potential cross-reaction with other protozoa
Molecular diagnosis	Whole blood PCR has a sensitivity and specificity of 93.1% and 95.6%, respectively. However, asymptomatically infected individuals may present a positive PCR test without any further disease development; thus, a positive PCR result does not always indicate disease. Quantitative PCR may represent an important tool in early diagnosis and a tool to monitor relapse
Ag detection	Leishmanial antigens can be detected in serum or urine samples with a wide range of sensitivity and specificity. The usefulness of this detection method in transplant recipient populations remains to be determined

References: [14, 15, 53, 54]
∗If possible, use a combination of methods

For this reason, one should take into consideration that even in areas with a high prevalence of asymptomatic infection, if the screening is considered, the ideal method has not been established. Infection could be recognized by serology or PCR performed on peripheral blood, but these methods may show a poor rate of agreement [56, 60]. The PCR assay presents higher sensitivity and specificity, but nevertheless intermittent parasitemia can lead to a false-negative result. Thus, in the case of a positive serology or antigen test, or if there is a clinical suspicion, a biopsy from liver, spleen, intestine, or skin lesions is recommended [55].

Risk for the Recipient: Infection and Reactivation

Whether an infection remains asymptomatic or progresses toward VL likely results from the complex interaction between genetic susceptibility and the immune response of the host, combined with parasitic, socioeconomic, and demographic

factors. At present, it is not possible to determine among asymptomatic people those who will develop VL disease and when [62–64].

In transplant recipients on immunosuppressive treatment, as well as other immunocompromised hosts, the impaired capability of the immune response to resolve infection allows disease development or reactivation [65]. There is no apparent association between the type of transplant and the risk of developing VL. The number of reported VL cases is greater in kidney transplant recipients than in other organ transplants, but this higher prevalence could be related to the predominance of this type of transplant. Nevertheless, it has been suggested that renal insufficiency and hemodialysis may be factors that contribute to the increased risk [16]. Overall, the major risk factor for VL appears to be related to the prevalence of the disease, as suggested by two studies involving SOT patients from endemic areas [16, 66]. Additionally, in a recent outbreak of leishmaniasis in Spain, the risk of developing VL was 135 times higher in SOT patients than in immunocompetent individuals from the same area [18]. Regarding the immunosuppression (IS) regimen, the risk of VL increases with the use of high-dose steroids [16].

Treatment

VL treatment in transplant recipients requires a systemic approach, and an immunosuppression dose reduction is often advisable. However, such decisions must be made on an individual basis. The choice of therapy depends on various factors, such as patient characteristics (e.g., age, immune status, and renal/liver function), *Leishmania* species, disease extent, drug availability, concomitant infections, and previous treatments.

Currently, liposomal amphotericin B (L-AmB) is considered the drug of choice for VL. The recommended regimen is 4 mg/kg/day IV on days 1–5, 10, 17, 24, 31, and 38 (total dose of 40 mg/kg). Nevertheless, this treatment depends on the patient response, and if necessary, both the dose and duration of treatment may be increased [52]. Other lipid-associated formulations of amphotericin B are less well studied, have not been approved by the FDA for the treatment of VL, and are not generally recommended. Patients who do not respond to initial treatment with L-AmB should be treated with an alternative drug or with a longer course of L-AmB. Higher doses of L-AmB can be applied, but this is usually not necessary. Furthermore, the geographical variability in response to treatment should be considered. Amphotericin resistance has been reported, despite being very uncommon. Notwithstanding, in this situation, pentavalent antimonials or miltefosine might be considered for use alone or in combination, but this has not yet been well studied [52, 67].

Pentavalent antimonials (PAs) have been the first-line drugs for VL in many countries. Overall, they are the second most commonly used drugs for the treatment of VL in transplant patients. These drugs require long courses of administration (up to 30 days). Before treatment, patients should be evaluated for underlying conditions that contraindicate the use of antimonials (such as renal or hepatic insufficiency, arrhythmia, and the use of beta-blockers or antiarrhythmic drugs). Adverse effects include acute pancreatitis, hepatitis, arthropathy, anasarca and oliguria, renal tubular acidosis, bone marrow toxicity and electrocardiographic changes, agranulocytosis, and neurological alterations. Currently, its use is limited in the transplant setting [52, 67].

Miltefosine was approved by the Food and Drug Administration (FDA) for the treatment of leishmaniasis in 2014. It is an oral antileishmanial drug used for the treatment of visceral, mucosal, and cutaneous leishmaniasis. Miltefosine is likely to interact with membrane lipids, inhibit cytochrome c oxidase (mitochondrial function), and cause an apoptosis-like cell response. The drug is administered at 50–100 mg/day for 28 days to treat VL caused by *L. donovani* (for patients over 12 years of age, who weigh at least 30 kg and are not pregnant or breastfeeding, as miltefosine is considered to be teratogenic). Gastrointestinal disorders such as vomiting and diarrhea associated with hepatotoxicity and thrombocytopenia can be seen. There are differences in the therapeutic response based on geographical area and the *Leishmania* species involved; in addition, the evidence for the use of miltefosine for VL caused by *L. infantum* is scarce, and the drug may not be useful for monotherapy in this situation. Some studies have described the development of resistance in India, Bangladesh, and Nepal. Despite the reports of treatment failure, especially for *L. infantum* infection, miltefosine could perhaps become a good option in the future for use in combined alternative therapy or for secondary prophylaxis [52, 67–70] (Table 3).

Secondary Prophylaxis and Follow-Up

The IDSA guidelines do not recommend secondary prophylaxis for initial management in non-HIV immunocompromised patients who have not manifested a relapse (weak, low) [52]. However, the relapse rate can be high, as observed in a series of 36 SOT cases that showed a relapse rate of ~26%. In this study, secondary prophylaxis was provided to 12 patients (34.3%). Relapse was observed in 1 (8.3%) of the 12 patients receiving prophylaxis and in 8 (34.8%) of the 23 patients not receiving prophylaxis (p 0.19, Fisher's exact test). In this series, secondary prophylaxis prevented relapse, but despite decreasing the number of relapse cases, the effect was not statistically significant. This lack of significance may be related to the limited number of patients studied. Moreover, different prophylaxis schemes have been used [67]. Thus, even if secondary prophylaxis is recommended, there is no consensus about the drug or scheme. In the case of secondary prophylaxis, periodic doses of parenteral L-AmB (3–5 mg/kg every 3–4 weeks) are usually prescribed; antimonials and pentamidine are other options [16].

Miltefosine may be a promising drug, but experience with its therapeutic use is limited [67]. As of yet, this issue remains unresolved due to a lack of research [16]. Furthermore, it is possible that patients who have already had recurrence should be under close surveillance or may need suppressive treatment. In this situation, it is reasonable to extend therapy or maintain the patient on secondary prophylaxis for longer periods (3–6 months). In addition, relapse can be related to the intensity of immunosuppression rather than drug failure (if L-AmB is used for primary treatment) [67].

The curative criteria of VL are essentially clinical, and with a favorable clinical evolution, parasitological control is dispensable. The first signs of response are usually nonspecific, such as improvements in appetite and general condition. Frequently, the disappearance of fever occurs between the second and fifth days of

Table 3 Current visceral leishmaniasis treatment in SOT and HSCT recipients

Drug	Mechanism of action	Route of administration	Recommended dosage	Comments
Liposomal amphotericin B (L-AmB)	Alters the parasite's membrane permeability	Intravenous	4 mg/kg/day on days 1 through 5 and 10, 17, 24, 31, and 38 If initial treatment fails, an alternative drug or a longer course of L-AmB should be used	Treatment of choice for VL Amphotericin B lipid complex bioequivalence with L-AmB has not been established
Pentavalent antimonials	Impair cellular energetics and nucleosynthesis	Intravenous (preferred) or intramuscular	[a]20 mg pentavalent antimony/kg/day for 28 days	Severe cardiotoxicity, nephrotoxicity, pancreatitis, and hepatotoxicity Compliance issues (length of treatment and painful intramuscular administration)
Miltefosine	Interacts with lipids (phospholipids and sterols)	Oral	2–2.5 mg/kg/day for 28 days; doses >150 mg/day have not been studied	High cure rates for VL caused by *L. donovani*, not as effective for *L. infantum* Teratogenic: cannot be administered to pregnant or breastfeeding women

References: [67–71]
[a]Sodium stibogluconate equivalent to 100 mg pentavalent antimony/mL

treatment. Hematological parameters improve after the second week. Weight gain and reduced spleen and liver volume can be seen in the first few weeks, although full regression may take a few months. The process of serum protein normalization occurs slowly and may last for months [67].

Ideally, patients should receive lifelong monitoring, at least for a minimum of 1 year after treatment or until the recovery of the immune response. If there is a clinical suspicion of relapse, this must be confirmed by parasitological methods [52].

Leishmaniasis outcome among transplant recipients depends on early diagnosis and treatment. If left untreated, the disease is fatal, with death being caused by intercurrent infections or bleeding. In a series published in 2008 among 68 transplant patients, death occurred in 22% of the patients, 5 of whom had not received any antileishmanial treatment, and the diagnosis was obtained postmortem [43]. More

recent publications show a lower mortality rate in SOT recipients than that observed in previous studies, likely as a result of accumulated experience.

Conclusion

Visceral leishmaniasis in transplant recipients (SOT and HSCT) is uncommon even in endemic areas, which partially explains the lack of randomized studies that might evaluate treatment and prophylaxis strategies. To date, the current knowledge is based on a few studies, on extrapolations from investigations conducted in the general population or in other immunocompromised conditions, and on expert opinions. Patients usually present with fever and/or cytopenia, and disease should be considered in the differential diagnosis. Visceral leishmaniasis can occur after primary infection through a sand fly bite, via transplanted organs and blood products, or as a consequence of reactivation of a latent infection. At present, diagnostic confirmation is based on the demonstration of *Leishmania* parasites. However, the preferable diagnostic strategy should be based on a combination of methods to increase performance, including molecular and serologic testing when available. L-AmB is still the best choice for treatment and prophylaxis.

Key Points

- Transmission through organ grafts has not been conclusively demonstrated, and there are relatively low rates of transfusion-transmitted leishmaniasis. Despite the potential risk, the best policy for screening and deferral of organs remains unclear.
- The disease presentation can be atypical. In visceral leishmaniasis (VL), fever and cytopenia are the most common symptoms.
- A combination of diagnostic methods is the best approach for investigation.
- L-AmB is the drug of choice and is typically effective and well tolerated.
- Secondary prophylaxis may prevent relapse, but this issue remains unresolved.
- Patients should be monitored for at least 1 year after treatment or until recovery of the immune response.
- If there is a clinical suspicion of relapse, this status must be confirmed by parasitological methods.

Cutaneous Leishmaniasis in Transplant Candidates and Recipients: Diagnosis and Management

Disease Epidemiology

Cutaneous leishmaniasis (CL) is the most common leishmanial syndrome in the general population and presents a worldwide distribution. However, 70–75% of the estimated global incidence is accounted for ten countries, including Brazil,

Colombia, North Sudan, Iran, Afghanistan, Algeria, Syria, Ethiopia, Costa Rica, and Peru. In addition, nearly 90% of MCL cases occur in Bolivia, Brazil, and Peru [3].

The disease usually occurs in people from endemic areas but can affect others, such as travelers, tourists, workers, and military troops, who may also inadvertently carry the disease to nonendemic areas. Anthroponotic CL (in which a disease-causing agent carried by humans is transferred to other animals, here with humans as the major reservoir) is mainly related to *L tropica*, which has a common cluster distribution in urban and peri-urban areas. Typically, in this situation, CL is characterized by large outbreaks, especially in war and conflict zones, in refugee camps, and in settings where there is a large-scale population migration [3].

In the immunocompetent patient, the initial clinical sign of CL is the appearance of small papules and an erythematous nodule, which may be single or multiple, usually located in an exposed region of the skin and progressing to ulcers after a few months. Commonly, the skin lesions have an erythematous base, which is infiltrated and firm in consistency; well-defined, high edges; a reddish background; and coarse granules. The skin lesions of CL are usually painless and chronic and with spontaneous healing as cell-mediated immunity develops as per the usual natural history, accelerated by antileishmanial therapy [1, 52].

Although CL is a self-limited disease, in some cases, it may be severe and lead to mucosal disease or disseminated leishmaniasis. Cutaneous infections caused by *Leishmania* (*Viannia* [*V.*]) *braziliensis* and related species in *Viannia* subgenus, and, less frequently, *L. amazonensis*, in a minority of cases, are associated with concomitant or late MCL, which can cause destructive lesions of the naso-oropharyngeal/laryngeal mucosa [52].

Another form of leishmaniasis with cutaneous presentation is post-Kala-azar dermal leishmaniasis (PKDL). PKDL is a complication of VL related to *Leishmania donovani*, occurring predominantly in Africa and Asia. Typical lesions (hypopigmented or hyperpigmented macules more frequently) present with a perioral distribution, and unlike CL, the lesions are not "volcanic" in shape. They usually appear 0–6 months after VL in Sudan and 2–3 years after VL in India. This form may be neglected and easily misdiagnosed for other skin conditions, and patient history should be reviewed for a prior history of VL [3] (Table 4).

In general, atypical skin lesions may include erythematous volcanic ulcers; diffuse, eczematous, lupoid, verrucous, dry, zosteriform, and nodular lesions; or erysipeloid, sporotrichoid, annular (resembling a ringworm infection), paronychial, palmoplantar, genital chancriform, and psoriatic forms. Leishmaniasis recidivans, characterized by small nodules that develop around a healed scar, has also been reported [72–74].

Clinical Impact in Transplants (SOT and HSCT)

CL or MCL, in addition to being the most common leishmanial syndrome worldwide, is rarely reported in SOT or HSCT recipients [12, 14, 44].

The clinical presentation of CL in immunocompromised patients will often be like that in immunocompetent individuals, and atypical features include parasite

Table 4 Species related to cutaneous leishmaniasis: geographical distribution and clinical manifestations

Species	Geographical region	Clinical manifestations
Old World		
L. aethiopica	Ethiopia, Kenya, Uganda	CL and diffuse forms
L. major	India, Pakistan, Turkey, Middle East, Central Southwest Asia	CL
L. tropica	India, Pakistan, Turkey, Greece, Middle East, Central Southwest Asia, North Africa	CL and VL
L. infantum	Mediterranean, North and sub-Saharan Africa, Balkans, China	VL and localized cutaneous lesions
L. donovani	India, Pakistan, China, North Africa	VL, PKDL, and CL
New World		
L. amazonensis	Panama, Amazon basin	CL, diffuse, and visceral forms
L. mexicana	Mexico	CL and diffuse forms
L. venezuelensis	Venezuela	CL
L. infantum	Central and South America	VL, occasionally cutaneous lesions
L. (V.) braziliensis	Central and South America	CL and MCL
L. (V.) guyanensis	Amazon basin and Guyana	CL and MCL
L. (V.) panamensis	Costa Rica, Panama, Colombia, Ecuador, Peru, and Venezuela	CL and MCL
L. (V.) peruviana	Andes (Peru and Argentina)	CL

Adapted from IDSA 2016 and WHO 2019
References: [3, 52]

dissemination with multiple lesions, clinical polymorphism, and visceralization, including in species where dissemination is unlikely to happen. Patients should be evaluated to exclude systemic impairment. Nonhealing skin lesions in patients with history of travel to an endemic region should arise suspicion. Whenever possible, treatment should be based on the species identification, as the treatment varies by parasite species [12], as in Table 5 [87, 95–98].

Disease Diagnostics and Clinical Evaluation

The best diagnostic approach in CL, MCL, and PKLD is the demonstration of the parasite through microscopy or molecular methods. As the clinical spectrum of CL varies and the list of differential diagnoses is long, a biopsy of lesions should be performed to demonstrate the presence of parasites. Biopsy samples should be

Table 5 Cutaneous leishmaniasis in transplant recipients: diagnosis, management, and outcome (2009–2018)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
1 KT	12 years	Italy	Single ulcer (tongue)	Biopsy: + Serology: – BM aspirate: –	Antimonials	0	Not informed	Baglieri and Scuderi [95]
1 LT	22 years	Spain	Single ulcer (lip)	Biopsy and PCR + *L. infantum*	Antimonials	0	No recurrence	Ramos et al. [96]
1 KT	1 year	Brazil	Simultaneous CL and MCL	Skin biopsy and culture: + PCR: *L. (V.) braziliensis*	L-AmB	Death due to infectious thrombophlebitis	–	Tuon et al. [87]
1 KT	8 months	Tunisia	Single ulcerated nodule	Skin biopsy *L. major*	Intradermal antimonial (two courses) with no response and then	0	No recurrence	Yaich et al. [97]

(continued)

Table 5 (continued)

Number of cases (SOT or HSCT)	Time after transplant	Country	Clinical manifestation	Diagnosis	Treatment	Mortality	Recurrence	Reference
					allopurinol + fluconazole			
1 KT and case series (#9), KT (#7), HT (#1), LT (#1)	1 month	Iran	Nodule + multiple lesions (DCL) Leishmaniasis form and species (series) *L. mexicana*: CL (1) *L. major*: DCL (2) *L. braziliensis*: CL and VL (3) *L. infantum*: PKDL (2) + CL and VL (1)	Skin biopsy: + Serology: – Leishmanin skin test: – PCR (skin): *L. major* BM biopsy and aspirate: –	Amphotericin B Treatment (series): amphotericin (5/9), antimonials (2/9), itraconazole (1/9), amphotericin + antimonial (1/9)		No recurrence	Zandieh et al. [98]

collected based on the type of exam that will be performed, for example, PCR or histology. In CL, *Leishmania* parasites, being quite superficial, are found in the epidermis and upper levels of the dermis. In ulcerative lesions, parasites are more concentrated at the base, although there is more tissue destruction in this location. Given these factors, for PCR and culture, the collection site should be at the base of the ulcer, but for histology, the indurated border of the lesion is recommended [54, 75].

Species identification is valuable, as multiple species can coexist in each area, and the choice of treatment and patient response are species dependent. Thus, parasite isolation through culture is usually required. However, the cultures are not highly sensitive, may be contaminated with skin flora, and may be slow, and the organisms may be nonviable, limiting the use of cultures in clinical practice [1, 75]. Given the low sensitivity of parasite culture, PCR can be particularly useful since it allows species identification [1, 76, 77]. However, there is great variability in the gene targets, performance characteristics, and methods/protocols, as well as a lack of commercial availability that currently limits the routine use of molecular testing for species identification. Recently, a *Leishmania* protein detected by matrix-assisted laser desorption ionization (MALDI) time of flight (TOF) has been used for species identification, which is fast and has lower cost [75], although a positive culture is required.

Histopathological findings depend on the stage of development of the lesion, the species of *Leishmania*, the host response, and the degree of cellular immunity. Immunohistochemical examination is useful as a supplementary tool but presents limitations related to inadequate selection sites for tissue sampling and cross-reactions with other protozoa [78–81]. The Montenegro skin test has limited diagnostic utility considering false positives, as positive results may be obtained in patients without active or cicatricial lesions; furthermore, the test can result in false negatives, especially in the context of anergy [82]. In CL, serological testing is not reliable because infection provokes a poor humoral response, which results in low sensitivity by this method.

Management

The treatment of CL (CL, MCL, and PKDL) for SOT and HSCT patients is not standardized. In general, treatment decisions must be made on an individual basis, and active drugs include amphotericin B, PAs, fluconazole, miltefosine, pentamidine, and paromomycin [15, 54, 62, 83–88]. Topical treatment (cryotherapy, thermotherapy, intralesional antimonials) or surgical excision has been advocated in cases involving a limited number of small lesions. However, taking into account the risk of visceralization, systemic treatment is usually prescribed for transplant patients, and immunosuppressive dose reduction has been recommended [12, 54, 62]. Most reported cases have been successfully treated with a lipid formulation of amphotericin or antimonials.

L-AmB is used for the treatment of CL, extrapolating the efficacy for VL treatment [75]. The majority of studies conducted in the general population

L-AmB demonstrated an overall efficacy of nearly 80–85% [89–92]. Therefore, in transplant patients, L-AmB is still the systemic treatment of choice. It is worth mentioning that patients with cutaneous or mucocutaneous lesions can present with visceral involvement. However, data does not allow further strong evidence-based recommendations.

The second most commonly used drug for CL treatment, including in transplant patients, is an antimonial. However, although antimonial formulations, such as sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®), are considered the gold standard for the treatment of leishmaniasis for immunocompetent individuals in some countries, they have limitations and substantial toxicity [67, 72].

Miltefosine is an FDA-approved oral option for the treatment of CL due to several *Viannia* subgenera. The susceptibility of cutaneous *Leishmania* species to miltefosine is lower than that of *L. donovani*, and this variable efficacy is related not only to *Leishmania* species but also to geographical heterogeneity within the same species. The treatment of *L. (V.) braziliensis* has varied by geographic region, and the usefulness of this drug has yet to be defined [54, 75]. A recent meta-analysis evaluated the cure rate after miltefosine treatment (>4 weeks) in 324 PKDL patients from South Asia. The results showed an estimated 90% cure rate 1 year after treatment, although nearly 20% of the PKDL patients suffered from side effects. Miltefosine was effective, although its efficacy has been observed to decline. Based on the limited data, longer treatment regimens with miltefosine are not proven to be safe [93]. To date, no trials in transplant recipients have been published, but in a recent small cohort, miltefosine was used in six SOT patients (five kidney transplants and one lung transplant) with VL after previous relapse with L-AmB. In this study, all patients had VL due to *L. infantum*, but one patient also presented with mucocutaneous lesions that responded to miltefosine [94].

Conclusion

CL and MCL in transplant recipients (SOT and HSCT) are a very rare event, and the scarcity of studies and data does not allow further strong evidence-based conclusions. Transplant clinicians should nonetheless be aware of this significant pathogen, which could be transmissed from either the organ donor, blood donor, or stem cell graft donor or be a de novo or reactivation infection in the recipient. At present, diagnostic confirmation is based on the demonstration of *Leishmania* parasites, and species identification should be attempted. Treatment is relatively effective, although may also bring significant toxicity.

Key Points

- Leishmaniasis is a rare disease in both SOT and HSCT, and cutaneous forms are even less frequent.

- Species usually related to CL and MCL in transplant patients can provoke visceral manifestations, and conversely species usually related to VL can cause cutaneous manifestations.
- Biopsy of lesions should always be performed, and species identification must be pursued. If microscopy is negative, PCR could be used, as it has the advantage of species identification.
- Treatment decisions must be made on an individual basis, but liposomal amphotericin (L-AmB) is usually recommended as the treatment of choice.

References

1. de Vries HJ, Reedijk SH, Schallig HD. Cutaneous leishmaniasis: recent developments in diagnosis and management. Am J Clin Dermatol. 2015;16:99–109.
2. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7:e35671.
3. WHO. Leishmaniasis. Available at: http://www.who.int/leishmaniasis. Accessed May 2019.
4. Thakur L, Singh KK, Shanker V, et al. Atypical leishmaniasis: a global perspective with emphasis on the Indian subcontinent. PLoS Negl Trop Dis. 2018;12:e0006659.
5. Khanra S, Datta S, Mondal D, et al. RFLPs of ITS, ITS1 and hsp70 amplicons and sequencing of ITS1 of recent clinical isolates of Kala-azar from India and Bangladesh confirms the association of *L. tropica* with the disease. Acta Trop. 2012;124:229–34.
6. Krayter L, Bumb RA, Azmi K, et al. Multilocus microsatellite typing reveals a genetic relationship but, also, genetic differences between Indian strains of *Leishmania tropica* causing cutaneous leishmaniasis and those causing visceral leishmaniasis. Parasit Vectors. 2014;7:123.
7. Karunaweera ND, Pratlong F, Siriwardane HV, Ihalamulla RL, Dedet JP. Sri Lankan cutaneous leishmaniasis is caused by *Leishmania donovani* zymodeme MON-37. Trans R Soc Trop Med Hyg. 2003;97:380–1.
8. Sharma NL, Mahajan VK, Kanga A, et al. Localized cutaneous leishmaniasis due to *Leishmania donovani* and *Leishmania tropica*: preliminary findings of the study of 161 new cases from a new endemic focus in Himachal Pradesh, India. Am J Trop Med Hyg. 2005;72:819–24.
9. Zhang WW, Ramasamy G, McCall LI, et al. Genetic analysis of *Leishmania donovani* tropism using a naturally attenuated cutaneous strain. PLoS Pathog. 2014;10:e1004244.
10. Kumar NP, Srinivasan R, Anish TS, Nandakumar G, Jambulingam P. Cutaneous leishmaniasis caused by *Leishmania donovani* in the tribal population of the Agasthyamala Biosphere Reserve forest, Western Ghats, Kerala, India. J Med Microbiol. 2015;64:157–63.
11. Guimaraes LH, Queiroz A, Silva JA, et al. Atypical manifestations of cutaneous leishmaniasis in a region endemic for *Leishmania braziliensis*: clinical, immunological and parasitological aspects. PLoS Negl Trop Dis. 2016;10:e0005100.
12. Clemente WT, Mourao PHO, Lopez-Medrano F, Schwartz BS, Garcia-Donoso C, Torre-Cisneros J. Visceral and cutaneous leishmaniasis recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102:S8–S15.
13. Ready PD. Epidemiology of visceral leishmaniasis. Clin Epidemiol. 2014;6:147–54.
14. WHO. Control of the Leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. Geneva: WHO; 2010.
15. van Griensven J, Carrillo E, López-Vélez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect. 2014;20:286–99.
16. Clemente W, Vidal E, Girao E, et al. Risk factors, clinical features and outcomes of visceral leishmaniasis in solid-organ transplant recipients: a retrospective multicenter case-control study. Clin Microbiol Infect. 2015;21:89–95.

17. Sanchez FC, Sanchez TV, Diaz MC, Moyano VE, Gallego CJ, Marrero DH. Visceral leishmaniasis in renal transplant recipients: report of 2 cases. Transplant Proc. 2018;50:581–2.
18. Carrasco-Antón N, López-Medrano F, Fernández-Ruiz M, et al. Environmental factors as key determinants for visceral leishmaniasis in solid organ transplant recipients, Madrid, Spain. Emerg Infect Dis. 2017;23:1155–9.
19. Mahesh E, Madhyastha PR, Varma V, Gurudev K. Mucocutaneous and visceral leishmaniasis in renal transplant patient from nonendemic region. Saudi J Kidney Dis Transpl. 2016;27:1059–62.
20. Opota O, Balmpouzis Z, Berutto C, et al. Visceral leishmaniasis in a lung transplant recipient: usefulness of highly sensitive real-time polymerase chain reaction for preemptive diagnosis. Transpl Infect Dis. 2016;18:801–4.
21. Desoubeaux G, Guéry E-A, Dujardin F, et al. Digestive bleeding in a Georgian liver transplant recipient. Clin Infect Dis. 2015;60:1131–3.
22. Pereira CM, Oliveira HM, Lopes V, et al. Visceral leishmaniasis after orthotopic liver transplantation: a rare cause of infection. Transpl Infect Dis. 2016;18:251–4.
23. de Silva AA, e Silva FA, Sesso RC, et al. Epidemiologic, clinical, diagnostic and therapeutic aspects of visceral leishmaniasis in renal transplant recipients: experience from thirty cases. BMC Infect Dis. 2015;15:96.
24. Drexler B, Holbro A. Unexpected bone marrow finding in a patient with pancytopenia after hematopoietic stem cell transplantation. Blood. 2014;124:678.
25. Duvignaud A, Receveur MC, Ezzedine K, Pistone T, Malvy D. Visceral leishmaniasis due to *Leishmania infantum* in a kidney transplant recipient living in France. Travel Med Infect Dis. 2015;13:115–6.
26. Pedroso JA, Salerno MP, Spagnoletti G, et al. Elderly kidney transplant recipient with intermittent fever: a case report of leishmaniasis with acute kidney injury during liposomal amphotericin B therapy. Transplant Proc. 2014;46:2365–7.
27. Radujkovic A, Hundemer M, Eisenbach C, et al. Visceral leishmaniasis in a patient with relapsed multiple myeloma receiving high-dose melphalan and autologous stem cell transplant. Leuk Lymphoma. 2014;55:2967–9.
28. Yucel S, Ozcan D, Seckin D, Allahverdiyev AM, Kayaselcuk F, Haberal M. Visceral leishmaniasis with cutaneous dissemination in a renal transplant recipient. Eur J Dermatol. 2013;23:892–3.
29. Acosta AMV, Segura EB, Caselles AM, Madrid RB, Minano JAP, Paricio PP. Diarrhea due to visceral leishmaniasis in a liver transplant recipient. Gastroenterol Hepatol. 2013;36:271–3.
30. Zumrutdal A, Erken E, Turunc T, et al. Delayed and overlooked diagnosis of an unusual opportunistic infection in a renal transplant recipient: visceral leishmaniasis. Turkiye Parazitol Derg. 2010;34:183–5.
31. Harzallah K, Belhadj R, Jemli B, et al. Visceral leishmaniasis in a renal transplant recipient treated with allopurinol. Saudi J Kidney Dis Transpl. 2010;21:105–8.
32. Dettwiler S, McKee T, Hadaya K, Chappuis F, van Delden C, Moll S. Visceral leishmaniasis in a kidney transplant recipient: parasitic interstitial nephritis, a cause of renal dysfunction. Am J Transplant. 2010;10:1486–9.
33. Veroux M, Corona D, Giuffrida G, et al. Visceral leishmaniasis in the early post-transplant period after kidney transplantation: clinical features and therapeutic management. Transpl Infect Dis. 2010;12:387–91.
34. Simon I, Wissing KM, Del Marmol V, et al. Recurrent leishmaniasis in kidney transplant recipients: report of 2 cases and systematic review of the literature. Transpl Infect Dis. 2011;13:397–406.
35. Rodriguez NC, Navas MS, Romero SG, Molero IG, Iglesias JM. Visceral leishmaniasis in a type 1 diabetic patient with isolated pancreas transplant. Endocrinol Nutr. 2011;58:375–7.
36. Postorino MC, Bellantoni M, Catalano C, et al. Visceral leishmaniasis reactivation in transplant patients: a minireview with report of a new case. J Nephrol. 2011;24:530–4.
37. Tatarelli P, Fornaro G, del Bono V, et al. Visceral leishmaniasis in hematopoietic cell transplantation: case report and review of the literature. J Infect Chemother. 2018;24:990–4.

38. Komitopoulou A, Tzenou T, Baltadakis J, Apostolidis J, Karakasis D, Harhalakis N. Is leishmaniasis an "unusual suspect" of infection in allogeneic transplantation? Transpl Infect Dis. 2014;16:1012–8.
39. Bautista G, Ramos A, Gil S. Visceral leishmaniasis in hematopoietic stem cell transplantation. Transpl Int. 2012;25:e83–5.
40. Martinez-Losada C, Martin C, Cuenca T, Torres A. Duodenal leishmaniasis after allogeneic hematopoietic SCT. Bone Marrow Transplant. 2013;48:614–5.
41. Brito MD, Campilho F, Branca R, et al. Visceral leishmaniasis: a differential diagnosis to remember after bone marrow transplantation. Case Rep Hematol. 2014;2014:587912.
42. Trabelsi S, Bouchekoua M, Aloui D, et al. Visceral leishmaniasis in renal transplant patient. Tunis Med. 2011;89:954.
43. Antinori S, Cascio A, Parravicini C, Bianchi R, Corbellino M. Leishmaniasis among organ transplant recipients. Lancet Infect Dis. 2008;8:191–9.
44. Gajurel K, Dhakal R, Deresinski S. Leishmaniasis in solid organ and hematopoietic stem cell transplant recipients. Clin Transplant. 2017;31:e12867.
45. Sakkas H, Gartzonika C, Levidiotou S. Laboratory diagnosis of human visceral leishmaniasis. J Vector Borne Dis. 2016;53:8–16.
46. Diro E, Yansouni CP, Takele Y, et al. Diagnosis of visceral leishmaniasis using peripheral blood microscopy in Ethiopia: a prospective phase-III study of the diagnostic performance of different concentration techniques compared to tissue aspiration. Am J Trop Med Hyg. 2017; 96:190–6.
47. Perez JL, Ayats J, de Ona M, Pumarola T. The role of the clinical microbiology laboratory in solid organ transplantation programs. Enferm Infecc Microbiol Clin. 2012;30(Suppl 2):2–9.
48. Elmahallawy EK, Martinez AS, Rodriguez-Granger J, et al. Diagnosis of leishmaniasis. J Infect Dev Ctries. 2014;8:961–72.
49. de Ruiter CM, van der Veer C, Leeflang MM, Deborggraeve S, Lucas C, Adams ER. Molecular tools for diagnosis of visceral leishmaniasis: systematic review and meta-analysis of diagnostic test accuracy. J Clin Microbiol. 2014;52:3147–55.
50. Sundar S, Singh OP. Molecular diagnosis of visceral leishmaniasis. Mol Diagn Ther. 2018;22:443–57.
51. Maia Z, Lirio M, Mistro S, Mendes CM, Mehta SR, Badaro R. Comparative study of rK39 *Leishmania* antigen for serodiagnosis of visceral leishmaniasis: systematic review with meta-analysis. PLoS Negl Trop Dis. 2012;6:e1484.
52. Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the infectious diseases society of America (IDSA) and the American society of tropical medicine and hygiene (ASTMH). Clin Infect Dis. 2016;63:1539–57.
53. de Paiva-Cavalcanti M, de Morais RC, Pessoa ESR, et al. Leishmaniases diagnosis: an update on the use of immunological and molecular tools. Cell Biosci. 2015;5:31.
54. Copeland NK, Aronson NE. Leishmaniasis: treatment updates and clinical practice guidelines review. Curr Opin Infect Dis. 2015;28:426–37.
55. ECQM. Guide for the quality and safety of organs for transplantation. Strasbourg: Council of Europe; 2018.
56. Michel G, Pomares C, Ferrua B, Marty P. Importance of worldwide asymptomatic carriers of *Leishmania infantum* (L. chagasi) in human. Acta Trop. 2011;119:69–75.
57. Clemente WT, Rabello A, Faria LC, et al. High prevalence of asymptomatic *Leishmania* spp. infection among liver transplant recipients and donors from an endemic area of Brazil. Am J Transplant. 2014;14:96–101.
58. Asfaram S, Fakhar M, Mohebali M, et al. Asymptomatic human blood donors carriers of *Leishmania infantum*: potential reservoirs for visceral leishmaniasis in Northwestern Iran. Transfus Apheresis Sci. 2017;56:474–9.
59. Ferreira-Silva MM, Teixeira LAS, Tibúrcio MS, et al. Socio-epidemiological characterisation of blood donors with asymptomatic *Leishmania infantum* infection from three Brazilian endemic regions and analysis of the transfusional transmission risk of visceral leishmaniasis. Transfus Med. 2018;28:433–9.

60. Mansueto P, Seidita A, Vitale G, Cascio A. Transfusion transmitted leishmaniasis. What to do with blood donors from endemic areas? Travel Med Infect Dis. 2014;12:617–27.
61. PAHO. Eligibility for blood donation: recommendations for education and selection of prospective blood donors. Washington, DC: PAHO; 2009.
62. La Hoz RM, Morris MI. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, Babesia, Acanthamoeba, Balamuthia, and Naegleria in solid organ transplant recipients- Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33(9):e13546. https://doi.org/10.1111/ctr.13546.
63. Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic *Leishmania* infection: a new challenge for *Leishmania* control. Clin Infect Dis. 2014;58:1424–9.
64. Chakravarty J, Hasker E, Kansal S, et al. Determinants for progression from asymptomatic infection to symptomatic visceral leishmaniasis: a cohort study. PLoS Negl Trop Dis. 2019;13: e0007216.
65. Saporito L, Giammanco GM, de Grazia S, Colomba C. Visceral leishmaniasis: host–parasite interactions and clinical presentation in the immunocompetent and in the immunocompromised host. Int J Infect Dis. 2013;17:e572–6.
66. Arce A, Estirado A, Ordobas M, et al. Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. Euro Surveill. 2013;18:20546.
67. Clemente WT, Mourao PHO, Aguado JM. Current approaches to visceral leishmaniasis treatment in solid organ transplant recipients. Expert Rev Anti-Infect Ther. 2018;16:391–7.
68. Singh K, Garg G, Ali V. Current therapeutics, their problems and thiol metabolism as potential drug targets in leishmaniasis. Curr Drug Metab. 2016;17:897–919.
69. Pagliano P, Esposito S. Visceral leishmaniosis in immunocompromised host: an update and literature review. J Chemother. 2017;29:261–6.
70. Zulfiqar B, Shelper TB, Avery VM. Leishmaniasis drug discovery: recent progress and challenges in assay development. Drug Discov Today. 2017;22:1516–31.
71. Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Am J Trop Med Hyg. 2017;96:24–45.
72. Hepburn NC. Cutaneous leishmaniasis: current and future management. Expert Rev Anti-Infect Ther. 2003;1:563–70.
73. Meireles CB, Maia LC, Soares GC, et al. Atypical presentations of cutaneous leishmaniasis: a systematic review. Acta Trop. 2017;172:240–54.
74. Neitzke-Abreu HC, Venazzi MS, Scodro RBDL, et al. Cutaneous leishmaniasis with atypical clinical manifestations: case report. IDCases. 2014;1:60–2.
75. Aronson NE, Joya CA. Cutaneous leishmaniasis: updates in diagnosis and management. Infect Dis Clin N Am. 2019;33:101–17.
76. Eiras DP, Kirkman LA, Murray HW. Cutaneous leishmaniasis: current treatment practices in the USA for returning travelers. Curr Treat Options Infect Dis. 2015;7:52–62.
77. Wong SS, Fung KS, Chau S, Poon RW, Wong SC, Yuen KY. Molecular diagnosis in clinical parasitology: when and why? Exp Biol Med (Maywood). 2014;239:1443–60.
78. Shirian S, Oryan A, Hatam GR, Panahi S, Daneshbod Y. Comparison of conventional, molecular, and immunohistochemical methods in diagnosis of typical and atypical cutaneous leishmaniasis. Arch Pathol Lab Med. 2014;138:235–40.
79. Kevric I, Cappel MA, Keeling JH. New world and old world *Leishmania* infections: a practical review. Dermatol Clin. 2015;33:579–93.
80. Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Rev Anti-Infect Ther. 2010;8:419–33.
81. Alves CF, Alves CF, Figueiredo MM, et al. American tegumentary leishmaniasis: effectiveness of an immunohistochemical protocol for the detection of *Leishmania* in skin. PLoS One. 2013;8:e63343.

82. Martins AL, Barreto JA, Lauris JR, Martins AC. American tegumentary leishmaniasis: correlations among immunological, histopathological and clinical parameters. An Bras Dermatol. 2014;89:52–8.
83. Sundar S, Chakravarty J. An update on pharmacotherapy for leishmaniasis. Expert Opin Pharmacother. 2015;16:237–52.
84. Faucher B, Pomares C, Fourcade S, et al. Mucosal *Leishmania infantum* leishmaniasis: specific pattern in a multicentre survey and historical cases. J Infect. 2011;63:76–82.
85. Mortazavi H, Salehi M, Kamyab K. Reactivation of cutaneous leishmaniasis after renal transplantation: a case report. Case Rep Dermatol Med. 2014;2014:251423.
86. Aliaga L, Cobo F, Mediavilla JD, et al. Localized mucosal leishmaniasis due to *Leishmania* (Leishmania) *infantum*: clinical and microbiologic findings in 31 patients. Medicine (Baltimore). 2003;82:147–58.
87. Tuon FF, Bombonatto GM, Battaglin ER, et al. Reactivation of mucosal and cutaneous leishmaniasis in a renal transplanted patient. Am J Trop Med Hyg. 2014;91:81–3.
88. Uliana SRB, Trinconi CT, Coelho AC. Chemotherapy of leishmaniasis: present challenges. Parasitology. 2018;145:464–80.
89. Mosimann V, Neumayr A, Paris DH, Blum J. Liposomal amphotericin B treatment of old World cutaneous and mucosal leishmaniasis: a literature review. Acta Trop. 2018;182:246–50.
90. Wortmann G, Zapor M, Ressner R, et al. Lipsosomal amphotericin B for treatment of cutaneous leishmaniasis. Am J Trop Med Hyg. 2010;83:1028–33.
91. Solomon M, Pavlotzky F, Barzilai A, Schwartz E. Liposomal amphotericin B in comparison to sodium stibogluconate for *Leishmania braziliensis* cutaneous leishmaniasis in travelers. J Am Acad Dermatol. 2013;68:284–9.
92. Guery R, Henry B, Martin-Blondel G, et al. Liposomal amphotericin B in travelers with cutaneous and muco-cutaneous leishmaniasis: not a panacea. PLoS Negl Trop Dis. 2017;11:e0006094.
93. Pijpers J, den Boer ML, Essink DR, Ritmeijer K. The safety and efficacy of miltefosine in the long-term treatment of post-kala-azar dermal leishmaniasis in South Asia – a review and meta-analysis. PLoS Negl Trop Dis. 2019;13:e0007173.
94. Asin MAPJ, Carrasco-Anton N, Fernandez-Ruiz M, et al. Experience with miltefosine for persistent or relapsing visceral leishmaniasis in solid organ transplant recipients: a case series from Spain. Transpl Infect Dis. 2017; 19. https://doi.org/10.1111/tid.12623.
95. Baglieri F, Scuderi G. A case of mucosal leishmaniasis of the tongue in a kidney transplant recipient. Int J Dermatol. 2012;51(5):597–00. https://doi.org/10.1111/j.1365-4632.2011.04981.x.
96. Ramos A, Múñez E, García-Domínguez J, et al. Mucosal leishmaniasis mimicking squamous cell carcinoma in a liver transplant recipient. Transpl Infect Dis. 2015;17:488–92.
97. Yaich S, Charfeddine K, Masmoudi A, et al. Atypical presentation of cutaneous leishmaniasis in a renal transplant recipient successfully treated with allopurinol and fluconazole. Ann Saudi Med. 2013;33:187–91.
98. Zandieh A, Zandieh B, Dastgheib L. Dissemination of localized cutaneous leishmaniasis in an organ transplant recipient: case report and literature review. Int J Dermatol. 2013;52:59–62.

Malaria in Transplant Candidates and Recipients: Diagnosis and Management

56

Pilar Martín-Dávila, Francesca F. Norman, and Rogelio López-Vélez

Contents

Introduction 1373
Clinical Impact 1374
Donor-Derived Malaria and Solid Organ Transplantation 1375
Malaria and Hematopoietic Stem Cell Transplantation (HSCT) 1378
Screening and Diagnosis 1384
Treatment 1385
Prevention in Travelers 1386
Recommendations in the Transplantation Setting 1386
Infected Donors 1387
Key Points 1387
References 1392

Introduction

The majority of human malaria cases are caused by four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. *P. knowlesi* also causes disease, although is considered principally a zoonotic malaria. Malaria occurs in tropical and subtropical areas, at altitudes below 1500 m, coinciding with the geographical distribution of the mosquito vector (various species of *Anopheles*). *P. falciparum* is the predominant species worldwide and is distributed mainly in areas of Africa, Southeast Asia, India, South America, and the Caribbean. *P. vivax* is distributed mainly in

P. Martín-Dávila (✉)
Transplant Infection Unit, Infectious Diseases Department, Ramón y Cajal University Hospital, Madrid, Spain
e-mail: pmartin.hrc@salud.madrid.org

F. F. Norman · R. López-Vélez
National Referral Unit for Tropical Diseases, Infectious Diseases Department, Ramón y Cajal University Hospital, Madrid, Spain
e-mail: fnorman@gmail.com; rogelio.lopezvelez@salud.madrid.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_55

Central America, South America, Asia, and the Middle East, while *P. ovale* occurs mainly in West Africa. *P. malariae* is widely distributed in tropical areas worldwide but is less prevalent. *P. knowlesi* is currently limited to areas of Southeast Asia.

Plasmodium species have two main phases in their life cycle: an asexual replicative phase in humans and a phase of sexual replication in the mosquito. The malaria-infected female mosquito inoculates sporozoites during a blood meal. The latter infect hepatocytes and then mature into schizonts which rupture to release merozoites. In the case of *P. vivax* and *P. ovale*, a proportion (hypnozoites) remain dormant inside the hepatocytes, and these forms would be responsible for future relapses.

Symptoms and signs are caused by the asexual erythrocytic stage of *Plasmodium* which involves the invasion of red blood cells by merozoites and replication over 2–3 days, and trophozoites mature into schizonts which rupture with reinvasion of normal erythrocytes by merozoites. Some parasites differentiate into gametocytes (sexual erythrocytic stages), which may be ingested by an Anopheles mosquito, thus completing the life cycle [1].

Increases in travel and migration have contributed to the global emergence of certain geographically restricted parasitic diseases such as malaria. Those at risk for acquiring malaria are people living or traveling in a geographical area where locally acquired malaria cases have occurred in the past 3 years. Special groups at risk include those travelers living in western countries that visit their friends and relatives (VFRs) in endemic areas. Travelers and migrants may import a variety of pathogens, but dissemination in non-endemic areas is usually limited as environmental conditions may be unfavorable and intermediate hosts and vectors may not be present. However, migrants with subclinical malaria may remain asymptomatic up to several years after arrival in the host country [2].

In the context of transplantation, malaria infections may occur as a consequence of a donor-derived infection (transfusion or transplant associated), a relapse of previous *P. vivax* or *P. ovale* infection if the recipient has traveled or lived in endemic areas, or due to newly acquired infection in endemic areas posttransplantation [3]. There is no evidence that the prevalence of arthropod-borne diseases is more common in immunocompromised hosts than in other groups of travelers. Malaria incidence is not increased in immunocompromised patients compared to the healthy population living in endemic areas [4].

See Box 1 for definitions according to the WHO malaria terminology [5].

Clinical Impact

Posttransplant malaria is a rare event, even in endemic areas. One reason may be that areas with greater malaria risk may not have the resources to support large transplant programs [6]. Donor-derived transmission of geographically restricted infections appears to be rare and has been estimated to occur in less than 1% of all transplant procedures [7, 8]; malaria would comprise only a small amount of that.

The incubation period (period between inoculation of malaria parasites and onset of clinical symptoms) ranges from 7 days for *P. falciparum* to 23 days for *P. malariae* malaria. The long incubation period for *P. vivax* and *P. ovale* malaria, from 3 weeks to 1 year, and rarely many years, is due to activation of hypnozoites. Clinicians must bear in mind that the incubation period may be much shorter (depending on the inoculum) in induced infections (produced through transfusion or transplantation from an infected donor). Hypnozoites only develop in vector-borne disease and are not produced in induced *P. vivax or P. ovale* malaria cases although relapses are possible if the liver from a donor infected with these species is transplanted.

Posttransplant malaria can be an insidious disease without the typical periodicity of fever and may be difficult to diagnose if there is no clinical suspicion. A high blood parasitemia, associated with an increased risk of severe malaria (malaria with signs of severe illness and/or evidence of vital organ dysfunction), has been reported in immunosuppressed patients. Severe malaria in SOT patients is mainly produced by *P. falciparum* malaria, but *P. vivax* may result in severe cases [9]. Risk of complicated malaria is significantly higher in patients over the age of 60 years than in younger patients. Respiratory distress with the need for mechanical ventilation is significantly associated with the risk of death [10]. Malaria appears to be associated with more severe outcomes in immunocompromised patients compared with the general population. Severe malaria associated with unfavorable outcomes and increased mortality in SOT recipients has been reported. Malaria can also be more severe in splenectomized patients due to the lack of clearance of intra-erythrocytic parasites. Other concomitant infections may also hamper the outcome [11–13].

Donor-Derived Malaria and Solid Organ Transplantation

Around 50 cases of donor-derived malaria post-solid organ transplantation (SOT) have been published, the majority in renal transplant recipients [8], although in some of the cases, transmission through infected blood products could not be excluded [6]. Transmission from donor products, tissues, or organs (due to infected red blood cells within the transplanted organ or *P. vivax*/*P. ovale* hypnozoites with liver transplantation) may occur, as *Plasmodium* spp. can survive at 4 °C for several days and cold preservation does not necessarily prevent transmission [14].

The first cases of possible donor-derived malaria were reported in the late 1970s/early 1980s [15–17], and the first case of donor-derived multiorgan transmission of malaria was reported in 1985 [18]. At least eight reports of multiorgan transmission of malaria have been published [11, 18–23]. Although rare cases of hospital-acquired malaria may occur, in these reports donor-to-host transmission via the graft may be considered confirmed [24]. Data from these cases are summarized in Table 1. Of note, initial screening of the donors prior to organ procurement was not done or was negative using microscopy in some instances. In some cases, transmission was only documented in some of the recipients, and other organ

Table 1 Cases of donor-derived organ transplant transmission of malaria. Adapted from [23]

Author (year, country of diagnosis) Donor's country of origin/travel *Plasmodium* species Malaria screening in donor	Organ transplant type	Presentation (days posttransplantation) Diagnosis Treatment Outcome
Holzer et al., 1985 (Switzerland) Zaire (recent immigration) *P. vivax* Blood smears negative	Renal transplant	Fever on day 9 Blood smears positive on day 25 Chloroquine Follow-up smears negative (persistent fever due to CMV) Outcome not specified
	Renal transplant	Fever on day 16 Blood smears positive on day 25 Chloroquine Favorable outcome
Babinet et al., 1991 (France) Cameroon (recent immigration) *P. falciparum* Retrospectively: positive serology and antigen	Heart transplant	Fever on day 12 Blood smears positive on day 18 Quinine Died on day 22
	Renal transplant	Died on day 45 due to CMV Retrospectively: weakly positive antigen on day 30
Fischer et al., 1999 (Germany) Cameroon (recent immigration) *P. vivax* Retrospectively: positive serology	Liver transplant	Fever on day 28 Blood smears positive Chloroquine + primaquine Died after 6 months due to progressive liver failure
	Renal transplant	Fever on day 42 Blood smears positive Mefloquine Favorable outcome
	Heart transplant	Asymptomatic Positive serology No treatment Favorable outcome
Chiche et al., 2003 (France) France (recent travel to Togo) *P. falciparum* Retrospectively: positive blood smear and serology	Liver transplant	Fever, coma, cholestasis on day 8 Blood smears positive Quinine + doxycycline Favorable outcome
	Heart transplant	Fever, low consciousness, acute renal failure on day 5 Blood smears positive Treatment not specified Died due to multiorgan failure on day 17
	Renal transplant	Asymptomatic Blood smear positive Treatment not specified Favorable outcome

(continued)

Table 1 (continued)

Author (year, country of diagnosis) Donor's country of origin/travel *Plasmodium* species Malaria screening in donor	Organ transplant type	Presentation (days posttransplantation) Diagnosis Treatment Outcome
Menichetti et al., 2006 (Italy) Ghana (recent travel to Ghana) *P. falciparum* Screening not done	Liver transplant	Fever on day 3 Blood smears positive on day 30 Quinine + doxycycline Favorable outcome
	Renal transplant	Asymptomatic Blood smear positive Mefloquine Favorable outcome
Rodríguez et al., 2007 (Spain) Bolivia (recent immigration from Colombia, treated for malaria 3 years previously) *P. vivax* Thick blood smear negative	Liver transplant	Fever on day 21 Blood smear positive Chloroquine + primaquine Relapse 2 months after treatment successfully treated with chloroquine + double dose primaquine
	Heart transplant	Asymptomatic Blood smear positive Treatment not specified Favorable outcome
	Renal transplant	Asymptomatic Blood smear positive Treatment not specified Favorable outcome
Sabé et al., 2014 (Spain) Mali (recent immigration) *P. falciparum* Blood smears negative Retrospectively: positive serology	Heart transplant	Fever, anemia on day 15 Blood smears positive Quinine + doxycycline changed to artesunate + atovaquone-proguanil Favorable outcome
	Lung transplant	Fever (day not specified) Blood smears positive Quinine + doxycycline Favorable outcome
	Liver transplant	Asymptomatic Positive antigen, blood smears negative Quinine + doxycycline Favorable outcome
Martín-Dávila et al., 2018 (Spain) Equatorial Guinea (recent travel to Equatorial Guinea) *P. malariae* + *P. ovale* Blood smears and rapid diagnostic test negative	Renal transplant	Fever, thrombocytopenia on day 30 Blood smears positive. Rapid diagnostic test negative PCR positive for *P. malariae* + *P. ovale curtisi* Chloroquine Favorable outcome
	Liver transplant	Asymptomatic Blood smears negative Rapid diagnostic test negative PCR positive for *P. malariae* + *P. ovale curtisi* Chloroquine + primaquine Favorable outcome

recipients were apparently not infected and/or received preemptive therapy. Based on previously published cases, the prognosis of posttransplant malaria appears to depend on the type of organ transplanted, the species of *Plasmodium* involved (*P. falciparum* being associated with more severe disease), the immunosuppressive therapy used, and the delay in diagnosis and initiation of adequate antimalarial treatment in the recipient [8].

Malaria and Hematopoietic Stem Cell Transplantation (HSCT)

A retrospective observational survey reported that more than one million of HSCT were performed by transplant centers from 75 countries over five continents. The numbers of HSCT differed by WHO region [25]. Worldwide, more than 70,000 hematopoietic stem cell transplants (HSCTs) are performed each year. Allogeneic bone marrow transplantation (allo-BMT) and allogeneic stem cell transplantation (allo-HSCT) are being increasingly used in countries where malaria is endemic [26–28]. In the setting of HSCT, malaria transmission may occur by natural exposure to infected mosquitoes or as a transmitted disease through contaminated blood products or grafts.

Malaria is an unusual complication after HSCT in non-endemic countries. Few cases of malaria infection in HSCT have been reported, and only approximately ten cases have been published in the medical literature [29–38] **(**Table 2**)**. The first case was reported in 1986 by Dharmasena et al. [29], a case of *P. falciparum* infection during a related allogenic bone marrow transplant in a Nigerian patient. The diagnosis was made on day 6 after transplant, but the patient died before treatment could be administered. Malaria infection was due to *Plasmodium falciparum* in seven cases and *P. vivax* in three cases. Only one case was an autologous BMT; the other cases were allogeneic HSCT (one case unrelated donor, eight cases related donors). The mode of acquisition was donor-derived in six cases, two cases were related with blood transfusions, and two other cases were considered recurrences of previous malaria episodes in the recipients. The median time to symptoms was 10.5 days after transplantation (6–70 days), and the median time to microbiological diagnosis was 14.5 days (6–70 days).

All the donor-derived cases in HLA-matched related allogenic HSCT [5] occurred in recipients born in malaria-endemic areas (Africa, four cases; Asia, one case). Screening for malaria infection in donors was performed with blood smears, and all were negative before stem cell harvesting. A donor-derived *Plasmodium vivax* infection occurred following unrelated bone marrow transplantation; the donor had documented *P. vivax* infection during a travel to Papua New Guinea, was treated, and was asymptomatic 11 months before marrow harvesting [33]. Transfusion-related malaria occurred in two cases, both from Tunisia, a country with no malaria transmission; the authors highlighted the importance of implementing policies for safe blood transfusions [36, 39]. All the cases were treated and cured, except for the first one reported. No recurrences were described after malaria treatment.

Table 2 Published cases of donor-derived malaria in HSCT patients [29–38]

Author, year (country of diagnosis). Reference number *Plasmodium* species	Type of BMT	Donor data Gender, age in years Relationship recipient Country of origin previous malaria infection/screening	Recipient data Gender, age Country of origin Hematology disease Conditioning regimen Previous malaria infection/ screening	Clinical presentation Malaria diagnosis Treatment Outcome	Mode of acquisition
Dharmasena, 1986 UK *P. falciparum*	Related Allo-BMT	Nigeria. Male (brother) Thin and thick blood films negative pretransplant	Female, 27 y-o Nigeria Living in the UK during 5 years Aplastic anemia Visited Nigeria 2 years before BMT Prophylaxis with proguanil chloroquine after travel No malaria screening previous to BMT Needed 2 BMT infusion due to graft failure	Loin pain and hemoglobinuria (after infusion) Graft failure (+26) Reinfused (+29) 2ndTX Fever (day +6) Blood films positive *P. falciparum* No treatment Death	Transplant-related (donor acquired)
Lefrere et al., 1996 France *P. falciparum*	Related allo-BMT	Comorian Islands Male (brother) Living in endemic area until 2 months before BMT Positive malaria serology Previous episodes of acute	Female, 30 y-o Comorian Islands AML Transfused 3 months before Blood smears negative previous to BMT	Fever (day +6) Blood smears + (day +12) (parasitemia 12.5%) Quinine (3d) + halofantrine Parasitemia cleared 3 d Symptoms free 1 y after BMT	Transplant-related (donor acquired)

(continued)

Table 2 (continued)

Author, year (country of diagnosis). Reference number *Plasmodium* species	Type of BMT	Donor data Gender, age in years Relationship recipient Country of origin previous malaria infection/screening	Recipient data Gender, age Country of origin Hematology disease Conditioning regimen Previous malaria infection/ screening	Clinical presentation Malaria diagnosis Treatment Outcome	Mode of acquisition
		malaria Blood smears negative	Conditioning regimen: BuCY		
Salutari et al., 1996 Italy *P. vivax*	Auto-BMT	–	Female, 20 y-o Bangladesh AML Auto-BMT	Fever (day +8) Day +14 blood smears *P. vivax* (parasitemia 4%) Chloroquine (3d) + primaquine (7d) Recovered day +30 (FU)	Recurrence-related
Tran et al., 1997 Taiwan *P. falciparum*	Related allo-BMT	Male, 16 y-o (brother) Vietnam No malaria screening	Female, 12 y-o Vietnam Related BMT Thalassemia minor Splenectomy intensive blood transfusion requirements previous month Conditioning regimen: BuCY2ME Malaria serology negative	Fever (day +6 and + 11) Blood smears positive (trophozoites and gametocytes) *P. falciparum* Quinine (x 7d) + mefloquine Parasitemia cleared on day +15	Transplant-related (donor acquired)

O Donell, 1998 UK *P. vivax*	Unrelated allo-BMT	Male Unrelated donor Germany Malaria infection during vacation in Papua New Guinea (11 months before) Incomplete compliance of antimalarial prophylaxis Halofantrine and tetracycline Blood smears negative previous to transplant *P. vivax* infection (quinine + halofantrine, 7d) Relapse 1 month later (parasitemia 1–2%)	Female, 14 y-o UK Unrelated allo-BMT LMC Splenectomy Conditioning: cyclophosphamide-etoposide-TBI	Fever day +40 Blood smears + *P. vivax* (parasitemia (1%) Chloroquine (7d) + primaquine (21d) Parasitemia cleared Discharged on day +69. No relapse during FU	Transplant-related (donor acquired)
Raina V et al., 1998 India *P. vivax*	Related allo-BMT	**Female. (sister)** **No data about donor**	Male, 20 y-o India CML Ph+ Conditioning regimen: BuCY Previous malaria 4–5 years before BMT	Day +70. Severe anemia and thrombocytopenia Blood smears positive (schizonts) *P. vivax* Chloroquine (3 d) + primaquine (15 days) Cured. Last FU 350d after BMT	Recurrence-related
Villeneuve L et al., 1999 France *P. falciparum*	Related allo-BMT	Male, 43 y-o (brother) Madagascar Thin smears negative Anti-*Plasmodium* antibodies positive (1:400) (day +18)	Male, 47 y-o Madagascar LLA Allo-HSCT conditioning regimen: CY etoposide, TBI Malaria serology negative	Fever and mental confusion (+9d) Buffy coat test for malaria + (+15d) Thin smear + (*P. falciparum*)	Transplant-related (donor acquired)

(continued)

Table 2 (continued)

Author, year (country of diagnosis). Reference number *Plasmodium* species	Type of BMT	Donor data Gender, age in years Relationship recipient Country of origin previous malaria infection/screening	Recipient data Gender, age Country of origin Hematology disease Conditioning regimen Previous malaria infection/ screening	Clinical presentation Malaria diagnosis Treatment Outcome	Mode of acquisition
			Transfusion (RBC and platelets) donors negative for malaria risk	i.v. quinine + doxycycline (7 days) Apiretic, aparasitemic (+18d)	
Abdelkefi A et al., 2004 Tunisia *P. falciparum*	Related allo-BMT	Male	Male, 25 y-o Tunisia MDS Conditioning regimen: TBI, CY	Day +19–21 pancytopenia hepatosplenomegaly, neurological signs Blood smears + (23% parasitemia) Bone marrow parasites and hemophagocytosis Quinine i.v. (10 d) Parasitemia cleared +6d Pancytopenia recovered +14d	Blood transfusion-related (single donor from endemic area 5 days before BMT)

Mejia et al., 2012 *US* *P. falciparum*	Related Allo-PBSCT	Male (brother) Sierra Leone In the US 3 months prior to donation Thick smears x3 negative pre-transplant Day +17: PCR and PfHRP2 + for *P. falciparum* Atovaquone/proguanil (3 d)	Female, 25 y-o Sierra Leone Sickle cells anemia In US >2 years Conditioning regimen: Alemtuzumab, TBI, sirolimus 5 U ∗RBC exchange, 2 ∗RBC transfusions (∗antimalarial antibodies negative)	Day +13. Routine peripheral blood smear, positive for *P. falciparum* (intracellular parasites (3.5% parasitemia). PCR + for *P. falciparum.* Atovaquone + proguanil (7d) Parasitemia cleared day +3	Transplant-related (donor acquired). ∗Blood donors: antimalarial antibodies negative
Ladeb S. et al., 2018 Tunisia *P. falciparum*	Related allo- BMT	Male. 30 y-o Brother BM negative for malaria	Male, 27 y-o Aplastic anemia Conditioning regimen: ATGAM, CY	Febrile neutropenia, hemolytic anemia, hemophagocytic syndrome (day +12) +26: thick blood film smears (parasitemia 20%) Quinine (20 doses) Fever vanished +28 Parasitemia cleared 3 d FU 1 year after BMT	Transfusion-related (*Plasmodium* PCR + in blood from donor native of endemic area)

ALL acute lymphocytic leukemia, *allo-BMT* allogenic bone marrow transplant, *auto-BMT* autologous bone marrow transplant, *BM* bone marrow, *BMT* bone marrow transplantation, *Bu* busulfan, *CML* chronic myelogenous leukemia, *CY* cyclophosphamide, *FU* follow-up, *MDS* myelodysplastic syndrome; *PCR* polymerase chain reaction, *TBI* total body irradiation, *2ME* 2-mercaptoethanol, *PBSCT* peripheral blood stem cell transplantation, *PfHRP2 P. falciparum* histidine-rich protein 2, *Ph* + Philadelphia chromosome positive, *RBC* red blood cells

Screening and Diagnosis

Diagnosis of malaria is based on microscopy (examination of thick and thin blood films) as the gold standard. If an initial blood smear is negative and the clinical suspicion is high, these should be repeated every 12–24 h on at least three occasions. As an adjunct to diagnosis, rapid diagnostic tests (RDTs) based on the detection of circulating *Plasmodium* antigens may be used. RDTs may detect one or more antigens, depending on the test, such as the histidine-rich protein 2 (HRP-2) which is specific for *P. falciparum*, parasite lactate dehydrogenase (pLDH), or aldolase. Antigenic tests may remain positive more than 1 month after successful treatment as clearance of antigens from the blood stream is slow. False-negative results may occur if parasitemia is very low or very high (prozone effect). In recent years, there have been numerous cases reporting false-negative RDT results linked to an antigenic variant form of *P. falciparum* HRP-2 [40]. PCR-based methods are highly sensitive and specific (with theoretical limits of detection of 0.0002–1 parasites/uL compared with 5–16 parasites/uL by microscopy) and appear to be more sensitive for the detection of mixed parasitemia [41]. PCR results will not generally be available on the same day in the majority of transplant centers, but these may allow early detection of subclinical malaria in donor-derived transmission and will aid in the correct identification of mixed infections with *P. ovale* or *P. vivax* which may need radical treatment of hypnozoites (see section "Treatment" below).

Malaria is recognized as the most significant transfusion-transmitted disease agent worldwide [42, 43] . The high requirements of blood transfusions before and after HSCT mean that the screening of *Plasmodium* infection in blood products must be thorough and utilize the most sensitive techniques. Depending on the risk of malaria infection (endemic or non-endemic areas), hemovigilance methods are defined by each country through their national regulation agencies and safety policies to avoid transfusion-transmitted malaria [44]. Non-endemic countries use several ways to prevent transfusion-malaria transmission, some with questionnaires, excluding or deferring donors who have had malaria, or resided in, or traveled to malarial areas. Other countries check malaria serology to detect malaria antibodies in the donated blood products; this confirms prior donor exposure and may be useful in more endemic regions, but does not specify whether the donor has active disease [45]. In malaria-endemic countries, transplant candidates, recipients, and donors frequently report previous episodes of malaria. Also, blood donors could been exposed to malaria infection many times. Serology-based screening is not useful in this context.

Considering the possibility of malaria transmission as reported here, the monitoring of donors and/or HSCT recipients from endemic areas by both microscopy and molecular tests may represent a safer strategy to prevent graft-transmitted malaria [46, 47].

In non-endemic countries, sensitive techniques like PCR-based tests in addition to malaria serology and peripheral blood and bone marrow smears must be applied to blood and grafts from HSCT donors and candidates with epidemiological risk of *Plasmodium* infection [48]. Molecular techniques could detect asymptomatic, mixed, and low parasitemias [46].

In case of unrelated donors, a detailed travel history and malaria screening in those at risk of malaria infection should be performed also using sensitive diagnostic methods.

For recipients, HSCT guidelines discourage autologous and allogeneic SCTRs from international travel to the developing world for up to ≥2 years after transplant [49].To reduce the risk of malaria infection after HSCT, they should be informed about the risks of international travel and the importance of seeking pre-travel health advice [50, 51].

Treatment

The choice of drug therapy is based on the *Plasmodium species* involved and case severity. If malaria is confirmed without species determination, effective treatment against *P. falciparum* must be initiated without delay. A combination of two or more classes of antimalarial drugs with unrelated mechanisms of action is preferred. Full details on malaria treatment are provided by the WHO [52] and CDC [53].

Uncomplicated *P. falciparum* malaria should be treated with oral artemisinin-based combination therapy (ACT): a fixed-dose combination of an artemisinin (artemether, dihydroartemisinin, etc.) derivative with a longer-acting antimalarial drug that has a different mode of action (lumefantrine, piperaquine, etc.). Severe *P. falciparum* malaria cases and any complicated/severe malaria cases must be treated with intravenous artesunate (the fastest parasite-clearing drug) and managed in an intensive care setting. (Boxes 2, 3). If artesunate is not available, use artemether in preference to quinine for treating severe malaria in adults and children. If only parenteral quinine is available, it should be given with careful monitoring [54].

P. vivax and *P. ovale* infections transmitted by blood/organs do not need primaquine for radical cure, because hypnozoites develop only from vector-borne disease. Nevertheless, liver hypnozoites may remain dormant in the transplanted liver and may cause relapses in the recipient. A course of primaquine (avoided in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals) may need to be added to the antimalarial regimen in order to obtain a complete cure. Also, single dose of tafenoquine on day 1 or day 2 decreases the risk of *P. vivax* recurrence noninferior to primaquine [55, 56]. Tafenoquine as primaquine is restricted to patients with normal G6PD activity. No experience with tafenoquine has been published yet in transplant population.

Drug toxicity and drug interactions (antimalarial and immunosuppressive drugs) must be evaluated [6] (see Table 3):

- Chloroquine, quinine, mefloquine, piperaquine, and lumefantrine are cardiotoxic and may increase the risk of arrhythmia. Chloroquine and mefloquine could cause prolonged QT interval with calcineurin inhibitor co-administration.
- An increase in the concentration of calcineurin inhibitors has been observed with doxycycline, chloroquine, mefloquine, and primaquine. Elevated mTOR inhibitor levels have been described with doxycycline and mefloquine.
- Everolimus, tacrolimus, mycophenolic acid, and rapamycin have shown in vitro and animal model intrinsic antimalarial activity against *P. falciparum* and against both chloroquine-sensitive and chloroquine-resistant parasites [57].

Table 3 Interactions between antimalarial drugs and immunosuppressive drugs used in transplantation. (Adapted from Buchan and Kotton. Clin Transplant 2019 [59])

Malarial drugs	Calcineurin inhibitors	m-TOR inhibitors
Atovaquone-proguanil	No significant interactions	
Chloroquine	May ↑ cyclosporine levels Can interact with tacrolimus to prolong QT	Everolimus may increase chloroquine levels
Doxycycline	No significant interactions	
Hydroxychloroquine	May ↑ cyclosporine levels	
Mefloquine	Can interact with tacrolimus to prolong QT	Everolimus may increase mefloquine levels
Primaquine	May ↑ cyclosporine levels Can interact with tacrolimus to prolong QT	

Prevention in Travelers

Transplant candidates and recipients traveling to the tropics should be encouraged to consult specialists before their travel. Review of cohorts of transplant recipients shows that they tend to have minimal or suboptimal preparation prior to travel, with limited pre-travel vaccination, medication, and education [58]. Malaria remains one of the most serious infections for travelers to tropical countries. The majority of patients with severe malaria are tourists or VFR migrants acquiring the infection in West Africa. Malaria prevention is strongly recommended for SOT travelers to areas at risk [59]. Insect repellents (N,N-diethyl-D-methylbenzamide (DEET) or picaridin) together with long-lasting insecticide-impregnated bed nets and chemoprophylaxis when indicated are the cornerstone of preventing malaria when traveling. However, routine chemoprophylaxis is not recommended for recipients staying longer or living in endemic countries. Full guidelines on immunosuppressed patients and malaria prevention are provided by the CDC [1].

The recommendations for malaria chemoprophylaxis for transplant recipients are the same as for conventional travelers. Due to drug interactions, transplant recipients should consider starting malaria chemoprophylaxis at least several days prior to travel in order to monitor the concentration of immunosuppressive drugs in case of interactions (see interactions in the text).

Recommendations in the Transplantation Setting

Recommendations include screening potential donors and candidates who are at risk for malaria infection (migrants/travelers who have been in endemic areas in the last 3 years) using microscopy (thick and thin blood films), RDTs, and PCR-based techniques (microscopy alone is not considered to be sensitive enough) [6].

Infected Donors

Recommendations about the deferral period for malaria "infected donors" vary among regions and countries, based on endemicity, availability of donor screening, and the level of risk of malaria transmission that is to avoided [6].

In some transplant centers, the time of deferral is similar to blood donors.

But in other countries and regions, due to organ shortage and the possibility of efficacious therapy and strict follow-up, national guidelines allow transplantation if the donor was asymptomatic and malaria is not the cause of death.

In deceased donors, if malarial tests are positive and malaria is not the cause of death, organs could be accepted, and prompt start of malaria treatment should be initiated in the recipients. Close follow-up of the recipient after transplantation is advised.

For living donors, if there are signs of recent malaria, the organ may be accepted only if adequate testing has been performed and antimalarial treatment has been given. Confirmation of negative parasitemia after treatment (peripheral blood tests, RTDs, and molecular tests) is needed to accept the organ. Follow-up in the recipient after transplantation is advised.

Preemptive treatment of malaria for transplant recipients is not recommended. If the donor has arrived recently (up to 3 weeks) from a high-risk area for malaria, preemptive treatment may be considered as the donor may be infected, but parasites are not detectable in blood because they are multiplying in the liver (hepatic schizogony). Another situation where preemptive therapy has been used is in asymptomatic SOT recipients from a multiorgan donor who transmitted malaria to other recipients. Preemptive treatment should follow the same drug regimens, dose, and duration as the treatment of symptomatic disease.

Presumptive treatment without laboratory confirmation should be considered for urgent and severe patients with a high clinical suspicion of malaria if there are difficulties in obtaining prompt laboratory confirmation. This practice may lead to wrong treatment of the underlying disease and is only acceptable in exceptional circumstances.

An *infected recipient* must be diagnosed (by blood smear or RDT) as soon as possible, as an early treatment usually results in prompt and uneventful recovery.

Key Points

- Malaria infection has been reported both in solid organ and hematopoietic stem cell transplant recipients.
- Modes of acquisition could be donor-derived, transfusion-transmitted, reactivation of previous malaria episode, and de novo infection.
- In the different steps of the transplant process (pretransplant donor and candidate screening; blood, tissue, and organ selection; and during the follow-up period after transplantation), malaria infection must be excluded in those at epidemiological risk of malaria (migrants/travelers/residents who have been in endemic

areas in the last 3 years) and/or signs or symptoms suggestive of *Plasmodium* sp. infection.

- Screening must be performed with microscopy (thick and thin blood films), RDTs, and PCR-based techniques.
- High sensitivity laboratory techniques for diagnostic are recommended (PCR-based techniques) to detect asymptomatic, low, and mixed parasitemia.
- *P. vivax* and *P. ovale* infection could reoccur due to hypnozoite stage of these species in the liver.
- Pre-travel health advice is strongly recommended in SOT and HSCT recipients who are traveling to malaria-endemic areas.

Box 1 Definitions According to the WHO Malaria Terminology [5]

A *malaria case* is the occurrence of malaria infection in a person in whom the presence of malaria parasites in the blood has been confirmed by a diagnostic test

Submicroscopic malaria is a case with low-density blood-stage malaria infection that is not detected by conventional microscopy but could potentially be transmitted.

A *chronic case* is the long-term presence of parasitemia that is not causing acute or obvious illness but could potentially be transmitted.

Recrudescence is the recurrence of asexual parasitemia due to incomplete clearance of asexual parasites of the same genotype that caused the original illness after antimalarial treatment.

Relapse is attributed to activation of hypnozoites of *P. vivax* or *P. ovale* acquired previously. Hypnozoites can remain dormant in host hepatocytes for variable periods (3 weeks–1 year or longer), before activation and development into a preerythrocytic schizont.

Recurrence is the reappearance of asexual parasitemia after treatment, due to recrudescence, relapse, or a new infection.

Cure is the elimination from an infected person of all malaria parasites that caused the infection. When applied to *P. vivax* and *P. ovale* malaria, the term is equivalent to *radical cure* (elimination of both blood-stage and latent liver hypnozoites).

Drug resistance is the ability of a parasite strain to survive and/or multiply despite the absorption of a medicine given in doses equal to or higher than those usually recommended. Drug resistance arises as result of genetic changes that confer reduced susceptibility. There are alarming reports of reduced susceptibility of *P. falciparum* to artemisinin compounds in the Greater Mekong subregion.

Treatment failure is the inability to clear malarial parasitemia or prevent recrudescence after administration of a normally effective dose of antimalarial medicine, regardless of whether clinical symptoms are resolved. Drug failure may be due to deficient absorption, unusual rate of degradation, or excretion of the drug and resistance of the parasite.

Box 2 Treatment of Uncomplicated Malaria in Adults [52]

Treatment of uncomplicated malaria

If species differentiation is not available or unclear treat as *P. falciparum* malaria

P. falciparum

Artemether/lumefantrine: 1 tablet = 20 mg artemether/120 mg lumefantrine. Standard 3-day regimen: 1 dose at 0, 8, 24, 36, 48, 60 h. dose adjusted to weight: ≥35 kg = 4 tablets, for 6 doses, total 24 tablets ∗∗ ***or***

Dihydroartemisinin/piperaquine: 1 tablet = 40 mg dihydroartemisinin and 320 mg piperaquine. Standard 3-day regimen: 1 dose at 0, 24, and 48 h. dose adjusted to weight: ≥75 kg = 4 tablets, for 3 doses, total 12 tablets; 36–74 kg = 3 tablets, for 3 doses, total 9 tablets ***or***

Atovaquone/proguanil: 1 tablet = 250 mg atovaquone and 100 mg proguanil. Standard 3-day regimen: 1 dose at 0, 24, and 48 h. dose adjusted to weight: >40 kg = 4 tablets, for 3 doses, total 12 tablets. Very high treatment failure rates due to resistant *Plasmodium falciparum* parasites have been reported in the greater Mekong region. Prefer to treat with A/P.

P. vivax/P. ovale

Dihydroartemisinin/piperaquine: Standard 3-day regimen: 1 dose at 0, 24, and 48 h. more rapid parasite clearance compared to chloroquine. Preferred drug in *P. vivax* as treatment failure after CQ treatment has been reported in many countries from all over endemic areas ***or***

Atovaquone/proguanil: Standard 3-day regimen: 1 dose at 0, 24, and 48 h ***or***

Artemether/lumefantrine: Standard 3-day regimen: 1 dose at 0, 8, 24, 36, 48, and 60 h ***or***

Chloroquine (CQ): Tablets of chloroquine are commercialized as different chloroquine salts: 250 mg chloroquine diphosphate, 200 mg hydroxychloroquine sulfate, 200 mg hydroxychloroquine all three equivalent to 155 mg chloroquine base per tablet and 136 mg chloroquine sulfate equivalent to 100 mg chloroquine base per tablet. Standard 2–3-day regimen: First dose of 10 mg base/kg (600 mg for adult) at 0 h, followed by 3 doses of 5 mg base/kg at 6–12, 24, and 48–36 h ***followed by***

Primaquine: 1 tablet = 26.3 mg or 13.2 mg primaquine phosphate equivalent to primaquine base 15 or 7.5 mg. Standard 14-day regimen: 0.5 mg base/kg (max 30 mg)/day for 14 consecutive days for *P. vivax* is mostly recommended, but 0.75 mg/base is preferred in eastern Indonesia and Papua New Guinea 0.25 mg base/kg (max 15 mg)/day for 14 consecutive days is enough for *P. ovale*. Check for glucose-6-phosphate-dehydrogenase (G6PD) deficiency.

P. malariae

Long half-life drugs as CQ or DHA/PPQ are preferred over short-life drugs as A/L or A/P due to the longer erythrocytic cycle.

(continued)

Box 2 (continued)

Chloroquine: Standard 2–3-day regimen: First dose of 10 mg base/kg (600 mg for adult) at 0 h, followed by 3 doses of 5 mg base/kg at 6–12, 24, and 48–36 h ***or***

Dihydroartemisinin/piperaquine: Standard 3-day regimen: 1 dose at 0, 24, and 48 h ***or***

Artemether/lumefantrine: Standard 3-day regimen: 1 dose at 0, 8, 24, 36, 48, and 60 h ***or***

Atovaquone/proguanil: Standard 3-day regimen: 1 dose at 0, 24, and 48 h

P. knowlesi

Artemether/lumefantrine: Standard 3-day regimen: 1 dose at 0, 8, 24, 36, 48, and 60 h ***or***

Dihydroartemisinin/piperaquine: Standard 3-day regimen: 1 dose at 0, 24, and 48 h ***or***

Chloroquine: Standard 2–3-day regimen: First dose of 10 mg base/kg (600 mg for adult) at 0 h, followed by 3 doses of 5 mg base/kg at 6–12, 24, 48–36 h ***or***

Atovaquone/proguanil: Standard 3-day regimen: 1 dose at 0, 24, and 48 h

**Doses may need to be adjusted for liver and kidney function. Check dosing before prescribing*

**Some authors now recommend to extend the standard 3-day regimen by adding 4 additional doses at 72, 84, 96, and 108 h: total 10 doses (40 tablets) over 5 days in male European patients [60]

Box 3 Treatment of Severe Malaria in Adults [52]

Treatment of severe malaria∗

***P. falciparum* (*P. knowlesi*)**

First-line treatment artesunate i.v.: Adults and children >20 kg: 2.4 mg/kg. No dose adjustment in the case of hepatic or renal impairment.

Switch to oral therapy as soon as the patient tolerates oral medication (but not before completing at least 48 h of i.v. treatment) and give a complete course of: Artemether/lumefantrine ***or*** dihydroartemisinin/piperaquine or ∗atovaquone/proguanil. ∗*preferred drug if infection has been acquired in Southeast Asia (risk of artemisinin-resistant P. falciparum strains)*.

If the situation does not allow for switching to oral therapy, continue i.v. artesunate for a total of 7 days and ***add*** doxycycline i.v. (100 mg every 12 h for a total of 7 days) ***or*** clindamycin i.v. (10 mg/kg loading dose, followed by 5 mg/kg every 8 h for a total of 7 days)

2nd-line treatment quinine dihydrochloride salt i.v. drug is labeled with the mass of drug expressed in salt rather than base (the active drug). Quinine

(continued)

Box 3 (continued)
dihydrochloride is available as solution of various strengths in distilled water: 500 mg salt = 413 mg base in vials at 1 ml; 600 mg salt = 496 mg base in vials at 2 ml; 1000 mg salt = 826 mg base in vials at 2 ml; 1200 mg salt = 1000 mg base in vials at 5 ml: Loading dose (must not be used if patient received mefloquine within preceding 24 h) of 20 mg/kg over 4 h followed by 10 mg/kg over 4 h starting 8 h after initiation of treatment [alternately 7 mg/kg over 30 min followed by 10 mg/kg over 4 h directly after the loading dose] and followed by 10 mg/kg over 4 h every 8 h [alternately 4 h drug administration and 4 h drug free interval], max. Dosage 600 mg every 8 h. switch to oral medication as soon as possible (but not before completing at least 48 h of i.v. treatment) and give a complete course of*:* **Artemether/lumefantrine *or* dihydroartemisinin/piperaquine *or* atovaquone/proguanil *or* quinine sulfate p.o.** (10 mg salt (=8.3 mg base)/kg every 8 h (max. Dosage: 600–650 mg every 8 h) for a total of 7 days) ADD either **doxycycline p.o.** (100 mg every 12 h for a total of 7 days) ***or*** **clindamycin p.o.** (5 mg/kg every 8 h for a total of 7 days)

P. vivax

As in P. falciparum, followed by **primaquine** for eradication of the hypnozoites

[*]***Doses may need to be adjusted for liver and kidney function. Check dosing before prescribing***

Criteria for the definition of severe malaria*:*

- *Cerebral malaria: Impaired consciousness (somnolence, coma), convulsions*
- *Respiratory distress: Hypoxia, pulmonary edema, acute respiratory distress syndrome ARDS*
- *Renal failure: Urine output <400 ml/24 h in adults (children: <12 ml/kg/24 h) or serum creatinine >265 μmol/l [>3.0 mg/dl] despite adequate volume repletion*
- *Liver failure: ASAT or ALAT >3 times upper normal limit or jaundice/bilirubin >50 μmol/l [>3 mg/dl]*
- *Acidosis: Arterial pH <7.3, plasma bicarbonate <15 mmol/l, BE >8 mmol/l, hyperlactatemia or hyperkalemia >5.5 mmol/l*
- *Hypoglycemia: Blood glucose level <2.2 mmol/l. Shock-circulatory collapse (<90/60 mmHg) despite adequate volume repletion*
- *Severe anemia: Hemoglobin <80 g/dl*
- *Impaired coagulation: Spontaneous bleedings, disseminated intravascular coagulation (DIC)*
- *Hyperparasitemia: ≥2% in patients from malaria non-endemic regions, ≥5% in patients from and permanently living in a malaria holo−/hyperendemic region*

Adjunct treatment of severe malaria: *If patient is unconscious, monitor Glasgow Coma Scale and consider cranial CT and lumbar puncture to rule out meningitis.*

Corticosteroids and mannitol are contraindicated in adult patients with cerebral malaria and cranial hypertension. Both drugs prolong coma and worsen prognosis. The use of prophylactic anticonvulsants is contraindicated as their use is associated with increased mortality. If the patient is in shock, consider blood culture and empirical coverage for gram-negative sepsis. Limit fluid replacement to diuresis and compensation for perspiration/fever. Malaria patients are prone to fluid overload and pulmonary edema. A central venous catheter (monitoring of CVP) or a PiCCO catheter may facilitate guiding fluid management. Avoid NSARDs because of the associated renal toxicity and risk of renal failure. Acetaminophen/paracetamol 1gr every 6 h for 72 h has renoprotective effect in patients with severe malaria treated with i.v. artesunate. Consider physical measures to control hyperpyrexia. Exchange transfusion/erythrocytapheresis (automated ET). If the patient is treated with i.v. artesunate, there is no evidence for additional efficacy of exchange transfusion. Some specialist still opts for ET in the case of extreme hyperparasitemia (>30%). If the patient is treated with i.v. quinine (because artesunate is not available), consider ET in severe malaria cases presenting with a parasitemia > 10% and organ complications. Switching from parenteral to oral therapy is mostly possible around the fourth day, after stabilization of the patient (able to swallow, no vomiting) and once the parasitemia has fallen below 1%.

References

1. Centers for Disease Control and Prevention. CDC – DPDx – Malaria [Internet]. [cited 30 Mar 2019]. Available from: https://www.cdc.gov/dpdx/malaria/index.html
2. Monge-maillo B, López-vélez R. Migration and malaria in Europe. Mediterr J Hematol Infect Dis. 2012;4(1):e2012014.
3. La Hoz RM, Morris MI. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, *Babesia*, *Acanthamoeba*, *Balamuthia*, and *Naegleria* in solid organ transplant recipients – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13546. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/ctr.13546
4. Elsharif M, Imam M, Elsharif E, Malik E, Omran M. Malaria incidence among kidney-transplanted recipients in an endemic malaria area, Sudan. Saudi J Kidney Dis Transplant. 2012;23(5):1099. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22982935
5. WHO. WHO | WHO malaria terminology [Internet]. WHO. World Health Organization; 2019 [cited 5 Jun 2019]. Available from: https://www.who.int/malaria/publications/atoz/malaria-terminology/en/
6. Pierrotti LC, Levi ME, Di Santi SM, Segurado AC, Petersen E. Malaria disease recommendations for solid organ transplant recipients and donors. Transplantation. 2018;102(2S Suppl 2): S16–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29381574
7. Grossi PA, Fishman JA. Donor-derived infections in solid organ transplant recipients. Am J Transplant. 2009;9(Suppl 4):S19–26.
8. Martín-Dávila P, Fortún J, López-Vélez R, Norman F, De Oca MM, Zamarrón P, et al. Transmission of tropical and geographically restricted infections during solid-organ transplantation. Clin Microbiol Rev. 2008;21(1):60–96. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2223841&tool=pmcentrez&rendertype=abstract
9. Kute VB, Vanikar AV, Shah PR. Postrenal transplant Plasmodium vivax malaria: neglected and not benign. Parasitol Res. 2013;112(4):1791–3.

10. Kurth F, Develoux M, Mechain M, Malvy D, Clerinx J, Antinori S, et al. Severe malaria in Europe: an 8-year multi-centre observational study. Malar J. 2017;16(1):57. Available from: http://malariajournal.biomedcentral.com/articles/10.1186/s12936-016-1673-z
11. Menichetti F, Bindi ML, Tascini C, Urbani L, Biancofiore G, Doria R, et al. Fever, mental impairment, acute anemia, and renal failure in patient undergoing orthotopic liver transplantation: posttransplantation malaria. Liver Transpl. 2006;12(4):674–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16555320
12. Wołyniec W, Sulima M, Renke M, Dębska-Ślizień A. Parasitic infections associated with unfavourable outcomes in transplant recipients. Medicina (B Aires). 2018;54(2):27. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6037257/
13. Rello J, Manuel O, Eggimann P, Richards G, Wejse C, Petersen JE, et al. Management of infections in critically ill returning travellers in the intensive care unit—II: clinical syndromes and special considerations in immunocompromised patients. Int J Infect Dis. 2016;48:104–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27134159
14. Chattopadhyay R, Majam VF, Kumar S. Survival of Plasmodium falciparum in human blood during refrigeration. Transfusion. 2011;51(3):630–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20849405
15. Cruz I, Mody V, Callender C, Hosten A. Malaria infection in transplant recipient. J Natl Med Assoc. 1978;70(2):105–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/359818
16. Lefavour GS, Pierce JC, Frame JD. Renal transplant-associated malaria. JAMA J Am Med Assoc. 1980;244(16):1820. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.1980.03310160036021
17. Johnston ID. Possible transmission of malaria by renal transplantation. Br Med J (Clin Res Ed). 1981;282(6266):780. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6783170
18. Holzer B, Glock Z, Zambelli D, Fey M. Transmission of malaria by renal transplantation. Transplantation. 1985;39(3):315. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3883597
19. Babinet J, Gay F, Bustos D, Dubarry M, Jaulmes D, Nguyen L, et al. Transmission of Plasmodium falciparum by heart transplant. BMJ. 1991;303(6816):1515–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1671824&tool=pmcentrez&rendertype=abstract
20. Chiche L, Lesage A, Duhamel C, Salame E, Malet M, Samba D, et al. Posttransplant malaria: first case of transmission of Plasmodium falciparum from a white multiorgan donor to four recipients. Transplantation. 2003;75(1):166–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12544892
21. Rodriguez M, Tome S, Vizcaino L, Fernandez-Castroagudin J, Otero-Anton E, Molina E, et al. Malaria infection through multiorgan donation: an update from Spain. Liver Transplant. 2007;13(9):1302–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17763382
22. Sabé N, González-Costello J, Oriol I, Sánchez-Salado JC, Ortega S, Oliver E, et al. Donor-transmitted malaria after heart transplant managed successfully with artesunate. Transpl Infect Dis. 2014;16(6):999–1002. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25272242
23. Martín-Dávila P, Norman F, Fortún-Abete J, Píris M, Lovatti R, Rubio JM, et al. Donor-derived multiorgan transmission of mixed *P. malariae* and *P. ovale* infection: impact of globalization on post-transplant infections. Transpl Infect Dis. 2018;20(5):e12938. Available from: http://doi.wiley.com/10.1111/tid.12938
24. Ecdc. Hospital acquired malaria infections in the European Union [Internet]. 2018 [cited 29 Mar 2019]. Available from: https://ecdc.europa.eu/sites/portal/files/documents/2018-04-30-RRA-Hospital-acquired-Malaria-European-Union-with%20erratum.pdf
25. Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foeken L, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. Lancet Haematol. 2015;2(3):e91–100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26687803
26. Jaimovich G, Martinez Rolon J, Baldomero H, Rivas M, Hanesman I, Bouzas L, et al. Latin America: the next region for haematopoietic transplant progress. Bone Marrow Transplant. 2017;52(5):671–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28112744

27. Baldomero H, Aljurf M, Zaidi SZA, Hashmi SK, Ghavamzadeh A, Elhaddad A, et al. Narrowing the gap for hematopoietic stem cell transplantation in the East-Mediterranean/African region: comparison with global HSCT indications and trends. Bone Marrow Transplant. 2019;54(3):402–17. Available from: http://www.nature.com/articles/s41409-018-0275-5
28. Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, et al. Hematopoietic stem cell transplantation: a global perspective. JAMA. 2010;303(16):1617. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20424252
29. Dharmasena F, Gordon-Smith EC. Transmission of malaria by bone marrow transplantation. Transplantation. 1986;42(2):228. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3526663
30. Lefrère F, Besson C, Datry A, Chaibi P, Leblond V, Binet JL, et al. Transmission of Plasmodium falciparum by allogeneic bone marrow transplantation. Bone Marrow Transplant. 1996;18(2):473–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8864468
31. Salutari P, Sica S, Chiusolo P, Micciulli G, Plaisant P, Nacci A, et al. Plasmodium vivax malaria after autologous bone marrow transplantation: an unusual complication. Bone Marrow Transplant. 1996;18(4):805–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8899200
32. Tran V-B, Tran V-B, Lin K-H. Malaria infection after allogeneic bone marrow transplantation in a child with thalassemia. Bone Marrow Transplant. 1997;19(12):1259–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9208123
33. O'Donnell J, Goldman J, Wagner K, Ehinger G, Martin N, Leahy M, et al. Donor-derived Plasmodium vivax infection following volunteer unrelated bone marrow transplantation. Bone Marrow Transplant. 1998;21(3):313–4. Available from: http://www.nature.com/articles/1701073
34. Raina V, Sharma A, Gujral S, Kumar R. Case report Plasmodium vivax causing pancytopenia after allogeneic blood stem cell transplantation in CML. Bone Marrow Transplant. 1998;22(2):205–6. Available from: http://www.stockton-press.co.uk/bmt
35. Villeneuve L, Cassaing S, Magnaval JF, Boisseau M, Huynh A, Demur C, et al. Plasmodium falciparum infection following allogeneic bone-marrow transplantation. Ann Trop Med Parasitol. 1999;93(5):533–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10690250
36. Abdelkefi A, Ben Othman T, Torjman L, Ladeb S, Lakhal A, Belhadj S, et al. Plasmodium falciparum causing hemophagocytic syndrome after allogeneic blood stem cell transplantation. Hematol J. 2004;5(5):449–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15448674
37. Mejia R, Booth GS, Fedorko DP, Hsieh MM, Khuu HM, Klein HG, et al. Peripheral blood stem cell transplant-related *Plasmodium falciparum* infection in a patient with sickle cell disease. Transfusion. 2012;52(12):2677–82. Available from: http://doi.wiley.com/10.1111/j.1537-2995.2012.03673.x
38. Ladeb S, Ben Abdejlil N, Fakhfakh N, Lakhal A, Belloumi D, Ben Hamed L, et al. *Plasmodium falciparum* infection transmitted by transfusion: a cause of hemophagocytic syndrome after bone marrow tranplantation in a non-endemic country. Transpl Infect Dis. 2018;20(3):e12887. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29573075
39. Chavada R, Keighley C, Quadri S, Asghari R, Hofmeyr A, Foo H. Uncommon manifestations of Listeria monocytogenes infection. BMC Infect Dis. 2014;14(1):641. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25466213
40. Berhane A, Anderson K, Mihreteab S, Gresty K, Rogier E, Mohamed S, et al. Major threat to malaria control programs by *Plasmodium falciparum* lacking histidine-rich protein 2, Eritrea. Emerg Infect Dis. 2018;24(3):462–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29460730
41. Roth JM, Korevaar DA, Leeflang MMG, Mens PF. Molecular malaria diagnostics: a systematic review and meta-analysis. Crit Rev Clin Lab Sci. 2015;1–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26376713

42. Verra F, Angheben A, Martello E, Giorli G, Perandin F, Bisoffi Z. A systematic review of transfusion-transmitted malaria in non-endemic areas. Malar J. 2018;17(1):36. Available from: https://doi.org/10.1186/s12936-018-2181-0
43. Justiz Vaillant AA, Sticco KL. Transfusion transmitted disease [Internet]. StatPearls. StatPearls Publishing; 2019 [cited 28 Mar 2019]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30860698
44. Brien SFO, Delage G, Seed CR, Pillonel J, Fabra CC, Davison K, et al. The epidemiology of imported malaria and transfusion policy in 5 nonendemic countries. Transfus Med Rev. 2015;29(3):162–71. https://doi.org/10.1016/j.tmrv.2015.03.004.
45. Allain J-P, Stramer SL, Carneiro-Proietti ABF, Martins ML, Lopes da Silva SN, Ribeiro M, et al. Transfusion-transmitted infectious diseases. Biologicals. 2009;37(2):71–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19231236
46. Inoue J, Machado CM, de Lima GFMC, de Nascimento MJC, Colturato VR, Di Santi SM. The monitoring of hematopoietic stem cell transplant donors and recipients from endemic areas for malaria. Rev Inst Med Trop Sao Paulo. 2010;52(5):281–4. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0036-46652010000500012&lng=en&tlng=en
47. Roberts DJ, Field S, Delaney M, Bates I. Problems and approaches for blood transfusion in the developing countries. Hematol Oncol Clin North Am. 2016;30(2):477–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27040966
48. Batista-dos-Santos AS, Roberto Freitas DC, Raiol M, Cabral GF, Cecília Feio A, Póvoa MM, et al. Strategy to improve malaria surveillance system preventing transfusion-transmitted malaria in blood banks using molecular diagnostic. Malar J. 2018;17:344. Available from: https://doi.org/10.1186/s12936-018-2486-z
49. Kotton C, Kroger A, Freedman. Approach to the immunocompromised traveller [Internet]. CDC Yellow Book. 2020 [cited 22 Sep 2019]. p. chapter 5. Available from: https://wwwnc.cdc.gov/travel/yellowbook/2020/travelers-with-additional-considerations/immunocompromised-travelers
50. Mikati T, Griffin K, Lane D, Matasar M, Shah MK. International travel patterns and travel risks for stem cell transplant recipients. J Travel Med. 2015;22(1):39–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25327693
51. Aung AK, Trubiano JA, Spelman DW. Travel risk assessment, advice and vaccinations in immunocompromised travellers (HIV, solid organ transplant and haematopoeitic stem cell transplant recipients): a review. Travel Med Infect Dis. 2015;13(1):31–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25593039
52. For the Treatment of Malaria Guidelines [Internet]. 2015 [cited 20 Jun 2019]. Available from: www.who.int
53. For Disease Control C. Guidelines for treatment of malaria in the United States (Based on drugs currently available for use in the United States) [Internet]. [cited 2019 Jun 20]. Available from: https://www.cdc.gov/malaria/resources/pdf/treatmenttable.pdf
54. Health Organization W. Management of severe malaria – a practical handbook. Third edition [Internet]. [cited 22 Sep 2019]. Available from: https://apps.who.int/iris/bitstream/handle/10665/79317/9789241548526_eng.pdf?sequence=1
55. Lacerda MVG, Llanos-Cuentas A, Krudsood S, Lon C, Saunders DL, Mohammed R, et al. Single-dose tafenoquine to prevent relapse of *Plasmodium vivax* malaria. N Engl J Med. 2019;380(3):215–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30650322
56. Llanos-Cuentas A, Lacerda MVG, Hien TT, Vélez ID, Namaik-larp C, Chu CS, et al. Tafenoquine versus primaquine to prevent relapse of *Plasmodium vivax* malaria. N Engl J Med. 2019;380(3):229–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30650326
57. Veletzky L, Rehman K, Lingscheid T, Poeppl W, Loetsch F, Burgmann H, et al. In vitro activity of immunosuppressive drugs against Plasmodium falciparum. Malar J. 2014;13(1):476. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25476467

58. Kotton CN. Travel and transplantation: travel-related diseases in transplant recipients. Curr Opin Organ Transplant. 2012;17(6):594–600. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23147910
59. Buchan CA, Kotton CN, AST Infectious Diseases Community of Practice. Travel medicine, transplant tourism, and the solid organ transplant recipient – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33(9):e13529. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/ctr.13529
60. Sondén K, Wyss K, Jovel I, Vieira da Silva A, Pohanka A, Asghar M, et al. High rate of treatment failures in nonimmune travelers treated with Artemether-Lumefantrine for uncomplicated *Plasmodium falciparum* malaria in Sweden: retrospective comparative analysis of effectiveness and case series. Clin Infect Dis. 2017;64(2):199–206. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27986683

Pathogenic Intestinal Parasites in Transplant Recipients

57

Joelle I. Rosser and Brian G. Blackburn

Contents

Introduction 1398
Intestinal Protozoa 1399
Cryptosporidium spp. 1399
Cryptosporidiosis in Transplant Recipients 1407
Cyclospora cayetanensis 1408
Cystoisospora belli 1411
Giardia spp. 1414
Blastocystis spp. 1416
Entamoeba histolytica 1418
Dientamoeba fragilis 1422
Balantidium coli 1424
Intestinal Helminths 1425
Intestinal Nematodes 1426
Soil-Transmitted Helminths 1426
Ascaris lumbricoides 1426
Hookworm Infection 1428
Trichuris trichiura (Whipworm) 1431
Strongyloides stercoralis 1433
Other Intestinal Nematodes 1434
Capillaria philippinensis 1434
Intestinal Cestodes 1435
Taenia saginata (Beef Tapeworm) 1435
Taenia solium (Pork Tapeworm) 1436
Hymenolepis nana 1438
Diphyllobothrium latum 1440
Dipylidium caninum 1441
Intestinal Flukes 1442

J. I. Rosser (✉) · B. G. Blackburn
Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA
e-mail: jrosser@stanford.edu; blackburn@stanford.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_57

Conclusion 1443
Key Points 1443
References 1444

Introduction

Intestinal parasitic infections are an uncommon cause of diarrheal illness in transplant recipients in the United States. However, the frequency is higher in developing countries and places with poor sanitation because transmission primarily occurs via consumption of contaminated food or water. In a series of 60 renal transplant recipients in India, 20% tested positive by stool examination for *Cryptosporidium* spp., while 12% were positive for *Giardia* [1]; in the United States, the prevalence in similar cohorts is generally less than 1%. One meta-analysis demonstrated that *Cryptosporidium* spp. are by far the most common intestinal protozoans reported worldwide in transplant recipients, followed by *Blastocystis* spp., with *Giardia* spp. the third most common [2]. *Strongyloides stercoralis* is the most common intestinal helminth reported in this cohort, with only a few case reports of other helminths.

Increasing globalization and travel places transplant recipients at higher risk for intestinal parasites in several ways, including (1) acquiring de novo parasitic infections during posttransplant travel to developing settings; [3] (2) exposure to a close contact who became infected with an intestinal parasite during travel; or (3) living in/receiving a transplant in a highly endemic country, through de novo infection of the recipient or a donor-derived infection. Donor-derived intestinal parasitic infections are important considerations, especially given that patients infected with intestinal parasites are often asymptomatic and routine screening for them is not always performed. Intestinal or liver transplants are of particular concern for the transmission of intestinal parasites, and transmission of *Cryptosporidium* spp., *Giardia* spp., and *Cystoisospora belli* in this manner has been reported [4–7]. Finally, exposure in early life or subsequent travel to a highly endemic area prior to transplant can result in asymptomatic infection which can reactivate after transplant; this can even occur years after initial infection.

While many intestinal parasites are more prevalent in developing settings, others have a global distribution. For example, *Cryptosporidium* spp. are common worldwide, and *Giardia* spp. are frequently acquired from drinking contaminated stream water even in the United States. Furthermore, imported food products can be a source of domestic acquisition of an intestinal parasite, as has occurred repeatedly with imported produce-associated outbreaks of *Cyclospora*.

In some cases, intestinal parasitic infections cause more severe disease in transplant recipients than in immunocompetent hosts, including prolonged and severe diarrhea, dehydration, and malnutrition. In others, intestinal parasites can even cause extra-intestinal manifestations such as biliary, hepatic, or pulmonary disease.

Diagnosis and appropriate treatment of intestinal parasitic infections are often delayed, in part because the low prevalence of parasitic infections in the United

States leads to decreased clinical suspicion. In addition, many conventional diagnostic tests do not specifically evaluate for intestinal parasites and are often insensitive and nonspecific. Fortunately, the increasing availability of molecular diagnostics has improved the accuracy of the assays used for some of the intestinal protozoa (e.g., *Cryptosporidium* spp., *Giardia,* spp., and *Entamoeba histolytica*), but these panels remain far from comprehensive. Diagnostic delays may also stem from the nonspecific nature of the pathological findings seen on intestinal biopsies in transplant recipients infected with intestinal parasites, which can also resemble the findings of rejection or graft-versus-host disease (GVHD).

Intestinal Protozoa

Protozoa are unicellular eukaryotes which feed off other organisms and alternate between proliferative trophozoite and dormant cyst stages. Infections with most protozoa do not cause eosinophilia. Of note, microsporidia can cause intestinal disease in transplant recipients; however, although formerly classified as protozoa, microsporidia are now classified as fungi and are thus instead discussed in ► Chap. 46, "Management of Mucorales Infections in Transplant Patients."

Cryptosporidium spp.

Epidemiology

Cryptosporidium spp. are coccidian protozoan parasites that typically infect the small intestine. In developing countries this is the second most common infectious cause of diarrhea and malnutrition in children. *Cryptosporidium* spp. are distributed globally; though transmission is more common in tropical areas, outbreaks and sporadic cases are also frequent in the United States. *Cryptosporidium* spp. are spread by fecal-oral transmission. Typically, this occurs through ingestion of oocysts in contaminated water and occasionally food. A very small inoculum (only about 10–50 oocysts) is required for productive infection, and a single individual can shed billions of oocysts during the illness.

Immunocompromised hosts often shed *Cryptosporidium* oocysts for prolonged periods. Furthermore, oocysts can live in the environment for months and are resistant to some filtration systems and disinfectants, including chlorine and iodine [8]. Outbreaks among immunocompetent hosts commonly occur in the United States due to contaminated drinking water supplies, swimming pools, and daycare centers, with the most famous example being the 1993 *Cryptosporidium* outbreak in Milwaukee that sickened over 400,000 people due to contamination of the drinking water supply.

Additionally, unlike *Cyclospora cayetanensi*s and *Cystoisospora belli*, *Cryptosporidium* oocysts do not require incubation in the environment before becoming infectious but rather are excreted as mature cysts. This results in the capacity for direct transmission between hosts and contributes to the highly infectious nature of

Cryptosporidium spp. This has important implications for potential nosocomial spread, particularly given the large number of oocysts shed by ill individuals, the low required inoculum, and the environmental resistance of cysts to disinfection. Additionally, in the case of *C. hominis* (which predominantly infects humans), direct person-to-person transmission can occur. *C. parvum* is found in both humans and other mammals and can therefore also be spread from close contact with infected animals. Humans are also rarely infected with other species in the *Cryptosporidium* genus [9].

Cryptosporidium infection is best prevented through avoidance of contaminated water and food. This includes the avoidance of swallowing water from streams, lakes, or swimming pools, as well as safe food handling practices. While chlorine-resistant, *Cryptosporidium* can be killed by boiling or can be filtered by water filters with <1 micron-sized pores [10].

Clinical Presentation

The incubation period for *Cryptosporidium* spp. infection is typically 7–10 days; the clinical illness is largely the same for the different species in the genus. In immunocompetent individuals, *Cryptosporidium* infection typically causes a self-limited illness lasting 1–2 weeks consisting of watery diarrhea, abdominal pain, nausea, and vomiting. In immunocompromised hosts (best described in patients with HIV/AIDS), infection can cause a more prolonged and severe watery diarrhea with associated malabsorption and weight loss.

Extra-intestinal complications rarely occur and typically involve the biliary tract. As infection spreads up the biliary system, it can result in cholecystitis, cholangitis, and pancreatitis. Very rarely, *Cryptosporidium* has also been identified in respiratory secretions; while it is uncertain whether this represents pathogenic infection, *Cryptosporidium* is a postulated cause of respiratory disease in some immunocompromised patients. Finally, *Cryptosporidium hominis* (but not *C. parvum*) has also been associated with a reactive arthropathy [11–13].

Diagnosis

Classically, *Cryptosporidium* spp. are diagnosed by microscopic examination of stool specimens stained with a modified acid-fast stain, as the oocysts are partially acid-fast (Fig. 1). However, immunofluorescence microscopy appears to be a more sensitive and specific means of diagnosis via stool examinations. Stool antigen detection is also nearly as accurate as immunofluorescence microscopy (and superior to acid-fast examination). A modified acid-fast stain can also be used to evaluate for oocysts in biliary samples when there is a suspicion for biliary disease; notably stool samples can be negative for oocysts in such cases. Finally, although not a specific finding, stool specimens typically lack fecal leukocytes or blood in patients infected with *Cryptosporidium* spp. [14].

Although previously limited to research settings, stool polymerase chain reaction (PCR) assays are becoming more widely available and are the most sensitive means of diagnosing cryptosporidiosis. The commercially available BioFire FilmArray GI Panel evaluates for 22 of the most common pathogens responsible for diarrheal

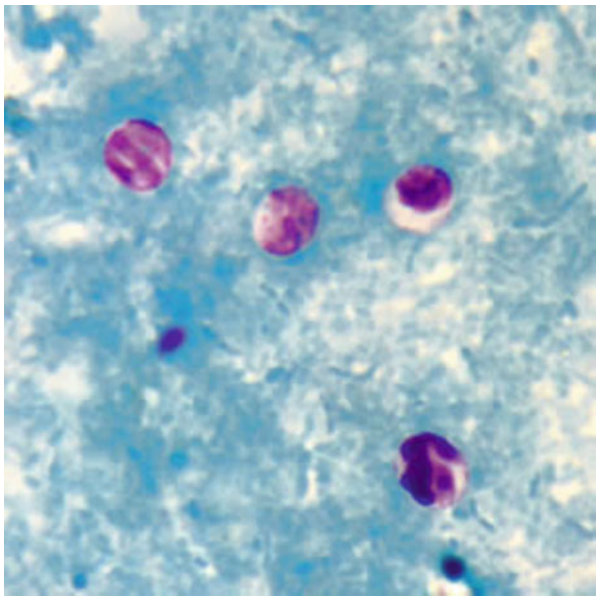


Fig. 1 Multiple small (4–6 μm) *Cryptosporidium* spp. oocysts stained red on modified acid-fast stain. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/cryptosporidiosis/index.html)

illness, including *Cryptosporidium* spp., and in one validation study had a sensitivity of 100% and specificity of >99% and can be performed in just 1 h [15]. The BD MAX Enteric Parasite Panel and the Luminex xTAG GI Pathogen Panel had slightly lower sensitivity and similar specificity values when compared to the BioFire assay (Table 1) [17].

Treatment

In immunocompetent hosts, *Cryptosporidium* infection is typically self-limited, and treatment is generally unnecessary. If antiparasitic treatment is considered, nitazoxanide is preferred; paromomycin is an alternative (Table 2) [18, 19]. However, the efficacy of antiparasitic therapy is uncertain. In some studies, treatment has resulted in an apparent clinical benefit; for example, in one RCT involving patients with diarrhea due to *Cryptosporidium* in Egypt, symptoms resolved after 7 days in 80% of patients treated with nitazoxanide compared to only 40% of patients who received placebo, and *Cryptosporidium* oocyst clearance was significantly faster in the nitazoxanide arm [20]. However, a Cochrane review found that neither nitazoxanide nor paromomycin treatment resulted in symptomatic improvement compared to placebo among patients with cryptosporidial diarrhea, although faster oocyst clearance was observed in some groups after treatment with nitazoxanide [21]. The side effects of these and other antiparasitic medications are outlined in Table 3.

In patients with HIV/AIDS, the mainstay of treatment is reconstitution of the immune system via antiretroviral therapy (ART). Antiparasitic treatment is also typically given to HIV-infected patients, and first-line therapy is nitazoxanide for 14 days, although the data supporting a clinical benefit are sparse.

Table 1 Diagnostic testing of protozoa infections

Protozoan	Microscopic stool examination	Trichrome or modified acid-fast stain recommended[a]	Stool Antigen	Serology	Peripheral Eosinophilia	Detected by BioFire FilmArray GI Panel	Detected by Luminex xTAG Gastrointestinal Pathogen Panel	Detected by BD MAX Enteric Parasite Panel
Cryptosporidium spp.	X	A	X	X		X	X	X
Cystoisospora belli	X	A			X			
Cyclospora cayetanensis	X	A				X		
Giardia spp.	X		X	X		X	X	X
Blastocystis spp.	X	T						
Entamoeba histolytica	X		X[b]	X		X	X	X
Dientamoeba fragilis	X	T			X			
Balantidium coli	X		X					

A acid-fast stain, *T* trichrome stain

[a]Difficult to visualize on unstained wet mount. Additional testing with modified acid-fast or trichrome stain is often necessary

[b]Ag detection assays can also be used on stool, serum, or abscess material

Table 2 Treatment of intestinal protozoa infections. (Adapted from Kappagoda et al. [18] and Gilbert et al. [19])

Protozoan	Preferred regimens	Alternative regimens	Additional treatment regimens for transplant recipients
Cryptosporidium spp.	Consider Nitazoxanide 500mg PO BID for 3 days (not clearly superior to placebo)		1. Nitazoxanide 500–1000mg PO BID for 14 days *OR* 2. Nitazoxanide + azithromycin for 6–8 weeks *OR* 3. Paromomycin + azithromycin ~Decrease immunosuppression if possible
Cyclospora cayetanensis	TMP-SMX-DS 1 tab PO BID for 7–10 days	1. Ciprofloxacin 500mg PO BID for 7 days *OR* 2. Nitazoxanide 500 mg PO BID for 3 days (limited anecdotal success)	1. Consider increasing TMX-SMX-DS dose to 1 DS tab PO QID and/or increasing duration to 4 weeks ~Decrease immunosuppression if possible 2. Consider secondary prophylaxis with TMP-SMX 1 SS tab PO daily
Cystoisospora belli	TMP-SMX-DS 1 tab PO BID for 7–10 days	1. Pyrimethamine 50–75 mg PO daily (+ folinic acid 10–25 mg PO daily) for 3–4 weeks *OR* 2. Ciprofloxacin 500mg PO BID for 7 days	1. Consider increasing TMX-SMX-DS dose to 1 DS PO QID and/or increasing duration to 4 weeks. ~Decrease immunosuppression if possible 2. Consider secondary prophylaxis with TMP-SMX 1 SS tab PO daily
Giardia spp.	1. Tinidazole 2g PO single dose *OR* 2. Metronidazole 500mg PO TID for 5–7 days	1. Nitazoxanide 500mg PO BID for 3 days *OR* 2. Albendazole 400mg daily for 5 days	Consider combination therapy for refractory disease: 1. Metronidazole plus paromomycin *OR* 2. Albendazole plus metronidazole *OR* 3. Nitazoxanide plus a second agent

(continued)

Table 2 (continued)

Protozoan	Preferred regimens	Alternative regimens	Additional treatment regimens for transplant recipients
Blastocystis spp.	Identify and treat concomitant pathogens. Consider: 1. Metronidazole 500 mg PO TID for 10 days, *OR* 2. Tinidazole 2g PO once, *OR* 3. Nitazoxanide 500 mg PO TID for 3 days	1. Iodoquinol 650 mg PO TID for 20 days *OR* 2. TMP-SMX-DS 1 tab BID for 7 days *OR* 3. Paromomycin 8–12 mg/kg PO TID for 7 days	
Entamoeba histolytica – asymptomatic carrier	Luminal agent: 1. Paromomycin 8–12 mg/kg PO TID for 7 days *OR* 2. Iodoquinol 650 mg PO TID for 20 days	Diloxanide furoate 500 mg PO TID for 10 days	
Entamoeba histolytica – amebic colitis	1. Tinidazole 2 g PO daily for 3–5 days *OR* 2. Metronidazole 500–750 mg PO TID for 7–10 days followed by luminal agent (see above)	Nitazoxanide 500 mg PO BID for 3 days	
Entamoeba histolytica – amebic liver abscess or other disseminated disease	1. Tinidazole 2 g PO daily *OR* 2. Metronidazole 500–750 mg PO TID; length dependent on clinical response followed by luminal agent (see above)		
Dientamoeba fragilis	1. Iodoquinol 650 mg PO TID for 20 days *OR* 2. Paromomycin 8–12 mg/kg PO TID for 7 days	1. Metronidazole 500–750 mg PO TID for 10 days 2. Treatment failure: combination iodoquinol plus paromomycin or tetracycline. Search for and treat concomitant pinworm infection	
Balantidium coli	1. Tetracycline 500 mg PO QID for 10 days *OR* 2. Metronidazole 750 mg PO TID for 5 days	1. Iodoquinol 650 mg PO TID for 20 days *OR* 2. Nitazoxanide 500 mg PO BID for 3 days	

Abbreviations: *PO* oral administration, *BID* twice a day, *TMP-SMX* trimethoprim-sulfamethoxazole, *TID* three times a day, *QID* four times a day, *DS* double strength, *mg* milligrams, *kg* kilograms

Table 3 Common adverse effects of antiparasitic medications. (Adapted from Kappagoda et al. [18])

	Gastrointestinal adverse effects	Non-gastrointestinal adverse effects	Other considerations
Metronidazole	Nausea, anorexia	Metallic taste, myelosuppression, peripheral or optic neuropathy, seizures, disulfiram reaction if taken with alcohol	
Tinidazole	Nausea, anorexia	Metallic taste, peripheral neuropathy, disulfiram reaction	Similar to but better tolerated than metronidazole
Nitazoxanide	Nausea, vomiting, abdominal pain, diarrhea	Headache, yellow sclera	
Paromomycin	Nausea, vomiting, diarrhea, abdominal cramps	Nephrotoxicity or ototoxicity if intestinal absorption occurs (rare)	
Iodoquinol	Nausea, abdominal cramps	Acne, iododerma (papular/pustular rash), thyroid gland enlargement, optic neuritis or peripheral neuropathy	Use with caution in patients with liver or thyroid disease; contraindicated in patients with iodine intolerance. Currently unavailable commercially in the United States; may be available from Expert Compounding Pharmacy
Diloxanide	Nausea, vomiting, diarrhea, flatulence		Currently unavailable commercially in the United States; may be available from Expert Compounding Pharmacy
Albendazole	Nausea, vomiting, abdominal pain, hepatitis	Headache, myelosuppression, alopecia	Take with a fatty meal to improve absorption. Possible teratogenicity
Mebendazole	Nausea, vomiting, diarrhea		Possible teratogenicity. Currently unavailable commercially in the United States; may be available from Expert Compounding Pharmacy

(continued)

Table 3 (continued)

	Gastrointestinal adverse effects	Non-gastrointestinal adverse effects	Other considerations
Ivermectin	Nausea, vomiting, diarrhea, hepatitis	Dizziness	
Praziquantel	Abdominal pain, nausea, hepatitis	Mild dizziness, headache, rash	Reduce dose in patients with liver disease
Niclosamide	Nausea, vomiting, abdominal pain	Headache	Currently unavailable commercially in the United States; may be available from Expert Compounding Pharmacy
Pyrantel pamoate	Nausea, vomiting, abdominal pain, diarrhea	Headache, dizziness, rash	
Trimethoprim-sulfamethoxazole	Nausea, vomiting, diarrhea, pancreatitis	Myelosuppression, hemolysis with G6PD deficiency, renal injury, hyperkalemia and cardiac arrhythmias, rash	Should not be given to pregnant women in the first trimester or near term
Ciprofloxacin	Nausea, vomiting	Tendinopathy, neuropsychiatric symptoms, arrhythmias	Should not be taken with dairy products, antacids, multivitamins, or other substances containing divalent cations which inhibit absorption. Often avoided in pregnant women

Much of the practice regarding the treatment of *Cryptosporidium* infections in transplant recipients is based upon the experience with AIDS patients, where re-establishment of a functioning immune system is the cornerstone to successful therapy. In contrast, it is not usually possible to achieve this goal entirely in the transplant population. If possible, immunosuppression should be decreased. There are no clinical trials evaluating the efficacy of antiparasitic therapy in transplant recipients. However, given the severity of disease in this population, the challenges of reversing immunosuppression, and the evidence that antimicrobials may speed oocyst clearance, antiparasitic therapy is usually recommended for transplant recipients with cryptosporidiosis (typically with prolonged courses and combination therapy). Treatment options include nitazoxanide monotherapy for at least 14 days or either nitazoxanide or paromomycin combined with azithromycin (Table 2) [11, 22].

Cryptosporidiosis in Transplant Recipients

Overview

Cryptosporidium is the most common intestinal parasite diagnosed in transplant recipients. Despite likely underdiagnosis, the reported incidence of symptomatic cryptosporidiosis is higher in transplant recipients than that of the general population, and in some regions, *Cryptosporidium* spp. are implicated in a quarter of cases of infectious diarrhea in transplant recipients [11, 23–25]. *Cryptosporidium* has been identified in the stool of over half of renal transplant recipients with acute diarrhea in Pakistan (although coinfection with another gastrointestinal pathogen was present in 12.5% of these patients) [26].

Cryptosporidiosis can be mistaken for other causes of posttransplant diarrhea, including drug toxicity [27] and graft-versus-host disease (GVHD). Furthermore, transplant recipients with cryptosporidiosis are at risk for both more prolonged diarrhea and extra-intestinal complications than immunocompetent hosts.

Cryptosporidium as a GVHD Mimic

Cryptosporidium infection is recognized as a complication after HSCT and is a frequent mimic of GVHD or can occur contemporaneously with GVHD [28]. Pathologic findings in both GVHD and *Cryptosporidium* infection consist of inflammation and apoptosis of epithelial crypt cells. The presence of cysts in tissue specimens can confirm the diagnosis, but these are not always seen [29–33]. PCR is highly sensitive and can be helpful in determining the presence of this protozoal infection in this population [30, 31].

It is important to identify *Cryptosporidium* as a cause of diarrhea since management involves tapering immunosuppression along with antiparasitic treatment, in contrast to the increased immunosuppression that is typically employed in the treatment of GVHD.

Cryptosporidium and Renal Failure

Although *Cryptosporidium* has been reported after liver, pancreas, and heart transplants, the majority of cases reported in SOT recipients have occurred after kidney transplants. In one review of 47 cases of cryptosporidiosis in SOT recipients (41 of which were kidney transplants) in France, watery diarrhea occurred in most patients, but acute kidney injury also occurred in 15 (32%) of 47 cases. The median time from transplant to infection was 3.4 years, and the median time from symptom onset to diagnosis was 10 days (range 2–110 days); this report emphasizes the importance of maintaining a high index of suspicion for the possibility of cryptosporidiosis, even years after transplant [34].

Tacrolimus use seems to increase the risk of cryptosporidiosis compared to cyclosporine use among transplant recipients. Tacrolimus use also seems to predispose transplant recipients with cryptosporidiosis to a higher risk of graft dysfunction [11, 23, 35].

Small case-series have also suggested that higher rates of cryptosporidiosis are observed in non-transplant patients on hemodialysis compared to controls [36, 37]; it

is unclear whether this is due to a relative immunocompromised state, nosocomial acquisition, or other risk factors. Regardless, this highlights a potential role for screening for *Cryptosporidium* spp. prior to renal transplant.

Extra-intestinal Manifestations of *Cryptosporidium*

Cryptosporidium spp. can also cause several extra-intestinal manifestations, including cholangitis, pancreatitis, reactive arthritis, and pneumonia [38]. Although rare in immunocompetent hosts, extra-intestinal complications are more common in immunocompromised hosts. Biliary infection is the most common extra-intestinal manifestation and can lead to sclerosing cholangitis [39]. In a report of 47 cases of cryptosporidiosis in SOT recipients, 9% had extra-intestinal involvement (three with biliary involvement and one with pulmonary involvement), a higher rate than seen in immunocompetent patients [34]. Sclerosing cholangitis has also been repeatedly documented as a complication of cryptosporidiosis after liver transplant, particularly in children [40, 41]. The diagnosis of biliary involvement should be based not only on elevated liver enzymes but also upon radiographic evidence of biliary duct abnormalities. The biliary tree is a potential reservoir for *Cryptosporidium* spp., leading to late relapse; nitazoxanide is the preferred agent for biliary cryptosporidiosis, given its biliary excretion [11].

HSCT and *Cryptosporidium* in Hyper-IgM Syndrome (HIGM1)

Patients with hyper-IgM syndrome (and thereby CD40 ligand deficiency) are highly susceptible to recurrent and severe *Cryptosporidium* infections, often complicated by sclerosing cholangitis (along with other infections such as *Pneumocystis* pneumonia, histoplasmosis, and leishmaniasis). HSCT has been used as a curative treatment for HIGM1; however, immunosuppression after allogeneic HSCT itself poses a risk of infection, including from *Cryptosporidium* spp. Patients with HIGM1 have died after HSCT from severe cryptosporidiosis, and other patients have recovered from persistent, severe *C. parvum* infection after HSCT [42]. Another case report describes a child with CD40 ligand deficiency complicated by liver failure from sclerosing cholangitis secondary to *Cryptosporidium* infection who underwent successful liver transplant followed by HSCT with no recurrence of cryptosporidiosis [43].

Cyclospora cayetanensis

Epidemiology

Cyclospora cayetanensis is a coccidian protozoan that parasitizes the small intestine. *Cyclospora* is spread by fecal-oral transmission and has a worldwide distribution but is more common in Latin America, India, and Southeast Asia. Humans are the only known host. The organism is transmitted sporadically in the United States, where outbreaks have been associated with contaminated food often traced to produce imported from Latin America, such as contaminated raspberries, basil, lettuce, and cilantro [44]. In the United States, most cases are reported during the spring and summer months, and outbreaks have occurred

almost every year since the mid-1990s. Similar to *Cystoisospora* (but in contrast to *Cryptosporidium* spp.), oocysts shed in the stool must incubate in the environment for several days before becoming infectious, so direct human-to-human transmission does not occur. Additionally, cysts mature best under hot, humid conditions, so transmission is facilitated in the tropics and during warm, rainy months in temperate regions. *Cyclospora* is relatively resistant to chlorine, so public water supplies are susceptible to contamination [45].

Clinical Presentation

Patients infected with *Cyclospora* can be asymptomatic or can develop a self-limited illness in immunocompetent hosts. The infection is often more severe in young children or elderly adults. The median incubation period is seven (range 2–14) days. Though usually self-limited, a prolonged waxing and waning course can occur; in one series, the average duration of symptoms was >3 weeks, and in another, symptoms typically lasted 10–12 weeks. Common symptoms include watery diarrhea, nausea, flatulence, abdominal cramping, fever, and weight loss. Patients may develop steatorrhea secondary to malabsorption. In immunocompromised hosts, *Cyclospora* typically causes a chronic, watery diarrhea which can lead to dehydration and malnutrition; occasionally patients can also have mucus or blood in the stool. It can also mimic irritable bowel syndrome (IBS) [45]. Biliary disease is occasionally seen with *Cyclospora* infection, including acalculous cholecystitis in HIV/AIDS patients [46].

Diagnosis

PCR assays are a highly sensitive and specific method for detecting *Cyclospora* infection and are becoming more widely available. As with *Cryptosporidium*, *Cyclospora* is included in the commercially available BioFiire FilmArray GI Panel, and in one validation study, this assay had a sensitivity and specificity approaching 100% [15]. However, the Luminex xTAG and BD MAX panels do not include *Cyclospora* (Table 1). When PCR is not available, direct visualization with traditional laboratory techniques can be used. The diagnosis of cyclosporiasis is made by identifying oocysts on stool exams stained with a modified acid-fast stain; however, this technique is not sensitive, and multiple stool samples may be necessary in order to make a diagnosis. *Cyclospora* oocysts (diameter 8–10 microns) are round and partially acid-fast, similar in appearance to *Cryptosporidium* spp. oocysts (diameter 5 microns) which share these characteristics but are smaller (Fig. 2). Modified safranin staining or fluorescence microscopy is an alternative method that can be used to detect *Cyclospora* oocysts. There are no serologic assays for *Cyclospora* [14] (Table 1).

Treatment

Patients with symptomatic *Cyclospora* infections should be treated with antimicrobial therapy. First-line treatment for immunocompetent patients is with trimethoprim-sulfamethoxazole (TMP-SMX). One RCT of 40 expatriates in Nepal infected with *Cyclospora* reported that patients were clinically improved and

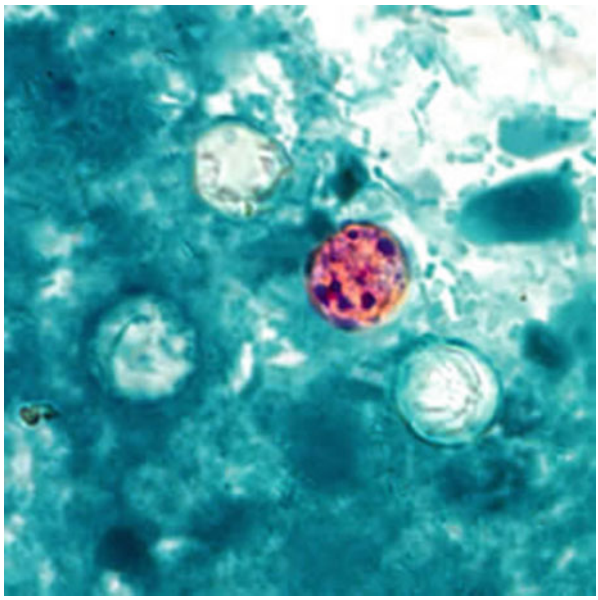


Fig. 2 Variable staining of only one of four *Cyclospora cayetanensis* oocysts (7.5–10 μm in size) on modified acid-fast stain. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/cyclosporiasis/index.html)

oocysts had become undetectable after 7 days of treatment in 94% of patients treated with TMP-SMX, compared to only 12% of patients in the placebo group [47].

Ciprofloxacin is also active against *Cyclospora*, although it appears to be inferior to TMP-SMX. In a small RCT of HIV-infected patients with cyclosporiasis, ciprofloxacin was compared to TMP-SMX. Over 95% patients who received TMP-SMX experienced resolution of diarrhea and clearance of oocysts after 7 days of therapy, compared to only 70% in the ciprofloxacin group [48]. Nitazoxanide is also an alternative, based on a study in which 71% of children infected with *Cyclospora* experienced clearance of oocysts from their stool after treatment with nitazoxanide [49] (Table 2).

Cyclospora in Transplant Recipients

Case reports suggest that infection with *Cyclospora* may be more severe and protracted in transplant recipients than in normal hosts [3, 50, 51]. One small outbreak of *Cyclospora* infection involved three individuals who traveled together to Indonesia and experienced acute diarrhea. Two of the individuals were immunocompetent and experienced 2–4 weeks of symptoms, which resolved without treatment. The third individual was a renal transplant recipient who was on tacrolimus, mycophenolic acid, and deflazacort; he developed protracted diarrhea lasting several months, lost 15 pounds of weight, and required two hospitalizations for dehydration. The patient did not respond to courses of ciprofloxacin or metronidazole, and his symptoms only resolved after treatment with TMP-SMX for 10 days [50]. Diarrhea lasting 2 months (associated with a 20-pound weight loss and multiple hospitalizations) was also observed in another renal transplant recipient who was infected with *Cyclospora*.

This patient improved after receiving 10 days of TMP-SMX [51]. In both cases, the diagnosis of *Cyclospora* was delayed and only made after colonoscopic biopsy revealed the parasite. Although there are few case reports involving *Cyclospora* in transplant recipients, these presentations support the idea that this cohort is more vulnerable to severe disease from this infection.

In immunosuppressed patients, higher doses and/or longer courses of antimicrobials may be needed to effectively treat cyclosporiasis, and reduction of immunosuppression is integral to successful treatment strategies. Most data come from the HIV/AIDS population; extrapolation to the transplant population is imperfect, but little primary data exist in the latter cohort. While most patients in one series of patients with HIV/AIDS who had cyclosporiasis initially responded to oral TMP-SMX therapy, recurrence was seen in 43% of cases within a month of the completion of therapy. The optimal dose and duration of TMP-SMX for immunosuppressed patients with cyclosporiasis are uncertain; after treatment, secondary prophylaxis with TMP-SMX should be considered to prevent relapse in those who remain substantially immunosuppressed (Table 2) [52].

Cystoisospora belli

Epidemiology

Cystoisospora (formerly *Isospora*) *belli* is a coccidian protozoan which parasitizes the small intestine. It is transmitted via the fecal-oral route, typically from consumption of contaminated food or water. While transmission occurs worldwide (including several outbreaks that have occurred in the United States), incidence is higher in developing settings and tropical and subtropical regions. Unlike *Cryptosporidium* and *Cyclospora* (which are commonly found in immunocompetent hosts as well), *Cystoisospora* is found mostly in immunocompromised individuals. Unlike *Cryptosporidium* spp. (but similar to *Cyclospora*), *Cystoisospora* oocysts require incubation in the environment in order to become infectious, so direct human-to-human transmission does not occur. However, oocysts can remain viable in the environment for months; institutional outbreaks have been reported related to contamination of the environment with oocysts. Sporadic cases among immunocompetent individuals are rare in the United States. Humans are the only known hosts for *C. belli* [9, 39].

Clinical Presentation

Many carriers of *C. belli* are asymptomatic. Among symptomatic individuals, the incubation period is typically 7–10 days, and symptoms in immunocompetent individuals include watery diarrhea with abdominal pain, nausea, vomiting, and weight loss. Fever is rare and extra-intestinal disease is very rare. More chronic cases occasionally occur and can mimic celiac sprue and irritable bowel syndrome or cause malabsorption [39]. While illness is self-limited in most immunocompetent individuals, immunocompromised patients are at particular risk for symptomatic and severe cystoisosporiasis.

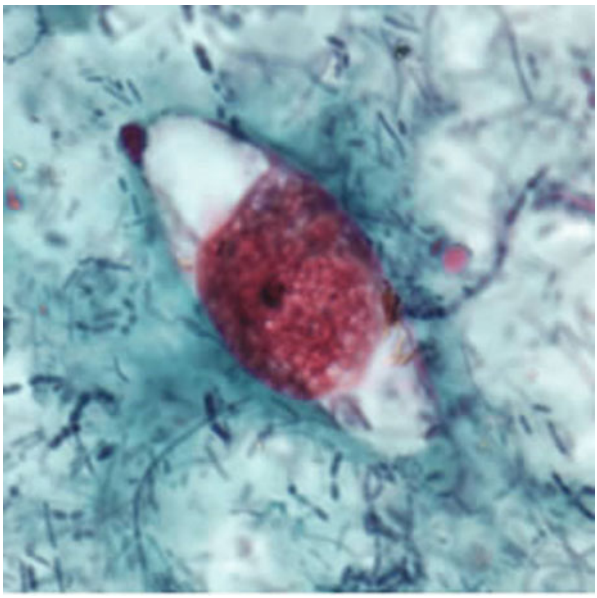


Fig. 3 Safranin stain of an immature *Cystoisospora belli* oocyst which is ellipsoidal containing a sporoblast and is much larger in size (25–30 μm) than the other intestinal coccidian oocysts. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/cystoisosporiasis/index.html)

Diagnosis

Diagnosis is traditionally made by identification of oocysts on stool examination stained with a modified acid-fast stain (or specific fluorescent techniques). *Cystoisospora* oocysts are elliptical and much larger (10–20 × 20–30 μm) than those of *Cryptosporidium and Cyclospora* (Fig. 3). PCR assays have been developed for detecting *Cystoisospora* DNA in stool samples, but they are not widely available and notably are not included in commercially available stool GI PCR panels; stool examination therefore remains the mainstay of diagnosis. In cases where intestinal biopsy is performed, histopathology classically shows mucosal atrophy, villous blunting, crypt hypertrophy, and lamina propria inflammation [14, 39]. Unlike most other protozoa (aside from *Dientamoeba fragilis*), *Cystoisospora* can also cause peripheral eosinophilia (Table 1).

Treatment

As with *Cryptosporidium* spp., infection with *Cystoisospora* is often self-limited in immunocompetent hosts and frequently resolves without treatment; when used, first-line treatment in immunocompetent patients is TMP-SMX (Table 2). Alternatives to TMP-SMX include ciprofloxacin or pyrimethamine (given with folinic acid), although these are considered second-line. In one small RCT of HIV-infected patients with *Cystoisospora*, symptom resolution and microbiologic cure were inferior among those patients treated with ciprofloxacin compared to those treated with TMP-SMX [48].

Immunocompromised patients infected with *Cystoisospora* should always be treated given the potential for severe disease. While there has been some success with short-course, twice-daily dosing regimens of TMP-SMX, the recommended

dose based on experience with HIV patients is one DS tab four times a day for up to 4 weeks. Secondary prophylaxis has been effective in decreasing the rate of recurrence in HIV-infected patients. In a trial of AIDS patients infected with *Cystoisospora*, secondary prophylaxis with TMP-SMX (one DS tab three times a week) or pyrimethamine/sulfadoxine (once weekly) was 100% effective in preventing recurrent diarrhea compared to a 50% recurrence rate in a placebo arm [53]. In HIV-infected patients with CD_4 counts <200, secondary prophylaxis is therefore recommended [54]. Although secondary prophylaxis has not been studied in the transplant population, extrapolation from consensus HIV guidelines and case reports of transplant patients suggests that secondary prophylaxis should be considered in such patients. In addition to antiparasitic medications, decreasing immunosuppression in transplant recipients when possible is another important component of the treatment of *Cystoisospora* infection and for preventing relapse [39].

Cystoisospora in Transplant Recipients

Cystoisospora can cause severe and recurrent disease in immunocompromised individuals and is a well-documented cause of prolonged diarrheal illness in patients with HIV/AIDS. Although uncommon, extra-intestinal cystoisosporiasis (such as cholecystitis and reactive arthritis) occurs in AIDS patients as well [55–57]. Although classically associated with HIV/AIDS, *Cystoisospora* infection causing prolonged diarrhea, weight loss, and recurrent infection has also been reported in patients with HTLV-1, non-Hodgkin's lymphoma, acute lymphoblastic leukemia, and thymoma [58–61].

There are two reported examples of suspected donor-derived infections of *Cystoisospora* in solid organ transplant recipients. In one case, infection occurred 1 month after intestinal transplant (both the donor and recipient were from a highly endemic region of northern Argentina). The recipient was treated with TMP-SMX four times daily for 10 days, then twice daily for 3 weeks, and then daily for 1 month with resolution of symptoms [7]. This case highlights the potential risk of donor-derived intestinal parasites with intestinal transplants [4, 5]. In another case, *Cystoisospora* organisms were incidentally found in the gallbladder of a combined liver-kidney donor. The recipient was treated with TMP-SMX four times a day for 10 days followed by twice daily for 3 weeks and did not develop clinical evidence of *Cystoisospora* infection [62].

Several other cases of cystoisosporiasis have been reported in liver and kidney transplant recipients, including three patients with a few weeks of diarrhea who had clinical and parasitologic response after just 7–10 days of TMP-SMX [63–65]. Another kidney transplant recipient presented with chronic diarrhea and was found to have both *Entamoeba histolytica* and *Salmonella enterica* serotype Typhi coinfections in addition to *C. belli*. This patient had persistent diarrhea despite treatment with both metronidazole and ciprofloxacin and only improved after receiving TMP-SMX [66], highlighting a failure of ciprofloxacin. Another case of cystoisosporiasis in a liver transplant recipient was treated with TMP-SMX at the low dose of one single strength tablet twice daily and experienced treatment failure [67].

Giardia spp.

Epidemiology

Giardia duodenalis (also known as *G. intestinalis* or as *G. lamblia*) is a protozoan flagellate which parasitizes the small intestine, causing both sporadic and epidemic diarrheal illness. Giardiasis is a water- and food-borne infection and an important cause of illness in international travelers. *Giardia* is found worldwide, although more commonly in developing settings with poor sanitation.

Giardia spp. have two morphological forms, cysts and trophozoites. Cysts are excreted in stool and can survive in moist environments for prolonged periods; they are the infectious form of the parasite. Trophozoites are pear-shaped, binucleate, multi-flagellated forms which are the active, invasive form of the parasite.

Infection occurs by fecal-oral transmission of cysts and in the United States is most often seen in outbreaks related to waterborne or daycare transmission, among international travelers, and in domestic hikers/campers exposed to contaminated water. Small mammals are common animal reservoirs and can maintain *Giardia* infection in the wild [9].

Giardia cysts are immediately infectious upon fecal excretion, so direct human-to-human transmission occurs, including via sexual transmission [68, 69]. *Giardia* is also resistant to chlorine, rendering waterborne transmission a major source of *Giardia* infection.

Humoral immunity is an important host defense against *Giardia* infection. Increased *Giardia* risk is associated with agammaglobulinemia, common variable immunodeficiency, and IgA deficiency. Although not well described in the literature, there is a theoretical increased risk of *Giardia* infection associated with medications that suppress humoral immunity.

Clinical Presentation

Half of individuals infected with *G. duodenalis* remain asymptomatic and clear of the infection; about 15% pass cysts while asymptomatic (sometimes for several months), and about 35% develop symptomatic infection. For this latter cohort, the incubation period averages 1–2 weeks, and acute infection with *Giardia* is classically characterized by foul-smelling diarrhea, bloating, flatulence, fatty (floating) stools, malaise, nausea, vomiting, and weight loss. Waxing and waning symptoms persist after the acute phase in half of symptomatic individuals and can last for months after clearance of the parasite due to acquired lactose intolerance and other etiologies. Chronic giardiasis can adversely affect growth and development in children, usually in the developing world.

Rarely, *Giardia* can cause biliary and pancreatic duct obstruction, leading to cholecystitis, cholangitis, pancreatitis, or granulomatous hepatitis. Hypersensitivity phenomena such as rash, urticaria, and reactive arthritis have also rarely been described in the setting of giardiasis. Previously infected individuals may report symptoms even years after effective treatment, sometimes leading to a diagnosis of irritable bowel syndrome [9, 13].

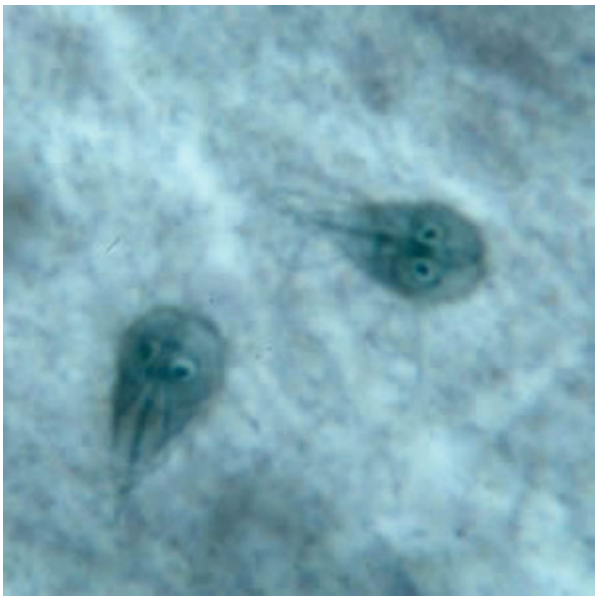


Fig. 4 Kohn stain of multiply flagellated, pear-shaped *Giardia* spp. trophozoites. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/giardiasis/index.html)

Diagnosis

Direct microscopic examination of stool samples to detect cysts or trophozoites is the classical means of diagnosis for *Giardia* infections (Fig. 4). However, low sensitivity and the requirement for technical expertise are limitations of this method. Several different antigen detection assays are commercially available, including direct immunofluorescent assays (DFA), immunochromatographic assays, and enzyme-linked immunosorbent assays (ELISAs). In general, these methods are more sensitive and have faster turn-around times than conventional stool microscopy. Specificity and cost are usually comparable; many commercial stool assays test for both *Giardia* and *Cryptosporidium* simultaneously. After treatment, *Giardia* stool antigen tests may remain positive for a prolonged period due to shedding of killed parasites [14] (Table 1).

PCR assays have also been developed to detect *Giardia* in stool samples; while some remain research tools, three commercially available panels include *Giardia:* the BioFire FilmArray, the Luminex xTAG Gastrointestinal Pathogen Panel, and the BD MAX Enteric Parasite Panel (Table 1). These tests are highly sensitive and specific for *Giardia* [17].

Patients with giardiasis usually do not have peripheral leukocytosis nor eosinophilia. White cells in stool specimens are usually absent. Fecal fat excretion and other laboratory tests of malabsorption may remain abnormal for months or longer after infection.

Treatment

There are several options for treating giardiasis. A single 2-gram dose of tinidazole is the preferred regimen, with cure rates that exceed 90%. Alternatives include a 3-day

course of nitazoxanide or 5–7 day courses of metronidazole (Table 2). Alternatives also include quinacrine, furazolidone, albendazole, or paromomycin, all of which have slightly lower cure rates than tinidazole [70]. Secnidazole is a newer single-dose medication available for the treatment of giardiasis from the same drug class as tinidazole and metronidazole and is associated with cure rates of 80–100%.

For immunocompromised patients or those with recurrent or persistent symptoms (e.g., >1 week after completion of initial therapy) and persistent *Giardia* identified on stool studies, therapy with an agent from a different drug class or prolonged combination therapy should be considered, such as paromomycin + metronidazole, albendazole + metronidazole, or nitazoxanide + a second agent. Combination therapy can spare patients from the adverse effects associated with high doses or prolonged therapies of a single agent and target *Giardia* by different mechanisms with resultant increased potency. Several studies show that combination therapies have increased activity against *Giardia* in vitro and suggest that combinations of anti-*Giardia* medications can effectively treat cases of refractory giardiasis [71–75]. For prolonged symptoms, it is also important to consider the contribution of acquired lactase deficiency, which 20—40% of patients with giardiasis develop.

Giardia in Transplant Recipients

Giardia is an uncommon but well-recognized cause of diarrhea in both HSCT and SOT recipients. The incidence of *Giardia* as a cause of gastrointestinal illness in transplant recipients is largely dependent on the local prevalence; in one retrospective study in Brazil of 657 renal transplant recipients with diarrhea, only 3 patients (0.4%) had *Giardia*, while in a study of 150 renal transplant patients in Iran, 11 patients (7.4%) had *Giardia* [76, 77]. There is insufficient data regarding whether *Giardia* is more common in transplant recipients or whether prevalence in that cohort simply parallels the general population locally.

Diagnosis of giardiasis is frequently delayed as the infection is often initially misdiagnosed as GVHD, pretransplant mucosal injury from conditioning therapy, or other noninfectious causes of diarrhea [6, 78, 79]. The low sensitivity of stool examinations can also make diagnosis difficult, and cases are often diagnosed only when cysts or trophozoites are seen on intestinal biopsy, as has been described in multiple case reports involving transplant recipients [76, 78–80].

Donor-derived infection with *Giardia* from at least one pancreas-kidney donor to a recipient (who had no epidemiologic risk factors for *Giardia*) also highlights the need to consider this possibility in transplant recipients who do not otherwise have known risk factors for *Giardia* [6].

Blastocystis spp.

Epidemiology

Blastocystis spp. are found worldwide but are more common in developing countries where sanitation is poor; in some settings, nearly 100% of the population is infected. Although previously referred to as *Blastocystis hominis*, this parasite is now

classified into multiple subtypes, so the organism is now referred to as *Blastocystis* species instead. Transmission likely occurs via the fecal-oral route, and *Blastocystis* spp. infect both human and animal hosts; some evidence supports zoonotic transmission. Cysts do not require environmental incubation, so person-to-person transmission is theoretically possible.

Clinical Presentation

Blastocystis spp. infect the human cecum and colon, but there is conflicting evidence regarding whether *Blastocystis* is a true pathogen or is only a commensal organism that does not cause symptoms. When *Blastocystis* is detected in patients with gastrointestinal symptoms such as diarrhea, nausea, abdominal cramps, and flatulence, some studies have suggested that it is more likely a marker for another unidentified pathogen (oftentimes *Giardia*). However, there is increasing evidence supporting the pathogenic importance of *Blastocystis*. Most reports refuting the pathogenicity of *Blastocystis* cite similar prevalence of the organism in asymptomatic and symptomatic patients; however, these data do not necessarily rule out the pathogenicity and may simply reflect that not all infections with the organism are symptomatic – a characteristic that is true of several intestinal parasites including *Giardia*, *Cryptosporidium*, and others. Furthermore, studies suggest that different subtypes of *Blastocystis* may be associated with different risks of symptomatic infection. Most convincingly, several studies have demonstrated that treatment targeting this organism can result in both symptomatic cure and organism clearance; for example, in a RCT of 76 patients infected with *Blastocystis* alone, 88% of patients treated with metronidazole had resolution of symptoms, and 80% had clearance of oocysts, compared to 14% and 3%, respectively, in the placebo group [81, 82]. *Blastocystis* is therefore becoming increasingly recognized as a cause of symptomatic disease. Some studies have also suggested an association between *Blastocystis* infection and irritable bowel syndrome.

Diagnosis

Blastocystis spp. are traditionally diagnosed by identification of cysts on microscopic examination of stool (Fig. 5), although technical issues often render this difficult. Additional stains such as trichrome can assist in identification. Fecal leukocytes are generally absent. Some research PCR panels can detect *Blastocystis*, but these are not widely available (Table 1). Additionally, although *Blastocystis* itself likely causes GI symptoms, identification of *Blastocystis* should prompt evaluation for other intestinal pathogens.

Treatment

Patients with asymptomatic infection do not require treatment. When *Blastocystis* is thought to be pathogenic, a therapeutic trial is reasonable. First-line treatment options include metronidazole, single-dose tinidazole, or nitazoxanide, all based on placebo-controlled trials demonstrating symptomatic improvement and parasite clearance with these agents [82, 83]. Trimethoprim-sulfamethoxazole, paromomycin, and iodoquinol have also been associated with symptomatic improvement in small studies [81] (Table 2).

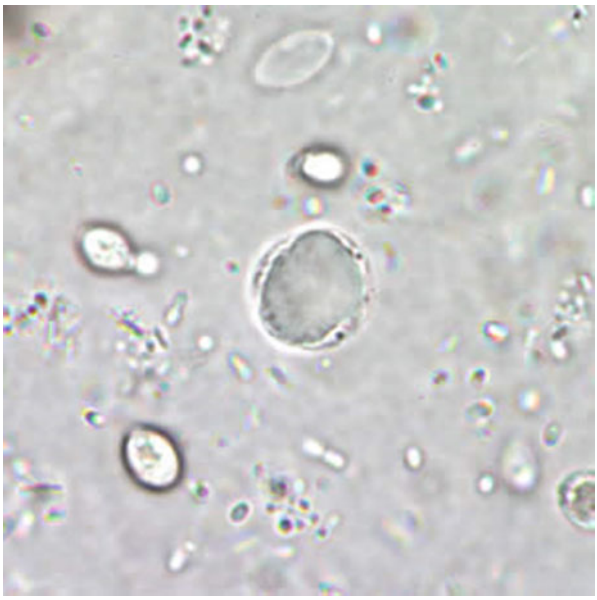


Fig. 5 Cyst-like forms of *Blastocystis* spp. on wet mount with central vacuole and multiple peripheral nuclei. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/blastocystis/index.html)

Blastocystis in Transplant Recipients

As with normal hosts, the pathologic significance of this organism in transplant recipients is often unclear and should prompt investigation for other infections. However, a handful of small cohort studies have shown that immunocompromised states (such as HIV infection, corticosteroid use, hematologic malignancy, and transplant) are associated with an increased risk of both *Blastocystis* infection and of such infections being symptomatic [84–87]. It is therefore reasonable to consider this as a possible infectious cause of diarrheal illness in transplant recipients that requires treatment, particularly if workup for other infections is negative. There have been a few reported cases of *Blastocystis*-associated diarrheal illness in transplant recipients, and in most such cases, GI symptoms and oocyst clearance were achieved after treatment with metronidazole (although in one patient, several weeks of metronidazole were required in order to achieve oocyst clearance and symptom response) [81, 84, 88, 89].

Entamoeba histolytica

Epidemiology

Entamoeba histolytica is a protozoan parasite that is a leading cause of diarrheal disease worldwide, particularly in developing countries where sanitation is poor. Globally, millions of people are infected with *E. histolytica*, with 55,000 deaths annually attributable to this parasite [90]. In developed countries, *E. histolytica* is seen mostly in institutionalized patients, homosexuals, and as an uncommon cause

of traveler's diarrhea, usually occurring in those who have spent weeks to months traveling. The parasite exists in a cyst stage (the metabolically less active but infectious form) and a trophozoite stage (the metabolically active form which causes invasive disease). Infection occurs by fecal-oral transmission via cysts in contaminated food or water. Cysts do not require environmental incubation and can also be sexually transmitted via fecal-oral contact [68, 69, 91]. Cysts can survive in the environment for weeks to months and are relatively resistant to chlorine but are susceptible to iodine.

Clinical Presentation

Infection with *E. histolytica* can lead to a wide spectrum of clinical presentations, including asymptomatic infection (90% of cases), mild diarrhea, dysentery, and toxic megacolon.

E. histolytica has tropism for the colon, and stools are often bloody. Malnutrition and weight loss occur in about half of cases, and about a third have fever. Patients who are very young, pregnant, immunosuppressed, malnourished, or alcoholic appear to be at higher risk for more severe disease. Amebiasis typically has a subacute onset, often over 1–3 weeks. Occasionally infection can result in chronic diarrhea mimicking IBS.

E. histolytica causes inflammation of the colon and can progress to fulminant colitis; a common complication is the formation of characteristic "flask-shaped ulcers" which can bleed or perforate. Fulminant colitis can also progress to toxic megacolon which has a high mortality rate. The formation of "amebomas" (intestinal masses caused by *E. histolytica* which can mimic a colonic neoplasm or appendicitis) is another possible complication of amebiasis.

E. histolytica infection can also lead to extra-intestinal complications, with the most common being liver abscesses. Amebic liver abscesses (ALA) are ten times more common in men than women, often in young adults and children; other risk factors include alcoholism, diabetes, pregnancy, malignancy, and corticosteroid use. Amebic liver abscesses typically cause fever and right upper quadrant abdominal pain, often without associated diarrhea. Hepatic dissemination can occur years after initial infection which is why remote exposures are still significant and even asymptomatic luminal disease should be treated (see below). Amebic liver abscesses are typically single lesions in the right hepatic lobe and have a classic "anchovy paste" appearance of necrotic debris on aspiration. Rarely hepatic abscesses can also rupture into the lung or pericardium [13, 39]. Leukocytosis is common, but eosinophilia does not occur and should prompt consideration of other diagnoses. Clinically, ALAs can be difficult to distinguish from pyogenic liver abscesses. Very rarely, *E. histolytica* can disseminate to the brain, skin, or other organs [13, 90].

Diagnosis

Available techniques for the diagnosis of amebiasis include nucleic acid detection, antigen detection, microscopy, and serology. When performed, colonoscopy can also aid in the diagnosis; colonoscopy can show flask-shaped ulcers grossly, a necrotic

ulcer base with surrounding minimal inflammation, and occasionally cysts or trophozoites microscopically.

PCR assays are the most sensitive and specific tests and can be performed on stool or other specimens (such as liver abscess fluid). PCR is highly specific and is 100 times more sensitive than fecal antigen tests [92]. PCR is also species-specific, detecting only *E. histolytica*, but not other nonpathogenic *Entamoeba* spp. Three commercially available panels (BioFire FilmArray, Luminex xTAG Gastrointestinal Pathogen Panel, and the BD MAX Enteric Parasite Panel) include assays for *E. histolytica* (Table 1). However, validation studies of sensitivity and specificity for BioFire and BD MAX were limited by low prevalence in the study setting [15, 16]. Luminex xTAG sensitivity was 100% and specificity was >99% [17].

Antigen detection assays are relatively easy to perform and are more sensitive and specific than stool microscopy but are still less sensitive than PCR. These assays are specific for *E. histolytica* and are not falsely positive in patients infected with nonpathogenic species such as *Entamoeba dispar*. Unlike serologic assays, they indicate active infection (not just prior exposure), which renders them useful in highly endemic areas. They can be performed on stool, serum, or liver abscess material [92].

Diagnosis of intestinal amebiasis was traditionally made by examination of stool specimens, but this method requires considerable expertise and is both insensitive and nonspecific. Examinations of wet mounts or stool stained with iron-hematoxylin or trichrome can be done to facilitate visualization of cysts or trophozoites (Fig. 6). However, several nonpathogenic *Entamoeba* species (*E. dispar*, *E. moshkovskii*, *E. bangladeshi*) are morphologically indistinguishable from *E. histolytica*. The finding of RBCs ingested within the cytoplasm of amebae suggests pathogenicity

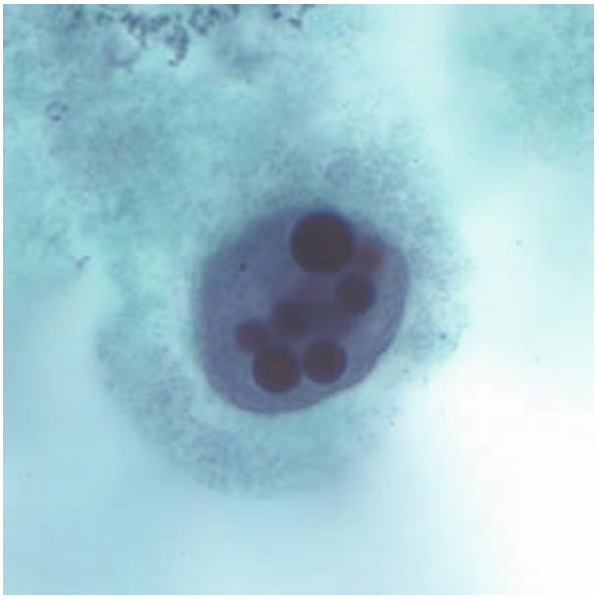


Fig. 6 Trichrome stain of *Entamoeba histolytica* containing multiple ingested dark erythrocytes. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/amebiasis/index.html)

and *E. histolytica* infection, although this finding is not entirely pathognomonic and can also be seen with *E. dispar*. In addition, WBCs are often absent in the stool of patients with *E. histolytica*, as the WBCs are destroyed by the trophozoites.

IgG antibodies against *E. histolytica* typically become detectable 5–7 days after infection and persist for years. However, serology is less useful for the diagnosis of acute intestinal disease because the sensitivity is not high early after infection, and a positive test is not specific for recent infection (as this can also indicate prior exposure). That said, serology can be helpful in travelers who live in developed countries with low epidemiologic risk of prior exposure. The sensitivity of serology is excellent in patients with amebic liver abscesses, rendering the negative predictive value in this clinical scenario quite high. Of note, *E. dispar* and other nonpathogenic species do not induce antibody development. Importantly, serologic assays can be falsely negative in immunosuppressed patients [92] (Table 1).

Treatment

All patients infected with *E. histolytica* should be treated with antimicrobials, including asymptomatic carriers because of the risks of subsequent invasive disease and of transmission to others. Different antimicrobials are required for treatment of the trophozoite (invasive) and cyst (luminal/infectious) forms of *E. histolytica*. Treatment of amebic colitis is with either tinidazole or metronidazole to kill the trophozoites; tinidazole is generally better tolerated (Table 2). Nitazoxanide might be an alternative to these agents, with high cure rates in a limited number of studies. To eradicate the luminal cyst forms, this course is followed by treatment with paromomycin; diloxanide and iodoquinol are alternatives but are not commercially available in the United States (except possibly from compounding pharmacies). Paromomycin commonly causes gastrointestinal side effects and so should not be started until after treatment with the anti-trophozoite agent is complete. Patients with amebic liver abscesses (and other forms of extra-intestinal amebiasis) are treated with the same agents, although often with longer courses; liver abscesses sometimes require drainage (e.g., those that are especially large, impending rupture, or responding poorly to medical therapy), although less commonly than with pyogenic liver abscesses. Patients who are asymptomatic cyst carriers only require treatment with a luminal agent such as paromomycin [90] (Table 2).

E. histolytica in Transplant Recipients

Although data are limited, immunosuppression may increase the risk for severe colitis from *E. histolytica* infection; one review described 24 cases of fulminant amebic colitis in patients who had all been exposed to corticosteroids. Although the rate of fulminant colitis in patients exposed to steroids or other forms of immunosuppression was not compared to immunocompetent patients, the majority of patients in this study received high-dose corticosteroids before they developed new or worsening symptoms, suggesting that the steroids contributed to the severity of the infection. All of the patients were given steroids due to an initial misdiagnosis of another cause of colitis or for another condition [93]. The clinical course of these patients varied from rapid clinical improvement in response to antiparasitic treatment

to prolonged diarrhea and even death in one patient [94–97]. Given the paucity of data regarding *E. histolytica* infection in transplant patients, it is unclear whether this cohort is at increased risk of severe infection. However, this review highlights the potential risk of adverse outcomes when patients with amebic colitis are treated with steroids, along with the potential pitfall of misdiagnosis of GVHD.

Data regarding the risk of extra-intestinal amebiasis in transplant recipients are even more scant. Extrapolating from another immunosuppressed population, there does not appear to be an increased risk of *E. histolytica* acquisition or extra-intestinal disease in HIV-infected patients [98].

Dientamoeba fragilis

Epidemiology

Dientamoeba fragilis is an intestinal protozoan flagellate that has a worldwide distribution, although it is more common in children and in developing countries. The organism infects the colon and is presumably spread by the fecal-oral route (although this has not yet been definitively determined), with incidence higher in areas with poor sanitation. *Enterobius vermicularis* (pinworm) has also been implicated as a possible transmission vector. Although *D. fragilis* infection can occur in the absence of pinworm, infection with pinworms may facilitate *D. fragilis* infection; therefore, identification and treatment of both infections may be necessary for cure [99, 100].

Clinical Presentation

As with *Blastocystis*, there are conflicting data regarding whether *D. fragilis* can cause symptomatic infection or is just a nonpathogenic colonizer. However, mounting evidence suggests that there is likely a spectrum of disease ranging from an asymptomatic carrier state to acute or chronic diarrheal illness. Intermittent diarrhea, abdominal discomfort, and fatigue are the most common symptoms. Associated symptoms can also include anorexia, weight loss, and nausea. Along with *Cystoisospora belli*, *Dientamoeba fragilis* is one of the few protozoa that can cause peripheral eosinophilia, which is reported in about half of infected patients; *D. fragilis* has also been identified as a cause of eosinophilic colitis [99, 100].

Diagnosis

Dientamoebiasis is traditionally diagnosed by stool examination. Cysts are rarely identified, and the trophozoite stage degrades after just 24–48 h outside the human body. Stool samples must therefore be quickly and carefully fixed and stained (e.g., with trichrome) to detect the fragile trophozoites (Fig. 7). Furthermore, stool concentration methods destroy the trophozoite, which is undetectable by these means. Overall, stool examinations are not sensitive for the diagnosis of *D. fragilis* [100]. Stool PCR assays for *D. fragilis* are the most sensitive and specific assays available, but this organism is not included in most commercially available GI PCR panels

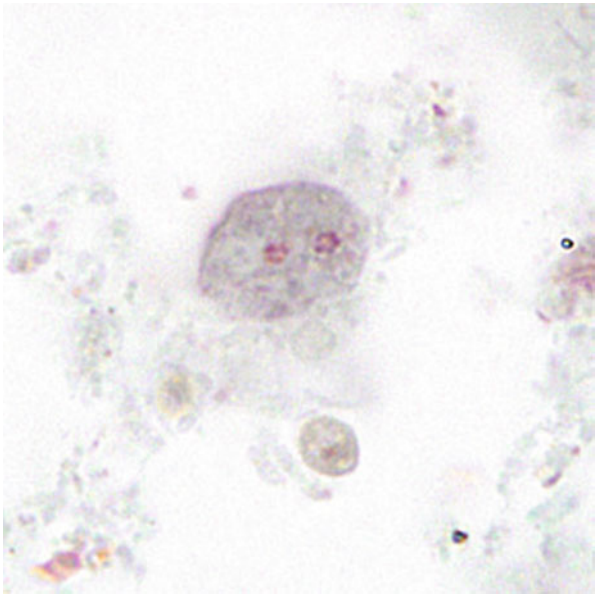


Fig. 7 Trichrome stain of the binucleate trophozoite of *Dientamoeba fragilis.* (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/dientamoeba/index.html)

including the BioFire FilmArray GI Panel. Furthermore, there is also a risk of false-positive results due to cross-reactivity with other trichomonada such as *Chilomastix mesnili*, a nonpathogenic intestinal protozoa [101] (Table 1).

Treatment

When *D. fragilis* is identified and considered etiologic in symptomatic patients, treatment options include paromomycin, iodoquinol, or metronidazole (Table 2). Secnidazole or ornidazole has also shown efficacy in single studies [100]. Treatment with paromomycin appears to result in reliable parasite clearance and symptom resolution; in one study, 98% of patients infected with *D. fragilis* who received paromomycin experienced clearance of the parasite, compared to a 41% clearance rate in patients who were not treated and a 57% clearance rate in patients treated with metronidazole [102].

Additionally, *D. fragilis* infection is frequently associated with (and perpetuated by) pinworm infection. Therefore, testing and treating for *E. vermicularis* are recommended in patients infected with *D. fragilis*.

D. fragilis in Transplant Recipients

There is minimal published literature that addresses *D. fragilis* infection in transplant recipients, aside from reports that simply describe the occasional identification of this organism in stool samples of immunocompromised patients [103]. One study in Argentina compared stool samples of 82 asymptomatic HIV-positive patients to 300 asymptomatic HIV-uninfected patients; the patients infected with HIV had higher rates of *D. fragilis* infection, but literature evaluating the risk of *D. fragilis* infection in HIV/AIDS is also limited [104]. Although it is unknown whether

immunocompromised patients are at higher risk of infection or symptomatic disease from *D. fragilis*, it should still be considered as a potential cause of diarrheal illness in transplant recipients.

Balantidium coli

Epidemiology

Balantidium coli is a ciliated protozoan parasite that is mostly found in tropical and subtropical regions. Transmission is by fecal-oral route via ingestion of cysts, and pigs and monkeys are common animal reservoirs. Close contact with pigs is therefore a risk factor for infection, although person-to-person transmission also occurs. *B. coli* cysts are resistant to chlorine [13, 105].

Clinical Presentation

B. coli infection causes a range of clinical presentations, from asymptomatic infection to acute colitis and rarely fulminant disease (e.g., severe dysentery with colonic ulcers, intestinal perforation, and sepsis). Case reports also describe pulmonary involvement from *B. coli*, presumed to be secondary to hematogenous spread in patients with fulminant disease. *B. coli* is uncommon and under-recognized. Occasionally, *B. coli* causes chronic colitis characterized by chronic abdominal pain and bloody diarrhea [13, 105].

Diagnosis

Diagnosis is by detection of trophozoites or cysts on stool examinations. *B. coli* trophozoites are large ciliates with a characteristic large vacuole which can be seen at low magnification (×100) on wet mount (Fig. 8). Repeated stool samples may be required to diagnose this infection due to intermittent excretion.

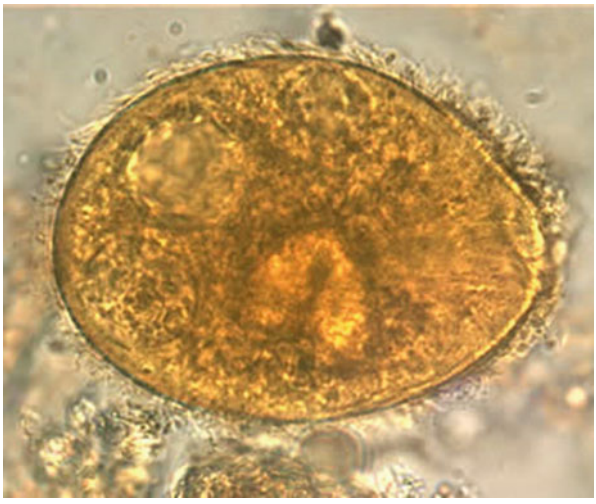


Fig. 8 Trophozoite of *Balantidium coli* on wet mount with characteristic cilia, bean-shaped nucleus, and large central vacuole. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/balantidiasis/index.html)

Treatment

First-line treatment is with tetracycline or metronidazole (Table 2). Alternatives include iodoquinol or nitazoxanide [106] (Table 2).

B. coli in Transplant Recipients

Although data in the transplant population is extremely limited, the risks of both infection and severe disease may be higher in immunocompromised hosts. Two cases of severe diarrhea due to *B. coli* in HIV-infected patients have been reported, and two other reports described *B. coli* infection leading to pulmonary disease in immunocompromised cancer patients [107–110].

Intestinal Helminths

Helminths are multicellular worms that range in size from microscopic to many meters in length. Tissue-invasive helminth infections usually cause peripheral eosinophilia, while helminth infections limited to the gastrointestinal tract usually result in low-grade eosinophilia at most (or no eosinophilia at all).

Over a quarter of people worldwide are infected with at least one helminth species. The most common intestinal helminths of humans are *Ascaris lumbricoides*, *Trichuris trichiura*, and the human hookworms [111]. The prevalence of helminths is highest in developing countries and in areas where sanitation is poor. In contrast, transplant services are more widely available in developed countries and are more accessible to people of higher socioeconomic status. Reports of helminths in transplant recipients are therefore uncommon. However, as transplants become more widely available globally, the number of transplant recipients with helminth infections will increase.

For transplant recipients with exposure to highly endemic areas, intestinal helminths should be considered in the appropriate clinical setting [112]. Recognition of exposure risk is critical since symptoms can be nonspecific, diagnostic tests are insensitive, and illness can occur even years after exposure.

In general, transplant is not a risk factor for the acquisition of intestinal helminth infections nor for severe disease once infected. Most helminths do not reproduce in humans, and adult worms die of senescence even in the absence of chemotherapy. Therefore, replication is not increased in immunosuppressed patients, and infection is not maintained after the natural lifespan of the adult worms passes, even in the absence of antiparasitic therapy. The major exception to this is *Strongyloides stercoralis,* as the risk of severe disease in transplant recipients with strongyloidiasis is much higher due to this organism's ability to "autoinfect" and thus replicate in humans, resulting in lifelong persistence of the infection in the absence of antiparasitic therapy. Dissemination of large numbers of organisms is therefore possible in the context of immunosuppression. Two other intestinal helminths with autoinfective cycles and the theoretical ability to cause hyperinfection are *Capillaria*

philippinensis and *Hymenolepis nana*, although hyperinfection in transplant recipients infected with these parasites has yet to be well documented.

Here we describe several intestinal helminths and review the few published case reports of helminthiases in transplant recipients.

Intestinal Nematodes

Intestinal nematodes (roundworms) are round, non-segmented worms with elongated, cylindrical bodies. The sexes are separate, with fertilized females producing eggs that subsequently give rise to larvae. After molting, larvae become adult worms.

Soil-Transmitted Helminths

Worldwide, the most common human intestinal helminths are *Ascaris lumbricoides*, *Trichuris trichiura* (whipworm), and the human hookworms *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus*. These organisms are also termed the *soil-transmitted helminths* (STHs) because their eggs or larvae must develop in soil before becoming infectious. The soil-transmitted helminths (*Strongyloides stercoralis*, which is sometimes grouped with the STHs, excepted) do not reproduce in humans, and thus infection burden is limited to the inoculum acquired during initial infection. Because of this, even in the absence of effective chemotherapy, patients living in non-endemic areas who have not traveled abroad for many years are unlikely to have active infections, since these parasites would have already died of senescence by then.

Ascaris lumbricoides

Epidemiology

Ascaris lumbricoides is the most common helminth infection of humans, with an estimated prevalence of around 800 million persons globally; infection is most common in children. Ascariasis is globally distributed but is most abundant in developing countries in the tropics. Adult *Ascaris* worms are typically creamy or pink-white, non-segmented, and 15–45 cm long and live in the small intestine; they are one of the few grossly visible intestinal nematodes (Fig. 9). Eggs are passed in the feces of infected persons; once they reach warm, moist soil, they become infectious after an average of 2–6 weeks. Humans usually acquire the infection by ingestion of these eggs via contaminated food (or water); eggs then hatch in the small intestine and release larvae that penetrate the intestine and migrate to the lungs a few days later. Over the week or so that follows, they ascend the tracheobronchial tree, are swallowed, and return to the intestines, where they mature into adult worms. Egg production begins approximately 2 months after infection, and adult worms have a

Fig. 9 Adult *Ascaris lumbricoides* worm, which can grow up to 20–45 cm long. Note round, smooth, non-segmented appearance. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/ascariasis/index.html)

natural lifespan of 1–2 years. Each adult female worm can produce over 200,000 eggs daily. Humans can also become infected by the closely related helminth *Ascaris suum*, a parasite primarily of pigs. Although there has been debate about whether this represents a unique species, current consensus is that it indeed does.

Clinical Presentation

Most people infected with *Ascaris* are asymptomatic, although a small proportion develop pulmonary symptoms (such as a cough) while larvae migrate through the lungs 1–2 weeks after infection. Eosinophilia may accompany this, as can eosinophilic pneumonitis/Loeffler's syndrome. With most established infections, adult worms in the small bowel lumen provoke no symptoms or produce only mild abdominal pain, nausea, or anorexia. However, heavier infections can cause malnutrition, especially in children living in endemic areas. Other complications of chronic ascariasis are rare and are largely due to mechanical issues related to the presence of the large adult worms in the gut, such as intestinal, biliary, or pancreatic obstruction, appendicitis, and intestinal perforation. When these complications occur, they are more common in children from endemic areas. Occasionally, adult worms can pass per rectum, through the mouth or nose or tear ducts.

Diagnosis

Because of the large numbers of eggs produced by each adult female worm, eggs can be readily identified on microscopic stool examinations. PCR assay availability is expanding, but these are not yet in widespread use. Adults are so large that they are grossly visible in the stool, particularly after treatment causes excretion of the worms (Fig. 9).

Treatment

Treatment of ascariasis is with albendazole; single-dose regimens are highly effective, with cure rates that surpass 90–95%; 1–3-day courses of mebendazole are also acceptable. Pyrantel pamoate and ivermectin are also alternatives (Table 4). Common side effects for these and other anti-helminth medications are outlined in Table 3.

Ascaris in Transplant Recipients

There are few reported cases of ascariasis in transplant recipients. In one report from India, *Ascaris* infection was documented in 4 (1.3%) of 297 patients that had undergone HSCT, although this was likely an underestimate [113]. There are no reports which describe an increased risk of infection or severity in transplant recipients. This is similar to the HIV/AIDS patient population, in whom no demonstrable increased risk of infection or severity has been observed [111, 114].

One case report described ascariasis in a renal transplant recipient who developed obstructive jaundice and pancreatitis due to migration of *Ascaris* into the biliary tree [115]. While this is a well-known complication of ascariasis and unlikely to be a result of the patient's underlying immune status, it is a reminder to consider biliary ascariasis in patients with obstructive jaundice or pancreatitis when epidemiologic risk factors are present.

Another case report described numerous *Ascaris* adult worms being excreted after whole body radiation during the preparative regimen for an HSCT [116].

Hookworm Infection

Epidemiology

The human hookworms *A. duodenale*, *A. ceylanicum*, and *N. americanus* are small, gray-white nematodes about 1 cm long that live in the upper small intestine. Globally, around 500 million people are infected with hookworms, mostly in developing tropical areas. *A. ceylanicum* was once considered a primarily zoonotic hookworm that rarely infected humans but has more recently been recognized as a common human hookworm, especially in Asia. As with the other soil-transmitted helminths, eggs passed in the stool of infected persons must develop on soil (ideally warm, moist, and shady); they hatch there within 1–2 days, becoming larvae. Larvae then molt over 5–10 days, subsequently becoming infectious. Following contact with human skin, larvae enter the body and are carried to the lungs. They then move up the trachea, are coughed up and ingested, and migrate to the small intestine. Females start egg deposition 4–6 weeks after infection. Although *N. americanus* can only be transmitted via this percutaneous route, *A. duodenale* and *A. ceylanicum* can also be transmitted orally as well as percutaneously. The adult lifespan averages 3–5 years for *N. americanus* and 1 year for *A. duodenale* and *A. ceylanicum*.

Table 4 Treatment of helminth infections. (Adapted from Kappagoda et al. [18] and Gilbert et al. [19])

Helminth	Preferred regimens	Alternative regimens
Ascaris lumbricoides	1. Albendazole 400 mg PO once *OR* 2. Mebendazole (500 mg PO once *OR* 100 mg PO BID for 3 days)	1. Ivermectin 150–200 mcg/kg PO once *OR* 2. Pyrantel pamoate 11 mg/kg (max 1 g) PO daily for 3 days
Ancylostoma spp. *and Necator americanus*	1. Albendazole 400 mg PO daily for 3 days *OR* 2. Mebendazole 100 mg PO BID for 3 days	Pyrantel pamoate 11 mg/kg (max 1 g) PO daily for 3 days
Trichuris trichiura	Mebendazole 100 mg PO BID ×3–7days	1. Albendazole 400 mg PO QD for 3–7 days *OR* 2. Albendazole 400 mg *plus* oxantel pamoate 20 mg/kg (both PO daily for 2 days) *OR* 3. Albendazole 400 mg *plus* ivermectin 200 mcg/kg (both PO once)
Strongyloides stercoralis	Uncomplicated infection: ivermectin 200 mcg/kg PO daily for 2 days; consider repeating 2 weeks later in immunocompromised individuals. For hyperinfection, many authorities recommend treatment with ivermectin 200 mcg/kg (SQ/veterinary preparation or PO) daily *plus* albendazole 400 mg PO BID until 7–14 days after parasite clearance and possible intermittent secondary ivermectin prophylaxis subsequently	1. Albendazole 400 mg PO BID for 7–14 days *OR* 2. Thiabendazole 25 mg/kg PO BID for 3–7 days
Capillaria philippinensis	1. Mebendazole 200mg PO BID for 20 days *OR* 2. Albendazole 400 mg PO BID for 10 days	
Taenia saginata	Praziquantel 5–10 mg/kg PO once	1. Niclosamide 2 g PO once *OR* 2. Nitazoxanide 500 mg PO BID for 3 days
Taenia solium (intestinal infection)	Praziquantel 5–10 mg/kg PO once	1. Niclosamide 2 g PO once *OR* 2. Nitazoxanide 500 mg PO BID for 3 days
Taenia solium (cysticercosis)	1. Albendazole 400 mg PO BID for 2–4 weeks 2. Praziquantel 33 mg/kg PO TID for 1 day followed by 15 mg/kg PO TID for 2–4 weeks 3. Corticosteroids given before, during, and after treatment to decrease seizure risk ~Decision of whether and how to treat NCC is complex and discussed further in the text and in other sources	

(continued)

Table 4 (continued)

Helminth	Preferred regimens	Alternative regimens
Hymenolepis nana	Praziquantel 25 mg/kg PO once	1. Niclosamide 2 g PO daily for 7 days *OR* 2. Nitazoxanide 500 mg PO BID for 3 days
Diphyllobothrium latum	Praziquantel 5–10 mg/kg PO once	1. Niclosamide 2 g PO once *OR* 2. Nitazoxanide 500 mg PO BID for 3 days
Dipylidium caninum	Praziquantel 5–10 mg/kg PO once	1. Niclosamide 2 g PO once *OR* 2. Nitazoxanide 500 mg PO BID for 3 days
Fasciolopsis buski	Praziquantel 25 mg/kg PO TID for 1 day	

Abbreviations: *PO* oral administration, *BID* twice a day, *TID* three times a day, *mcg* micrograms, *mg* milligrams, *kg* kilograms, *NCC* neurocysticercosis, *SQ* subcutaneous

Clinical Presentation

Hookworms latch onto the intestinal wall where they release an anticoagulant to assist in sucking blood. They therefore cause iron-deficiency anemia as their primary pathophysiologic effect on the human host. During their migration through the lungs, they can also cause an eosinophilic pneumonitis (Loeffler's syndrome), which is less common and less severe than it is with *Ascaris*. Similar to the other STHs, most people infected with hookworm harbor light infections and are asymptomatic. Initial infection may be characterized by "ground itch," a pruritic maculopapular rash at the site of skin penetration. Serpiginous tracks (cutaneous larva migrans) on the skin occur with infection by animal hookworms (e.g., *Ancylostoma braziliense*) but not human hookworms. Epigastric pain, diarrhea, and anorexia may occur about 6–12 weeks after penetration, as larvae begin attaching to the small bowel mucosa. There are important clinical differences between the hookworm species: *N. americanus* is shorter, removes 5–10× less blood, and produces fewer eggs than *A. duodenale*. Some who are infected with adult hookworms experience chronic abdominal pain and eosinophilia. However, the most important manifestations of hookworm disease are iron-deficiency anemia and malnutrition. The anemia from hookworm infection develops gradually but can become severe.

Diagnosis

Diagnosis of hookworm infection is made by identification of eggs on microscopic stool examinations (Fig. 10); concentration techniques may be necessary for the detection of light infections because egg output is not as high as with other STHs. The use of PCR assays for the diagnosis of hookworm infections is becoming more common, but these are not yet in widespread use.

Treatment

Albendazole is the preferred antiparasitic drug for the treatment of hookworm infection. Single-dose albendazole regimens are reasonably effective and are commonly used; however, 3-day regimens yield higher cure rates (Table 4).

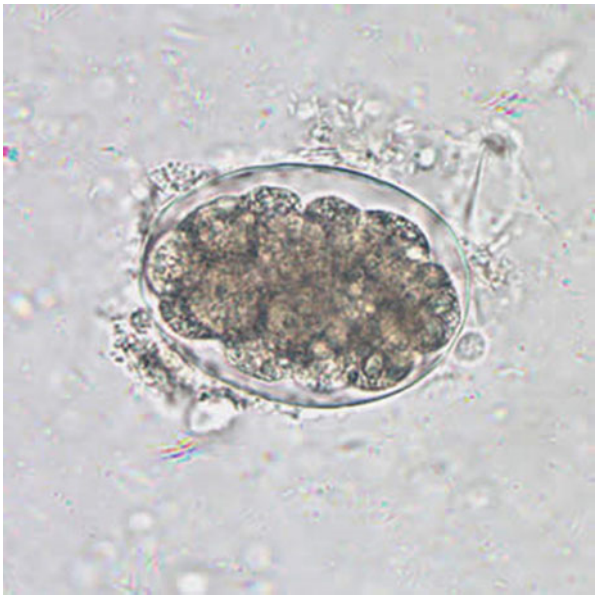


Fig. 10 Thin-walled hookworm egg on an unstained wet mount. *Ancylostoma* and *Necator* eggs are indistinguishable microscopically. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern, https://www.cdc.gov/dpdx/hookworm/index.html)

Hookworm in Transplant Recipients

As with most intestinal helminths, hookworm infection risk and severity are not known to be increased in transplant recipients. Published reports of hookworm infection in this population are scant, although one report describes an HSCT recipient who had a relatively severe case of colitis in the setting of hookworm infection. After developing diarrhea, this allogeneic HSCT recipient was initially thought to have GVDH; however, flexible sigmoidoscopy showed a live hookworm embedded in the intestinal mucosa with surrounding scattered erosions and mild distal sigmoid colitis. He received 7 days of mebendazole with rapid resolution of the diarrhea within 1–2 weeks and subsequent healing of colonic erosions [117]. The severity of diarrhea and colitis in this case was atypical for hookworm infection, which usually causes subacute symptoms and anemia. Furthermore, hookworm typically infects the small intestine, not the colon. This case raises the possibility that profound immunosuppression may worsen the severity of hookworm infection in some patients.

Trichuris trichiura (Whipworm)

Epidemiology

Trichuris trichiura is also known as the whipworm because of its long, whiplike head, which embeds into the intestinal mucosa. It is the third most common roundworm in humans. About 500 million people are infected with *Trichuris*, with highest prevalence in developing countries that have wet and warm tropical climates; children are more commonly infected than adults and are more likely to be

symptomatic from heavy infections. Adult worms are gray-to-pink and 3–5 cm long. They primarily reside in the cecum and ascending colon; with heavy infections, the distribution can extend to the entire colon. Eggs have a distinctive barrel-shaped appearance with a thick shell and bipolar plugs (Fig. 11). *Trichuris* eggs shed in the feces become infective within 2–4 weeks after reaching soil. After human infection occurs, oviposition begins 2–3 months later, and adult worms have a natural lifespan of 1–3 years. Fecal-oral transmission occurs by ingestion of eggs in contaminated food or water.

Clinical Presentation

Most patients with trichuriasis are asymptomatic, and some have mild eosinophilia. With heavy infections (particularly in children), chronic abdominal pain and diarrhea can result, as well as anemia, malnutrition, and growth retardation. The *Trichuris* dysentery syndrome can result, characterized by tenesmus and frequent stools containing mucus and blood. Recurrent rectal prolapse can occur, with adult worms often visible.

Diagnosis

Diagnosis is made by identification of characteristic barrel-shaped eggs with bipolar plugs on microscopic stool examination (Fig. 11). PCR assays using next-generation sequencing techniques are emerging for the diagnosis of trichuriasis but are not yet in widespread use.

Fig. 11 Egg of *Trichuris trichiura* on unstained wet mount; note the thick, barrel-shaped shell and bipolar plugs. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/trichuriasis/index.html)

Treatment

T. trichiura is more difficult to cure than the other STHs. Single-dose cure rates with albendazole or mebendazole are low; this parasite should therefore be treated with longer courses (at least 3–7 days) or combinations of antiparasitic drugs (Table 4). Overall, mebendazole is superior to albendazole for the treatment of *T. trichiura*, and standard courses are 3–7 days of therapy. Alternatives include combinations of albendazole plus oxantel pamoate or albendazole plus ivermectin; in theory, mebendazole could be substituted for albendazole in these regimens, but this has not yet been studied. Several studies suggest that combination regimens result in higher cure rates than single-dose regimens for trichuriasis (Table 4).

T. trichiura in Transplant Recipients

Infection risk and severity of trichuriasis are not known to be higher in transplant recipients, but data are limited. However, one report describes a renal transplant recipient who was treated for rejection with several immunosuppressive agents. He then developed chronic watery diarrhea, anemia, and acute kidney injury. Colonoscopy showed diffuse hyperemia and nonspecific colitis on biopsy, and stool examination showed *T. trichiura* eggs; symptoms resolved after treatment with mebendazole for 3 days [118]. This case raises the possibility that trichuriasis might be more severe in transplant recipients, especially when immunosuppression is increased in the setting of treatment for rejection.

Strongyloides stercoralis

S. stercoralis is an intestinal nematode that is primarily transmitted through skin contact with infested soil in much of the tropical and subtropical developing world. *S. stercoralis* infection is usually asymptomatic, but mild abdominal pain, diarrhea, or other gastrointestinal symptoms can be seen in a minority of infected patients; like with *Ascaris* and hookworm, acute infection can result in eosinophilic pneumonitis/Loeffler's syndrome. Unlike almost all other helminths, *S. stercoralis* can complete its life cycle in the human host through a process termed *autoinfection*, which results in several relatively unique characteristics among helminths including lifelong persistence in a host (in the absence of treatment), multiplication in the absence of exogenous reinfection, and potential person-to-person transmission. The primary medical importance of this parasite in the developed world lies in its potential to cause the hyperinfection syndrome (mostly in immunocompromised patients), wherein large numbers of larvae may disseminate throughout the body, with mortality rates of up to 70–80%; serologic screening of transplant candidates/recipients who have epidemiologic exposure is important, with treatment of all infected persons (with ivermectin; Table 4), in part to prevent the subsequent possibility of hyperinfection syndrome. Strongyloidiasis is a major concern in transplant recipients and is discussed in more detail in ► Chap. 53, "Strongyloides: Emerging Challenges and Prevention."

Other Intestinal Nematodes

Capillaria philippinensis

Epidemiology

Capillaria philippinensis is a parasite of fish-eating birds that is acquired through ingestion of undercooked or raw fish. Capillariasis is endemic mainly to Southern/Eastern Asia (especially Thailand and the Philippines), though infections have also occasionally been acquired elsewhere.

Clinical Presentation

The clinical presentation of patients infected with *C. philippinensis* can include chronic, watery diarrhea which can be severe and cause both dehydration and malnutrition. Fever, abdominal pain, and eosinophilia are often present. Untreated, the infection can be fatal within a few months. Similar to *Strongyloides*, *C. philippinensis* is one of the few intestinal helminths which can reproduce in humans via autoinfection (such that the burden of disease is not only a result of the initial inoculum). This can potentially result in hyperinfection with large numbers of worms disseminating throughout the body, especially in the setting of immune compromise, which makes this parasite a particular concern in transplant recipients.

Diagnosis

The diagnosis of *C. philippinensis* is made by identifying larvae or eggs on microscopic stool examinations. The eggs are oval and have bipolar plugs and can be confused with those of *T. trichiura* (Fig. 12).

Fig. 12 *Capillaria philippinensis* egg on unstained wet mount with bipolar prominences which can be confused with *Trichuris trichiura.* (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/intestinalcapillariasis/index.html)

Treatment

All infected patients should be treated, both to alleviate symptoms and prevent subsequent hyperinfection. Prolonged courses of albendazole or mebendazole are preferred; relapse is common (Table 4). Of note, a closely related helminth, *Capillaria hepatica*, also rarely infects humans through ingestion of food or water contaminated by infected animal stool and usually leads to hepatic egg deposition, resulting in fibrosis.

C. philippinensis in Transplant Recipients

Although the life cycle of *C. philippinensis* suggests that there is a risk of hyperinfection and dissemination in immunocompromised patients infected with this organism, there are no reported cases of capillariasis causing this syndrome in transplant recipients; this association therefore remains theoretical for now.

Intestinal Cestodes

Intestinal cestodes are tapeworms, characterized by long, flat, segmented bodies which reside primarily in the gastrointestinal tract. Cestodes attach via their head (scolex) to the intestinal wall, and distal to this are numerous segments (proglottids). These proglottids can detach from the rest of the worm in some species (e.g., *Taenia* spp.) and be excreted in the stool, where the grossly visible proglottids or the microscopic eggs they contain can be found; in other species (e.g., *Hymenolepis*), only the eggs are found in the stool.

Taenia saginata (Beef Tapeworm)

T. saginata occurs worldwide, with transmission intensity highest in developing countries and where consumption of undercooked beef is common. Human infection is acquired via consumption of undercooked beef infested with *T. saginata* cysticerci. About 50 million people are infected worldwide, with the highest prevalence in East Africa, Indonesia, and China. Humans infected with *T. saginata* usually only have a few tapeworms in their intestinal tract, although these can be 4 m in length or longer (Fig. 13). Adult *T. saginata* tapeworms begin oviposition 2–3 months after initial infection and have a natural lifespan of a few years. Most humans who are infected with *T. saginata* are asymptomatic. A minority develop abdominal pain, weight loss, or nausea, and some have low-grade eosinophilia. Some patients also notice proglottids in their stool or anus. Diagnosis is made through the identification of eggs or proglottids on stool examinations (Fig. 14); several specimens should be obtained to increase diagnostic yield, since excretion is intermittent. Molecular assays are being developed for the detection of taeniasis but are not yet in widespread use. Treatment is with praziquantel, which results in a high cure rate; niclosamide (and possibly nitazoxanide) is an alternative (Table 4).

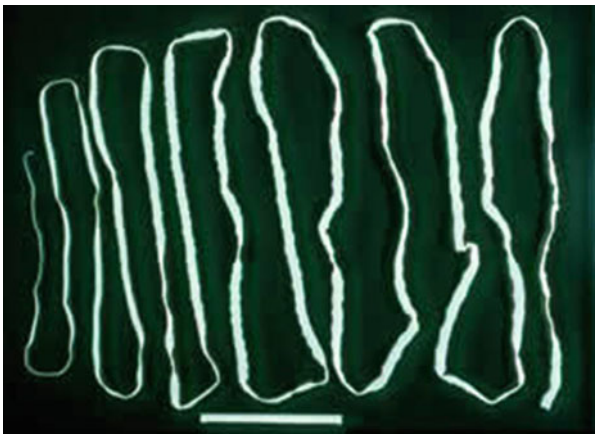


Fig. 13 *Taenia saginata* adult worm (about 4 m in length); note long, segmented, flat appearance. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/taeniasis/index.html)

Taenia solium (Pork Tapeworm)

Epidemiology

T. solium is endemic to much of the developing world, especially where free-range pigs are raised. Taeniasis, intestinal infection with the adult tapeworm, results from human consumption of undercooked pork infested with cysticerci (larval cysts). Cysticercosis (tissue infection with *T. solium* larval cysts) results from ingestion of *T. solium* eggs, which are spread by fecal-oral transmission from a person with intestinal taeniasis; the prevalence of cysticercosis is estimated at 50 million globally. Humans infected with *T. solium* usually have only a few tapeworms in their intestinal tract, although these are usually 3–6 m in length. Adult *T. solium* tapeworms begin oviposition 2–3 months after initial infection and have a natural lifespan of a few years.

Clinical Presentation

The clinical presentation of *T. solium* tapeworm carriers is similar to that of *T. saginata*. Cysticercosis involving the subcutaneous tissue or skeletal muscle is usually asymptomatic. With neurocysticercosis (NCC), cysts that disseminate to the CNS can cause seizures, hydrocephalus, or chronic meningitis.

Diagnosis

Diagnosis is by identification of eggs or proglottids on microscopic stool examinations for intestinal disease (Fig. 14). Of note, *T. solium* cannot be differentiated morphologically from *T. saginata*. Serology is an important means of diagnosis for cysticercosis due to *T. solium* and is highly sensitive, especially the enzyme-linked immunoelectrotransfer blot (EITB) assay. Brain imaging can be useful for the diagnosis of NCC, with CT scans revealing calcifications and MRIs revealing the cyst and sometimes the scolex; diagnostic criteria have been published that can aid with the diagnosis of NCC [119].

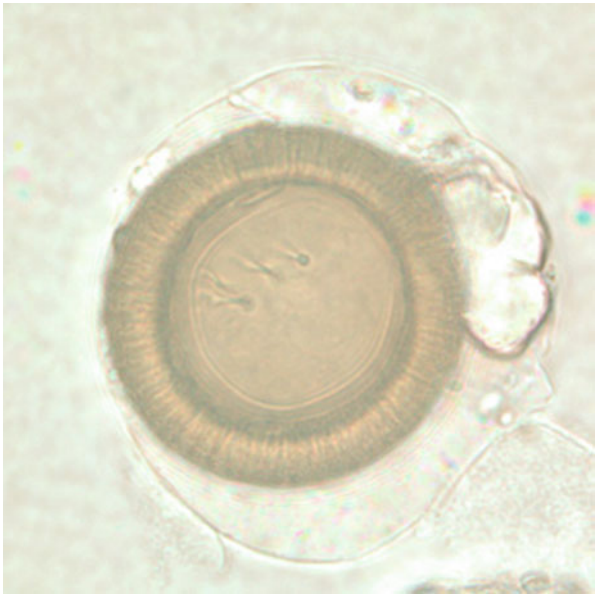


Fig. 14 Unstained wet mount of *Taenia* spp. egg with characteristic radially striated shell and refractile hooks. *T. saginata and T. solium* eggs are indistinguishable morphologically. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/taeniasis/index.html)

Treatment

Although intestinal *T. solium* infection is usually asymptomatic, patients should always be treated to prevent subsequent cysticercosis; most patients with intestinal taeniasis should be screened with brain imaging prior to treatment to identify potential NCC, which could change management. Praziquantel is the first-line treatment for intestinal taeniasis, with niclosamide (and possibly nitazoxanide) as alternative (Table 4). The decision to treat NCC with antiparasitic agents is complex and beyond the scope of this chapter; in general, the location, number, and type of cysts and clinical manifestations should be considered, with most authorities now recommending antiparasitic treatment for most forms of NCC. Corticosteroids are given concurrently with antiparasitic therapy to decrease the inflammatory response and risk of seizures as the parasite degenerates; patients should be screened for strongyloidiasis and tuberculosis prior to the administration of corticosteroids. In general, patients with intraparenchymal cysts should be treated with albendazole; emerging data indicate that albendazole plus praziquantel combination therapy may be superior to albendazole alone, especially if there are more than two cysts [120]. Patients with only intraparenchymal calcifications generally do not require antiparasitic therapy. Patients with subarachnoid cysts should generally receive prolonged courses of antiparasitic drugs plus corticosteroids. Surgical removal is indicated for intraventricular, intraocular, and spinal cysts. Intraocular cysts should be excluded before initiating antiparasitic therapy for cysticercosis. If present, intraocular cysts should be surgically removed before administration of antiparasitic treatment to avoid irreversible eye damage due to the resulting inflammatory response. Importantly, the index patient and close/household contacts should be

screened for intestinal taeniasis so that they may be treated with praziquantel to prevent ongoing transmission.

T. solium in Transplant Recipients

Globally, neurocysticercosis is common, and there have been cases reported in transplant recipients, including in two liver, one kidney, and two HSCT recipients. In one case, symptom onset was about 6 years after transplant [121], but in the others, neurologic symptoms began a few months after transplant [122–124]. Three of these five patients were treated with antiepileptics, antiparasitics, and corticosteroids with good clinical response. The other two had more complicated courses, as described below.

One liver transplant recipient who was found to have multiple small NCC cysts was treated with antiepileptics, steroids, and praziquantel 25 mg/kg three times a day. Four months later, the original cysts had regressed but new NCC cysts had developed. He was then treated for an additional 3 months with albendazole without recurrence of disease [123].

One HSCT recipient had a single NCC cyst incidentally noted on brain MRI 4 months after HSCT, with serologic testing also positive for *T. solium*. Antiparasitic/steroid treatment was deferred, and the cyst resolved spontaneously 7–8 months later [125].

The role of transplant in these cases is unclear, although the development of symptoms or new lesions shortly after transplant in some cases suggests the possibility of an etiologic role. However, more data are needed to evaluate the natural history of NCC in transplant recipients.

Hymenolepis nana

Epidemiology

Hymenolepis nana (the "dwarf tapeworm") is smaller than other cestodes (~0.5 cm long) and attaches to the ileum. Unlike other tapeworms, *H. nana* can complete its life cycle in a single host (through autoinfection). The natural lifespan of adult worms is only 4–6 weeks, but because of autoinfection, long-term persistence of the infection is possible within a given host. *H. nana* is one of the most common human tapeworm infections worldwide, with a prevalence of about 75 million. It is spread by the fecal-oral route and is thus most prevalent in warm, developing areas with poor sanitation; children are most commonly infected. Eggs are immediately infectious upon excretion in the stool and cannot survive more than 10 days in the external environment.

Clinical Presentation

Most patients infected with *H. nana* are asymptomatic, although with heavy infections (especially among children) chronic diarrhea or abdominal discomfort may be present.

Diagnosis

Diagnosis is made by the identification of eggs or proglottids on microscopic stool examinations which are evident about 3–4 weeks after initial infection (Fig. 15). Low-grade eosinophilia may be present in those infected with *H. nana*.

Treatment

Given the ability of *H. nana* to cause persistent or even increasing burden of infection with time (due to autoinfection), all infected patients should be treated. The preferred agent is praziquantel, although at higher doses than for other cestodes (Table 4). Niclosamide and nitazoxanide are alternatives, but longer courses are needed if one of these agents is used.

H. nana in Transplant Recipients

The risk of infection with *H. nana* does not seem to be increased in the transplant population. In a review of 706 stool samples from renal transplant recipients in Iran, only 2 (0.2%) samples were positive for *H. nana* [126].

Because *H. nana* is one of the few intestinal helminths which can complete its life cycle in the human host, hyperinfection is a theoretical possibility in immunocompromised patients. However, hyperinfection with *H. nana* in transplant recipients has not been described. One case report from Malaysia and another from India describe *H. nana* ova identified in the stool of renal transplant recipients. One patient had minimal symptoms [127], and the other had 4

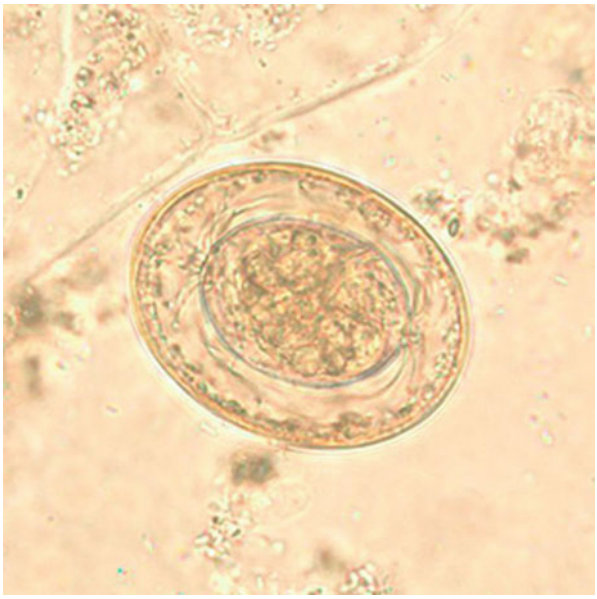


Fig. 15 *Hymenolepis nana* egg on unstained wet mount; note the bipolar filaments. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/hymenolepiasis/index.html)

weeks of abdominal pain and diarrhea, which resolved after treatment with nitazoxanide and praziquantel [128]. These reports are consistent with the natural history of *H. nana* infection in the general population, and there are no reports which suggest that transplant alters this. However, the potential risk that this tapeworm poses to immunocompromised patients is reinforced by a patient with AIDS (CD_4 count, 28 cells/mm^3) who developed malignant transformation of *H. nana* infection. Although extra-intestinal infection with *H. nana* is rare, this process involved his lungs, lymph nodes, and other organs and ultimately led to his death [129].

Diphyllobothrium latum

Epidemiology

D. latum (the "fish tapeworm") is the most common species in this genus that infects humans. Humans become infected through the ingestion of undercooked or raw freshwater fish that are infested with this parasite. *D. latum* is most commonly seen in northern Europe, Russia, North America, Chile, and Japan; the estimated worldwide human prevalence is about 10 million. *D. latum* is the largest human tapeworm, reaching sizes of over 10–15 m, and inhabits the ileum and jejunum; the natural lifespan of adult worms can exceed a decade.

Clinical Presentation

Many patients infected with *D. latum* are asymptomatic, but some develop weakness, dizziness, salt craving, and diarrhea. A minority develop abdominal pain or diarrhea. The parasite interferes with vitamin B12 absorption, so megaloblastic anemia may result from long-term infection. Infected patients not uncommonly see large proglottids pass in their stool.

Diagnosis

The diagnosis can be confirmed through the identification of eggs or proglottids on a microscopic stool examination (Fig. 16); oviposition begins about 4–6 weeks after infection. Some patients may develop mild eosinophilia.

Treatment

First-line treatment is with a single dose of praziquantel or, alternatively, niclosamide or nitazoxanide (Table 4).

D. latum in Transplant Recipients

Reports of *D. latum* in transplant recipients have been sparse. One report describes severe infection in an autologous HSCT recipient. In this case, the patient had mild abdominal pain and diarrhea for several months preceding transplant. Three days posttransplant, he developed severe diarrhea which lasted for over a month until stool examination ultimately revealed *D. latum* ova. He was treated with a single dose of praziquantel with improvement of diarrhea, but then had symptomatic and

Fig. 16 Unstained wet mount of a *Diphyllobothrium latum* egg which is characterized by an operculum on one end and a small knob on the other end. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/diphyllobothriasis/index.html)

parasitologic relapse 1 week later, which necessitated a second dose of praziquantel [130]. This case suggests the possibility that transplant recipients may be at higher risk of severe infection from *D. latum* and that longer treatment courses might be necessary for cure.

Dipylidium caninum

Epidemiology

D. caninum (also known as the "dog tapeworm") is the most common intestinal cestode of dogs (and is also found in cats) but occasionally also infects humans as accidental hosts. Part of its life cycle occurs in arthropods, and transmission to mammals (including humans) occurs through the accidental ingestion of infected fleas or lice. Human cases are therefore most commonly seen in young children who have close contact with dogs or cats. *D. caninum* adult worms are usually 15–20 cm in length.

Clinical Presentation

Parasite load is typically low because few infected fleas or lice are typically ingested, and most infected patients are asymptomatic. A minority of infected persons experience mild diarrhea or abdominal discomfort.

Diagnosis

Proglottids are periodically excreted and can occasionally be seen grossly in the diapers or underpants of infected children. Otherwise, the diagnosis is made by identifying ova or proglottids on microscopic stool examination (Fig. 17).

Fig. 17 Adult *Dipylidium caninum* worm made up of proglottid segments, which can break off; if found in a person's underwear, can be mistaken for pinworms. (Courtesy Division of Parasitic Diseases and Malaria/Centers for Disease Control and Prevention, DPDx – Laboratory Identification of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/dipylidium/index.html)

Treatment

Treatment is with single-dose praziquantel; an alternative is niclosamide (Table 4).

D. caninum in Transplant Recipients

There is one case reported of *D. caninum* in a renal transplant recipient who presented with prolonged diarrhea, ultimately diagnosed with *D. caninum* infection by identification of the organism of on stool examination. The patient was treated with niclosamide resulting in symptomatic resolution and clearance of stool [131]. The severity and length of symptoms in this case were unusual for a human infection with *D. caninum*, raising the possibility that this patient's immunocompromised state influenced the course of this infection.

Intestinal Flukes

Flukes (or trematodes) are flatworms that range in size from microscopic to several centimeters in length. There are scores of species of intestinal flukes that infect humans. One of the most common and largest (2–7 cm in length) is *Fasciolopsis buski*, which is endemic mostly to Asia, with highest prevalence in children. Pigs are also hosts of this parasite, and transmission to humans is typically associated with ingestion of contaminated water or aquatic plants (such as watercress or bamboo shoots), especially in areas where pigs are raised. Fasciolopsiasis is usually asymptomatic; when symptoms do occur, they are due to inflammation and ulceration in the intestine. Abdominal pain, diarrhea, eosinophilia, and malabsorption can occur. Diagnosis is made by the examination of microscopic stool examinations. Treatment is with praziquantel (Table 4). While fasciolopsiasis has not been reported in transplant recipients to date, this may become a more common infection in this population and should remain a consideration when risk factors are present.

Conclusion

The differential diagnosis in transplant recipients with a gastrointestinal illness is broad and includes GVHD, adverse effects from medications, bacterial infections, viral infections, and others, in addition to intestinal parasitic infections. Because parasitic infections are less common in the developed nations where transplantation is more common, they are often considered only after initial workup for other etiologies has been undertaken, and even then diagnosis often relies on insensitive stool examinations. Diagnostic delays are therefore common for these otherwise readily treatable infections. There are few reports of infections with intestinal parasites in transplant recipients, but with increasing globalization, these organisms are likely to become increasingly common in this population.

Key Points

- Many intestinal parasites have a worldwide distribution, but most predominate in tropical and subtropical areas where sanitation and hygiene are poor.
- Most intestinal parasites are transmitted by consumption of contaminated food and water; some protozoa such as *Cryptosporidium* spp., *Giardia*, and *E. histolytica* do not require incubation in the environment and can therefore also be transmitted directly from person-to-person.
- There is substantial clinical overlap among the intestinal parasites; most are associated with abdominal pain, diarrhea, or chronic GI symptoms. Some are associated with fulminant infection or extra-intestinal complications, while patients infected with others are often asymptomatic.
- Transplant recipients are at increased risk for severe or recurrent disease from many intestinal protozoal infections and possibly for some intestinal helminths, although data are limited.
- The diagnosis of intestinal parasites is often made through identification on microscopic stool examinations; stool preparations stained with modified acid-fast stains are needed to identify the intestinal coccidia (*Cryptosporidium* spp., *Cystoisospora*, and *Cyclospora*) which are usually missed on standard stool examinations.
- PCR has become increasingly available for the diagnosis of some intestinal protozoa such as *Cryptosporidium* spp., *Cyclospora*, *Giardia*, and *E. histolytica*. PCR testing is much more sensitive and specific than microscopy. However, PCR assays are not commercially available for most other intestinal protozoa and for none of the intestinal helminths, for which diagnosis still relies on insensitive stool examinations.
- Treatment of intestinal parasites in transplant recipients involves antiparasitic medications and in some cases decreasing immunosuppression. Prolonged or combination antiparasitic therapy can also be considered in some cases.

References

1. Minz M, Udgiri N, Heer M, Kashyap R, Malla N. Cryptosporidiosis in live related renal transplant recipients: a single center experience. Transplantation. 2004;77:1916–7. https://doi.org/10.1097/01.TP.0000132341.99938.C1.
2. Silvia F, Simona F, Fabrizio B. Solid organ transplant and parasitic diseases: a review of the clinical cases in the last two decades. Pathogens. 2018;7. https://doi.org/10.3390/pathogens7030065.
3. Kılbaş ZG, Yenİcesu M, Araz E, Tanyüksel M. Cyclospora cayetanensis infection in a patient with renal transplant. Türk Hij Ve Deney Biyol Derg. 2009;66:25–7.
4. Delis SG, Tector J, Kato T, Mittal N, Weppler D, Levi D, et al. Diagnosis and treatment of cryptosporidium infection in intestinal transplant recipients. Transplant Proc. 2002;34:951–2. https://doi.org/10.1016/S0041-1345(02)02712-4.
5. Pozio E, Rivasi F, Cacci SM. Infection with Cryptosporidium hominis and reinfection with Cryptosporidium parvum in a transplanted ileum. APMIS. 2004;112:309–13. https://doi.org/10.1111/j.1600-0463.2004.apm11204-0513.x.
6. Kristensen AA, Horneland R, Birn H, Svensson M. Giardia lamblia infection after pancreas-kidney transplantation. BMJ Case Rep. 2016;2016. https://doi.org/10.1136/bcr-2015-211515.
7. Gruz F, Fuxman C, Errea A, Tokumoto M, Fernandez A, Velasquez J, et al. Isospora belli infection after isolated intestinal transplant. Transpl Infect Dis. 2010;12:69–72. https://doi.org/10.1111/j.1399-3062.2009.00439.x.
8. Juranek DD. Cryptosporidiosis: sources of infection and guidelines for prevention. Clin Infect Dis Off Publ Infect Dis Soc Am. 1995;21(Suppl 1):S57–61.
9. Cama VA, Mathison BA. Infections by intestinal Coccidia and Giardia duodenalis. Clin Lab Med. 2015;35:423–44. https://doi.org/10.1016/j.cll.2015.02.010.
10. Prevention C-C for DC and. CDC – Cryptosporidiosis – fact sheets – a guide to water filters [Internet]. 7 Jan 2019 [cited 16 May 2019]. Available: https://www.cdc.gov/parasites/crypto/gen_info/filters.html.
11. Florescu DF, Sandkovsky U. Cryptosporidium infection in solid organ transplantation. World J Transplant. 2016;6:460–71. https://doi.org/10.5500/wjt.v6.i3.460.
12. Barsoum RS. Parasitic infections in transplant recipients. Nat Rev Nephrol. 2006;2:490–503. https://doi.org/10.1038/ncpneph0255.
13. Manson's Tropical Infectious Diseases [Internet]. Elsevier; 2014. https://doi.org/10.1016/C2010-0-66223-7.
14. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM. Detection of intestinal Protozoa in the clinical laboratory. J Clin Microbiol. 2014;52:712–20. https://doi.org/10.1128/JCM.02877-13.
15. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol. 2015;53:915–25. https://doi.org/10.1128/JCM.02674-14.
16. Madison-Antenucci S, Relich RF, Doyle L, Espina N, Fuller D, Karchmer T, et al. Multicenter evaluation of BD max enteric parasite real-time PCR assay for detection of Giardia duodenalis, Cryptosporidium hominis, Cryptosporidium parvum, and Entamoeba histolytica. J Clin Microbiol. 2016;54:2681–8. https://doi.org/10.1128/JCM.00765-16.
17. Claas EC, Burnham C-AD, Mazzulli T, Templeton K, Topin F. Performance of the xTAG® gastrointestinal pathogen panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. J Microbiol Biotechnol. 2013;23:1041–5.
18. Kappagoda S, Singh U, Blackburn BG. Antiparasitic therapy. Mayo Clin Proc. 2011;86:561–83. https://doi.org/10.4065/mcp.2011.0203.
19. Gilbert D, Eliopoulos G, Chambers H, Saag M, Pavia A. The Sanford guide to antimicrobial therapy. 48th ed. Sperryville: Antimicrobial Therapy, Inc.; 2018.

20. Rossignol J-FA, Ayoub A, Ayers MS. Treatment of diarrhea caused by Cryptosporidium parvum: a prospective randomized, double-blind, placebo-controlled study of Nitazoxanide. J Infect Dis. 2001;184:103–6. https://doi.org/10.1086/321008.
21. Abubakar II, Aliyu SH, Arumugam C, Hunter PR, Usman N. Prevention and treatment of cryptosporidiosis in immunocompromised patients. Cochrane Database Syst Rev. 2007; https://doi.org/10.1002/14651858.CD004932.pub2.
22. Schwartz BS, Mawhorter SD. Parasitic infections in solid organ transplantation. Am J Transplant. 2013;13:280–303. https://doi.org/10.1111/ajt.12120.
23. Bhadauria D, Goel A, Kaul A, Sharma RK, Gupta A, Ruhela V, et al. Cryptosporidium infection after renal transplantation in an endemic area. Transpl Infect Dis. 2015;17:48–55. https://doi.org/10.1111/tid.12336.
24. Ghoshal U, Ranjan P, Dey A, Ghoshal UC. Intestinal cryptosporidiosis in renal transplant recipients: prevalence, species detection and comparative evaluation of SSU rRNA and Cryptosporidium oocyst wall protein genes. Indian J Med Microbiol. 2018;36:247. https://doi.org/10.4103/ijmm.IJMM_18_179.
25. Dey A, Ghoshal U, Agarwal V, Ghoshal UC. Genotyping of Cryptosporidium species and their clinical manifestations in patients with renal transplantation and human immunodeficiency virus infection. J Pathog. 2016;2016:2623602. https://doi.org/10.1155/2016/2623602.
26. Raja K, Abbas Z, Hassan SM, Luck NH, Aziz T, Mubarak M. Prevalence of cryptosporidiosis in renal transplant recipients presenting with acute diarrhea at a single center in Pakistan. J Nephropathol. 2014;3:127–31. https://doi.org/10.12860/jnp.2014.25.
27. Frei P, Weber A, Geier A, Mertens JC, Kohler S, Rogler G, et al. Lessons from a transplant patient with diarrhea, cryptosporidial infection, and possible mycophenolate mofetil-associated colitis. Transpl Infect Dis. 2011;13:416–8. https://doi.org/10.1111/j.1399-3062.2011.00653.x.
28. Legrand F, Grenouillet F, Larosa F, Dalle F, Saas P, Millon L, et al. Diagnosis and treatment of digestive cryptosporidiosis in allogeneic haematopoietic stem cell transplant recipients: a prospective single centre study. Bone Marrow Transplant. 2011;46:858–62. https://doi.org/10.1038/bmt.2010.200.
29. Kagawa K, Fujino H, Miki H, Sogabe K, Takahashi M, Maruhashi T, et al. Cryptosporidiosis in a transplant recipient with severe intractable diarrhea: detection of Cryptosporidium oocysts by intestinal biopsies. Transpl Infect Dis. 2018;20:e12826. https://doi.org/10.1111/tid.12826.
30. Sebastián E, Martín J, McDonald GB, Flores T, Rodríguez A, Blanco A, et al. *Cryptosporidium parvum* infection vs GVHD after hematopoietic SCT: diagnosis by PCR with resolution of symptoms. Bone Marrow Transplant. 2011;46:612–4. https://doi.org/10.1038/bmt.2010.156.
31. Schiller J, Klein S, Engels M, Büttner R, Rybniker J, Fätkenheuer G, et al. Case report: a Cryptosporidium infection in a patient with relapsed T-lymphoblastic lymphoma undergoing allogeneic stem cell transplantation. Eur J Haematol. 2018;100:383–5. https://doi.org/10.1111/ejh.12998.
32. Gauchotte G, Bressenot A. Colonic cryptosporidiosis in allograft patients: a rare differential diagnosis of acute graft-versus-host disease. Hum Pathol. 2010;41:918–9. https://doi.org/10.1016/j.humpath.2010.01.017.
33. Müller C, Zeiser R, Grüllich C, Finke J, Bertz H, Schmitt-Gräff A, et al. Intestinal cryptosporidiosis mimicking acute graft-versus-host disease following matched unrelated hematopoietic stem cell transplantation. Transplantation. 2004;77:1478–9. https://doi.org/10.1097/01.TP.0000121806.42836.45.
34. Lanternier F, Amazzough K, Favennec L, Mamzer-Bruneel M-F, Abdoul H, Tourret J, et al. Cryptosporidium spp. infection in solid organ transplantation: the Nationwide "TRANSCRYPTO" study. Transplantation. 2017;101:826–30. https://doi.org/10.1097/TP.0000000000001503.
35. Bonatti H, Barroso LF, Sawyer RG, Kotton CN, Sifri CD. Cryptosporidium enteritis in solid organ transplant recipients: multicenter retrospective evaluation of 10 cases reveals an association with elevated tacrolimus concentrations. Transpl Infect Dis Off J Transplant Soc. 2012;14:635–48. https://doi.org/10.1111/j.1399-3062.2012.00719.x.
36. Chieffi PP, Sens YA, Paschoalotti MA, Miorin LA, Silva HG, Jabur P. Infection by Cryptosporidium parvum in renal patients submitted to renal transplant or hemodialysis. Rev Soc Bras Med Trop. 1998;31:333–7.

37. Turkcapar N, Kutlay S, Nergizoglu G, Atli T, Duman N. Prevalence of Cryptosporidium infection in hemodialysis patients. Nephron. 2002;90:344–6. https://doi.org/10.1159/000049072.
38. Nachbaur D, Kropshofer G, Feichtinger H, Allerberger F, Niederwieser D. Cryptosporidiosis after CD34-selected autologous peripheral blood stem cell transplantation (PBSCT). Treatment with paromomycin, azithromycin and recombinant human interleukin-2. Bone Marrow Transplant. 1997;19:1261–3. https://doi.org/10.1038/sj.bmt.1700826.
39. Parasitic infections. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2004;4 Suppl 10: 142–155. https://doi.org/10.1111/j.1600-6135.2004.00677.x.
40. Campos M, Jouzdani E, Sempoux C, Buts JP, Reding R, Otte JB, et al. Sclerosing cholangitis associated to cryptosporidiosis in liver-transplanted children. Eur J Pediatr. 2000;159:113–5.
41. Denkinger CM, Harigopal P, Ruiz P, Dowdy LM. Cryptosporidium parvum-associated sclerosing cholangitis in a liver transplant patient. Transpl Infect Dis. 2008;10:133–6. https://doi.org/10.1111/j.1399-3062.2007.00245.x.
42. Dimicoli S, Bensoussan D, Latger-Cannard V, Straczek J, Antunes L, Mainard L, et al. Complete recovery from *Cryptosporidium parvum* infection with gastroenteritis and sclerosing cholangitis after successful bone marrow transplantation in two brothers with X-linked hyper-IgM syndrome. Bone Marrow Transplant. 2003;32:733–7. https://doi.org/10.1038/sj.bmt.1704211.
43. Quarello P, Tandoi F, Carraro F, Vassallo E, Pinon M, Romagnoli R, et al. Successful sequential liver and hematopoietic stem cell transplantation in a child with CD40 ligand deficiency and Cryptosporidium-induced liver cirrhosis. Transplantation. 2018;102:823–8. https://doi.org/10.1097/TP.0000000000002114.
44. Casillas SM, Bennett C, Straily A. Notes from the field: multiple Cyclosporiasis outbreaks — United States, 2018. Morb Mortal Wkly Rep. 2018;67:1101–2. https://doi.org/10.15585/mmwr.mm6739a6.
45. Ortega YR, Sanchez R. Update on Cyclospora cayetanensis, a food-borne and waterborne parasite. Clin Microbiol Rev. 2010;23:218–34. https://doi.org/10.1128/CMR.00026-09.
46. Zar FA, El-Bayoumi E, Yungbluth MM. Histologic proof of acalculous cholecystitis due to Cyclospora cayetanensis. Clin Infect Dis. 2001;33:e140–1. https://doi.org/10.1086/324586.
47. Hoge CW, Shlim DR, Ghimire M, Rabold JG, Pandey P, Walch A, et al. Placebo-controlled trial of co-trimoxazole for Cyclospora infections among travellers and foreign residents in Nepal. Lancet Lond Engl. 1995;345:691–3.
48. Verdier RI, Fitzgerald DW, Johnson WD, Pape JW. Trimethoprim-sulfamethoxazole compared with ciprofloxacin for treatment and prophylaxis of Isospora belli and Cyclospora cayetanensis infection in HIV-infected patients. A randomized, controlled trial. Ann Intern Med. 2000;132:885–8.
49. Diaz E, Mondragon J, Ramirez E, Bernal R. Epidemiology and control of intestinal parasites with Nitazoxanide in children in Mexico. Am J Trop Med Hyg. 2003;68:384–5. https://doi.org/10.4269/ajtmh.2003.68.384.
50. Bednarska M, Bajer A, Welc-Falęciak R, Pawełas A. Cyclospora cayetanensis infection in transplant traveller: a case report of outbreak. Parasit Vectors. 2015;8:411. https://doi.org/10.1186/s13071-015-1026-8.
51. Visvesvara GS, Arrowood MJ, Qvarnstrom Y, Sriram R, Bandea R, Wilkins PP, et al. Concurrent parasitic infections in a renal transplant patient. Emerg Infect Dis. 2013;19:2044–5. https://doi.org/10.3201/eid1912.120926.
52. Pape JW, Verdier RI, Boncy M, Boncy J, Johnson WD. Cyclospora infection in adults infected with HIV. Clinical manifestations, treatment, and prophylaxis. Ann Intern Med. 1994;121:654–7.
53. Pape JW, Verdier RI, Johnson WD. Treatment and prophylaxis of Isospora belli infection in patients with the acquired immunodeficiency syndrome. N Engl J Med. 1989;320:1044–7. https://doi.org/10.1056/NEJM198904203201604.
54. HIV/AIDS Treatment Guidelines. In: AIDSinfo [Internet]. [cited 15 May 2019]. Available: http://aidsinfo.nih.gov/guidelines.

55. Benator DA, French AL, Beaudet LM, Levy CS, Orenstein JM. Isospora belli infection associated with acalculous cholecystitis in a patient with AIDS. Ann Intern Med. 1994;121:663–4.
56. Bernard E, Delgiudice P, Carles M, Boissy C, Saint-Paul MC, Le Fichoux Y, et al. Disseminated isosporiasis in an AIDS patient. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 1997;16:699–701.
57. González-Dominguez J, Roldán R, Villanueva JL, Kindelán JM, Jurado R, Torre-Cisneros J. Isospora belli reactive arthritis in a patient with AIDS. Ann Rheum Dis. 1994;53:618–9. https://doi.org/10.1136/ard.53.9.618-b.
58. Jayshree RS, Acharya RS, Sridhar H. Isospora belli infection in a patient with acute lymphoblastic leukaemia in India. J Diarrhoeal Dis Res. 1996;14:44–5.
59. Ud Din N, Torka P, Hutchison RE, Riddell SW, Wright J, Gajra A. Severe Isospora (Cystoisospora) belli diarrhea preceding the diagnosis of human T-cell-leukemia-Virus-1-associated T-cell lymphoma. Case Rep Infect Dis. 2012;2012:1. https://doi.org/10.1155/2012/640104.
60. Resiere D, Vantelon JM, Bourée P, Chachaty E, Nitenberg G, Blot F. Isospora belli infection in a patient with non-Hodgkin's lymphoma. Clin Microbiol Infect. 2003;9:1065–7. https://doi.org/10.1046/j.1469-0691.2003.00742.x.
61. Meamar AR, Rezaian M, Mirzaei AZ, Zahabiun F, Faghihi AH, Oormazdi H, et al. Severe diarrhea due to Isospora belli in a patient with thymoma. J Microbiol Immunol Infect Wei Mian Yu Gan Ran Za Zhi. 2009;42:526–9.
62. Akateh C, Arnold CA, Benissan-Messan D, Michaels A, Black SM. Cystoisospora belli gallbladder infection in a liver transplant donor. Case Rep Infect Dis. 2018;2018:1. https://doi.org/10.1155/2018/3170238.
63. Atambay M, Bayraktar MR, Kayabas U, Yilmaz S, Bayindir Y. A rare diarrheic parasite in a liver transplant patient: Isospora belli. Transplant Proc. 2007;39:1693–5. https://doi.org/10.1016/j.transproceed.2007.02.080.
64. Yazar S, Tokgöz B, Yaman O, Sahin I. Isospora belli infection in a patient with a renal transplant. Turk Parazitolojii Derg. 2006;30:22–4.
65. Marathe A, Parikh K. Severe diarrhoea due to Cystoisospora belli in renal transplant patient on Immunosuppressive drugs. Indian J Med Microbiol. 2013;31:185. https://doi.org/10.4103/0255-0857.115227.
66. Koru O, Araz RE, Yilmaz YA, Ergüven S, Yenicesu M, Pektaş B, et al. Case report: Isospora belli infection in a renal transplant recipient. Turk Parazitolojii Derg. 2007;31:98–100.
67. Usluca S, Inceboz T, Unek T, Aksoy U. Isospora belli in a patient with liver transplantation. Turk Parazitolojii Derg. 2012;36:247–50. https://doi.org/10.5152/tpd.2012.58.
68. Levine GI. Sexually transmitted parasitic diseases. Prim Care. 1991;18:101–28.
69. Moldwin RM. Sexually transmitted protozoal infections. Trichomonas vaginalis, Entamoeba histolytica, and Giardia lamblia. Urol Clin North Am. 1992;19:93–101.
70. Granados CE, Reveiz L, Uribe LG, Criollo CP. Drugs for treating giardiasis. Cochrane Database Syst Rev. 2012;12:CD007787. https://doi.org/10.1002/14651858.CD007787.pub2.
71. Nash TE, Ohl CA, Thomas E, Subramanian G, Keiser P, Moore TA. Treatment of patients with refractory giardiasis. Clin Infect Dis. 2001;33:22–8. https://doi.org/10.1086/320886.
72. Escobedo A, Lalle M, Hrastnik N, Rodriguez-Morales AJ, Castro Sanchez EM, Cimerman S, et al. Combination therapy in the management of giardiasis: what laboratory and clinical studies tell us, so far. 2016:205. https://doi.org/10.1016/j.actatropica.2016.06.026.
73. Lalle M, Hanevik K. Treatment-refractory giardiasis: challenges and solutions. Infect Drug Resist. 2018;11:1921–33. https://doi.org/10.2147/IDR.S141468.
74. Lopez-Velez R, Batlle C, Jiménez C, Navarro M, Norman F, Perez-Molina J. Short course combination therapy for giardiasis after Nitroimidazole failure. Am J Trop Med Hyg. 2010;83:171–3. https://doi.org/10.4269/ajtmh.2010.09-0742.
75. Mørch K, Hanevik K, Robertson LJ, Strand EA, Langeland N. Treatment-ladder and genetic characterisation of parasites in refractory giardiasis after an outbreak in Norway. J Infect. 2008;56:268–73. https://doi.org/10.1016/j.jinf.2008.01.013.

76. Valar C, Keitel E, Dal Prá RL, Gnatta D, Santos AF, Bianco PD, et al. Parasitic infection in renal transplant recipients. Transplant Proc. 2007;39:460–2. https://doi.org/10.1016/j.transproceed.2007.01.025.
77. Azami M, Sharifi M, Hejazi SH, Tazhibi M. Intestinal parasitic infections in renal transplant recipients. Braz J Infect Dis. 2010;14:15–8. https://doi.org/10.1016/S1413-8670(10)70004-0.
78. Bromiker R, Korman SH, Or R, Hardan I, Naparstek E, Cohen P, et al. Severe giardiasis in two patients undergoing bone marrow transplantation. Bone Marrow Transplant. 1989;4:701–3.
79. Ajumobi AB, Daniels JA, Sostre CF, Trevino HH. Giardiasis in a hematopoietic stem cell transplant patient. Transpl Infect Dis. 2014;16:984–7. https://doi.org/10.1111/tid.12272.
80. Mukku KK, Raju S, Yelanati R. Refractory giardiasis in renal transplantation: a case report. Nephrology. 2015;20:44. https://doi.org/10.1111/nep.12336.
81. Tan KSW. New insights on classification, identification, and clinical relevance of Blastocystis spp. Clin Microbiol Rev. 2008;21:639–65. https://doi.org/10.1128/CMR.00022-08.
82. Nigro L, Larocca L, Massarelli L, Patamia I, Minniti S, Palermo F, et al. A placebo-controlled treatment trial of Blastocystis hominis infection with metronidazole. J Travel Med. 2003;10:128–30. https://doi.org/10.2310/7060.2003.31714.
83. Rossignol J-F, Kabil SM, Said M, Samir H, Younis AM. Effect of nitazoxanide in persistent diarrhea and enteritis associated with Blastocystis hominis. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2005;3:987–91.
84. Ok UZ, Cirit M, Uner A, Ok E, Akçiçek F, Başçi A, et al. Cryptosporidiosis and blastocystosis in renal transplant recipients. Nephron. 1997;75:171–4. https://doi.org/10.1159/000189527.
85. Cirioni O, Giacometti A, Drenaggi D, Ancarani F, Scalise G. Prevalence and clinical relevance of Blastocystis hominis in diverse patient cohorts. Eur J Epidemiol. 1999;15:389–93.
86. Taşova Y, Sahin B, Koltaş S, Paydaş S. Clinical significance and frequency of Blastocystis hominis in Turkish patients with hematological malignancy. Acta Med Okayama. 2000;54:133–6. https://doi.org/10.18926/AMO/32298.
87. Noureldin MS, Shaltout AA, El Hamshary EM, Ali ME. Opportunistic intestinal protozoal infections in immunocompromised children. J Egypt Soc Parasitol. 1999;29:951–61.
88. Ghosh K, Ayyaril M, Nirmala V. Acute GVHD involving the gastrointestinal tract and infestation with Blastocystis hominis in a patient with chronic myeloid leukaemia following allogeneic bone marrow transplantation. Bone Marrow Transplant. 1998;22:1115–7. https://doi.org/10.1038/sj.bmt.1701488.
89. Rao K, Sekar U, Iraivan KT, Abraham G, Soundararajan P. Blastocystis hominis–an emerging cause of diarrhoea in renal transplant recipients. J Assoc Physicians India. 2003;51:719–21.
90. Shirley D-AT, Farr L, Watanabe K, Moonah S. A review of the global burden, new diagnostics, and current therapeutics for Amebiasis. Open Forum Infect Dis. 2018;5. https://doi.org/10.1093/ofid/ofy161.
91. CDC – DPDx – Amebiasis [Internet]. 9 Jan 2019 [cited 13 Apr 2019]. Available: https://www.cdc.gov/dpdx/amebiasis/index.html.
92. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for Entamoeba species. Clin Microbiol Rev. 2007;20:511–32. https://doi.org/10.1128/CMR.00004-07.
93. Shirley D-A, Moonah S. Fulminant amebic colitis after corticosteroid therapy: a systematic review. PLoS Negl Trop Dis. 2016;10:e0004879. https://doi.org/10.1371/journal.pntd.0004879.
94. Numata A, Itabashi M, Kishimoto K, Motohashi K, Hagihara M, Kuwabara H, et al. Intestinal amoebiasis in a patient with acute graft-versus-host disease after allogeneic bone marrow transplantation successfully treated by metronidazole. Transpl Infect Dis Off J Transplant Soc. 2015;17:886–9. https://doi.org/10.1111/tid.12460.
95. Palau LA. First report of invasive amebiasis in an organ transplant recipient. Transplantation. 1997;64:936–7.
96. Perret C, Harris P, Rivera M, Vial P, Duarte I, Barriga F. Refractory enteric Amebiasis in pediatric patients with acute graft-versus-host disease after allogeneic bone marrow transplantation. J Pediatr Gastroenterol Nutr. 2000;31:86–90.

97. Bavaro P, Di Girolamo G, Di Bartolomeo P, Angrilli F, Olioso P, Papalinetti G, et al. Amebiasis after bone marrow transplantation. Bone Marrow Transplant. 1994;13:213–4.
98. Hung C-C, Ji D-D, Sun H-Y, Lee Y-T, Hsu S-Y, Chang S-Y, et al. Increased risk for Entamoeba histolytica infection and invasive Amebiasis in HIV seropositive men who have sex with men in Taiwan. PLoS Negl Trop Dis. 2008;2:e175. https://doi.org/10.1371/journal.pntd.0000175.
99. Garcia LS. Dientamoeba fragilis, one of the neglected intestinal Protozoa. J Clin Microbiol. 2016;54:2243–50. https://doi.org/10.1128/JCM.00400-16.
100. Stark D, Barratt J, Chan D, Ellis JT. Dientamoeba fragilis, the neglected Trichomonad of the human bowel. Clin Microbiol Rev. 2016;29:553–80. https://doi.org/10.1128/CMR.00076-15.
101. Autier B, Belaz S, Razakandrainibe R, Gangneux J-P, Robert-Gangneux F. Comparison of three commercial multiplex PCR assays for the diagnosis of intestinal protozoa. Parasite. 2018;25:48. https://doi.org/10.1051/parasite/2018049.
102. van Hellemond JJ, Molhoek N, Koelewijn R, Wismans PJ, van Genderen PJJ. Is paromomycin the drug of choice for eradication of Dientamoeba fragilis in adults? Int J Parasitol Drugs Drug Resist. 2012;2:162–5. https://doi.org/10.1016/j.ijpddr.2012.03.002.
103. Esteghamati A, Khanaliha K, Bokharaei-Salim F, Sayyahfar S, Ghaderipour M. Prevalence of intestinal parasitic infection in Cancer, organ transplant and primary immunodeficiency patients in Tehran, Iran. Asian Pac J Cancer Prev APJCP. 2019;20:495–501. https://doi.org/10.31557/APJCP.2019.20.2.495.
104. Méndez OC, Szmulewicz G, Menghi C, Torres S, González G, Gatta C. Comparison of intestinal parasite infestation indexes among HIV positive and negative populations. Medicina (Mex). 1994;54:307–10.
105. Schuster FL, Ramirez-Avila L. Current world status of Balantidium coli. Clin Microbiol Rev. 2008;21:626–38. https://doi.org/10.1128/CMR.00021-08.
106. CDC – DPDx – Balantidiasis – Treatment Information [Internet]. [cited 20 May 2019]. Available: https://www.cdc.gov/dpdx/balantidiasis/tx.html.
107. Cermeño JR, Hernández De Cuesta I, Uzcátegui O, Páez J, Rivera M, Baliachi N. Balantidium coli in an HIV-infected patient with chronic diarrhoea. AIDS Lond Engl. 2003;17:941–2. https://doi.org/10.1097/01.aids.0000060335.12269.8f.
108. Clyti E, Aznar C, Couppie P, el Guedj M, Carme B, Pradinaud R. A case of coinfection by Balantidium coli and HIV in French Guiana. Bull Soc Pathol Exot. 1998;91:309–11.
109. Anargyrou K, Petrikkos GL, Suller MTE, Skiada A, Siakantaris MP, Osuntoyinbo RT, et al. Pulmonary Balantidium coli infection in a leukemic patient. Am J Hematol. 2003;73:180–3. https://doi.org/10.1002/ajh.10336.
110. Vasilakopoulou A, Dimarongona K, Samakovli A, Papadimitris K, Avlami A. Balantidium coli pneumonia in an immunocompromised patient. Scand J Infect Dis. 2003;35:144–6.
111. Pawełczyk A, Welc-Falęciak R, Bajer A. Selected aspects of helminth infections Schistosoma sp., Ascaris lumbricoides, Strongyloides stercoralis in individuals diagnosed with human immunodeficiency virus (HIV) infection. Przegl Epidemiol. 2018;72:349–61. https://doi.org/10.32394/pe.72.3.11.
112. Cooper AJR, Dholakia S, Holland CV, Friend PJ. Helminths in organ transplantation. Lancet Infect Dis. 2017;17:e166–76. https://doi.org/10.1016/S1473-3099(16)30533-3.
113. George B, Mathews V, Srivastava A, Chandy M. Infections among allogeneic bone marrow transplant recipients in India. Bone Marrow Transplant. 2004;33:311. https://doi.org/10.1038/sj.bmt.1704347.
114. Amoo JK, Akindele AA, Amoo AOJ, Efunshile AM, Ojurongbe TA, Fayemiwo SA, et al. Prevalence of enteric parasitic infections among people living with HIV in Abeokuta, Nigeria. Pan Afr Med J. 2018;30:66. https://doi.org/10.11604/pamj.2018.30.66.13160.
115. Sunil P, Tribhuvan G, Anil M. Ascariasis as a cause of obstructive jaundice in a renal transplant patient. J Nephrol. 2004;17:449–51.
116. Jurcic JG, Koll B, Brown AE, Crown JP, Yahalom J, Gulati SC. Excretion of Ascaris lumbricoides during total body irradiation. Bone Marrow Transplant. 1994;13:491–3.

117. Ozdemir E, Molldrem JJ. Hookworm infection of sigmoid colon masquerading as graft-versus-host disease in an allogeneic stem cell transplant recipient after donor lymphocyte infusion for refractory acute promyelocytic leukemia. Bone Marrow Transplant. 2006;37:785–6. https://doi.org/10.1038/sj.bmt.1705309.
118. Huang N-C, Fang H-C, Chou K-J, Chung H-M. Trichuris trichiura: an unusual cause of chronic diarrhoea in a renal transplant patient. Nephrol Dial Transplant. 2003;18:2434–5. https://doi.org/10.1093/ndt/gfg328.
119. White AC, Coyle CM, Rajshekhar V, Singh G, Hauser WA, Mohanty A, et al. Diagnosis and treatment of Neurocysticercosis: 2017 clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. 2018;66:e49–75. https://doi.org/10.1093/cid/cix1084.
120. Garcia HH, Lescano AG, Gonzales I, Bustos JA, Pretell EJ, Horton J, et al. Cysticidal efficacy of combined treatment with Praziquantel and Albendazole for parenchymal brain Cysticercosis. Clin Infect Dis Off Publ Infect Dis Soc Am. 2016;62:1375–9. https://doi.org/10.1093/cid/ciw134.
121. Barra Valencia V, Moreno Elola-Olaso A, Fundora Suárez Y, Meneu Díaz JC, de los Galanes SF J, Pérez Saborido B, et al. Second case of neurocysticercosis in a patient with liver transplantation (first case in Spain): a case report. Transplant Proc. 2007;39:2454–7. https://doi.org/10.1016/j.transproceed.2007.07.049.
122. Illade L, Molina Angulo B, Solís I, Diaz MÁ, Gonzalez-Vicent M. Neurocysticercosis: an unusual seizure etiology in a hematopoietic stem cell transplanted patient. Pediatr Hematol Oncol. 2018;35:20–2. https://doi.org/10.1080/08880018.2018.1426667.
123. Hoare M, Gelson WTH, Antoun N, Alexander GJM. Early recurrence of neurocysticercosis after orthotopic liver transplant. Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc. 2006;12:490–1. https://doi.org/10.1002/lt.20643.
124. Gordillo-Paniagua G, Muñoz-Arizpe R, Ponsa-Molina R. Unusual complication in a patient with renal transplantation: cerebral cysticercosis. Nephron. 1987;45:65–7. https://doi.org/10.1159/000184074.
125. Purvey S, Lu K, Mukkamalla SK, Anandi P, Dumitriu B, Kranick S, et al. Conservative management of neurocysticercosis in a patient with hematopoietic stem cell transplantation: a case report and review. Transpl Infect Dis. 2015;17:456–62. https://doi.org/10.1111/tid.12392.
126. Rostami MN, Keshavarz H, Eskandari E, Kia EB, Rezaeian M. Intestinal parasitic infections in renal transplant recipients. Iran J Parasitol. 2007;2:16–23.
127. Syafinaz AN, Hamat RA, Malina O, Siti NM, Niazlin MT, Jamal F. Hymenolepis nana in a renal transplant recipient: to treat or not to treat? Med J Malaysia. 2011;66:259–60.
128. Gupta A, Upadhay BK, Khaira A, Bhowmik D, Tiwari SC. Chronic diarrhea caused by Hymenolepis nana in a renal transplant recipient. Clin Exp Nephrol. 2009;13:185–6. https://doi.org/10.1007/s10157-008-0125-7.
129. Muehlenbachs A, Bhatnagar J, Agudelo CA, Hidron A, Eberhard ML, Mathison BA, et al. Malignant transformation of Hymenolepis nana in a human host. N Engl J Med. 2015;373:1845–52. https://doi.org/10.1056/NEJMoa1505892.
130. Detrait M, Poirier L, Roy D-C, Kiss T, Busque L, Cohen S, et al. Diphyllobothriasis, a rare cause of profuse diarrhea following autologous transplantation. Bone Marrow Transplant. 2009;44:131–2. https://doi.org/10.1038/bmt.2008.439.
131. Sahin I, Köz S, Atambay M, Kayabas U, Piskin T, Unal B. A rare cause of diarrhea in a kidney transplant recipient: Dipylidium caninum. Transplant Proc. 2015;47:2243–4. https://doi.org/10.1016/j.transproceed.2015.05.011.

Acanthamoeba, *Naegleria*, and *Balamuthia* in Transplant Patients: An Emerging Threat

58

Yoram A. Puius, Margaret L. Aldrich, and Jennifer R. Cope

Contents

Introduction 1452
Disease Epidemiology 1453
Acanthamoeba 1453
Naegleria fowleri 1454
Balamuthia 1455
Clinical Evaluation and Disease Diagnostics 1455
Acanthamoeba 1455
Naegleria fowleri 1457
Balamuthia 1458
Clinical Impact in Transplant 1460
Acanthamoeba 1460
Naegleria fowleri 1460
Balamuthia 1462
Management 1463
Drug Interactions and Toxicities 1463

Y. A. Puius (✉)
Division of Infectious Diseases, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY, USA
e-mail: ypuius@montefiore.org

M. L. Aldrich
Division of Pediatric Infectious Diseases, Department of Pediatrics, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY, USA
e-mail: maldrich@montefiore.org

J. R. Cope
Waterborne Disease Prevention Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA
e-mail: bjt9@cdc.gov

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_58

Acanthamoeba 1463
Naegleria fowleri 1465
Balamuthia 1466
Key Points 1467
References 1469

Introduction

Free-living amoebae (FLAs) are protists that are ubiquitous in the environment but rarely cause human infections. Three of these are most often associated with clinical disease: *Naegleria fowleri*, which causes primary amebic meningoencephalitis (PAM); *Acanthamoeba* spp., which can cause granulomatous amebic encephalitis (GAE), *Acanthamoeba* keratitis (AK), and a variety of other forms of disseminated cutaneous, pulmonary, or sinus disease; and *Balamuthia mandrillaris*, which also causes a form of GAE and cutaneous lesions. The pathogenesis and epidemiology of these organisms are varied, with the portal of entry playing an important role in pathogenesis. Strategies for diagnosis and treatment of FLAs are hampered by the rarity of the infections, and it is unknown how many cases are undiagnosed.

FLAs present a twofold challenge to the field of transplantation. First is the consideration of the organ donor potentially infected with an FLA and the need to decide whether to accept or reject the organ in a short time frame, typically based on incomplete information. The second is diagnosis and treatment of FLAs – possibly donor-derived – in a solid organ transplant (SOT) or stem cell transplant recipient already at risk for rare infections that are difficult to diagnose and treat. In addition, a more susceptible host such as a transplant patient often provides greater opportunity for an atypical presentation and disseminated disease.

The transplant clinician needs to maintain a high index of suspicion for FLAs given the appropriate history and epidemiology. An added caution when considering these diagnoses in the transplant population is that recommendations for diagnosis and treatment are based on case reports and series, and best practices are yet to be satisfactorily defined.

The American Society of Transplantation has recently published guidelines for diagnosis and management that briefly address FLAs [1] and donor-derived FLA infections [2]. This chapter will focus on the clinical implications of FLA in the solid organ and bone marrow transplant populations. A reader who is currently caring for a patient with a suspected or confirmed FLA infection may consider going to the Postscript at the end of this chapter for information on obtaining immediate assistance from the Centers for Disease Control and Prevention (CDC).

Disease Epidemiology

Acanthamoeba

Ecology and Pathobiology

Acanthamoeba was first identified in 1930 as a contaminant in a yeast culture. The first description of *Acanthamoeba* associated granulomatous amebic encephalitis (GAE) was reported in 1960, and reports of *Acanthamoeba* keratitis (AK) followed in 1970. Many species of *Acanthamoeba* have caused human infection, including *A. culbertsoni*, *A. polyphaga*, *A. castellanii*, *A. astronyxis*, *A. hatchetti*, *A. rhysodes*, *A. divionensis*, *A. lugdunensis*, and *A. lenticulata*. These protists are found throughout the world and have been identified in soil, water, and air samples. In certain regions of the world, there are reports of 85-100% of healthy populations with evidence of a serologic response to *Acanthamoeba*, suggesting prior exposure [3].

Acanthamoeba spp. grow best at neutral pH and temperatures of approximately 30 °C [3]. However, they are able to survive in a variety of environments, including tissue culture media, salt water, body fluids, and distilled water. Viable amoebae have been recovered from a fresh and salt water sources, water associated with buildings and facilities, and various forms of refuse.

There are two stages to the life cycle of this protist: the active trophozoite stage and the dormant cyst stage. *Acanthamoeba* keratitis is thought to start with trophozoite invasion through a break in the epithelial barrier of the cornea, aided by secreted proteases, followed by a cascade of events to include stromal infiltration, depletion of the keratocytes, and activation of the host inflammatory response, which ultimately leads to photophobia, stromal necrosis, and blindness [4]. In contrast, primary transmission in GAE and disseminated cutaneous disease are thought to occur via inhalation of cysts or cutaneous inoculation. Microscopic findings at autopsy suggest that the amoeba invade the alveolar blood vessels and then spread hematogenously, crossing the blood-brain barrier to infect the CNS [3].

Risk Factors and Incidence

Acanthamoeba keratitis has been associated with contaminated contact lens solution, with a multistate outbreak identified between 2005 and 2007 [5]. The infected patients were otherwise healthy with a median age of 40 years. In contrast, disseminated *Acanthamoeba* infections and GAE occur in immunocompromised patients.

Though historically associated with the AIDS epidemic, transplant recipients are also at risk for invasive disease; infections have been reported in patients with transplants of the heart [6–8], kidney [9–14], and lung [15–18]. In addition, there have been reports of disease in patients with hematologic malignancies [19–21] and in stem cell transplant recipients [22–30]. Immunocompromising conditions with increased risk of infection include diabetes, renal failure, liver cirrhosis, and chronic use of steroids or other immunosuppressive therapies [3]. Due to

the ubiquitous nature of the organism, environmental risk factors have not been definitively identified, although nasal irrigation with contaminated water may have been the source of infection in several cases [31].

Naegleria fowleri

Ecology and Pathobiology

In 1965, an amoeba was identified as the cause of a case of meningitis in Australia [32]. The following year, examination of American cases revealed their cause to be a novel amoeba species distinct from *Acanthamoeba*. The clinical entity was termed primary amebic meningoencephalitis or PAM [33]. *Naegleria fowleri* is the only pathogenic species in the genus *Naegleria*. It exists in three different forms: the trophozoite, which predominates in a warm, high-nutrient aquatic environment; a transient motile flagellate stage, which occurs in a low-nutrient aquatic environment (to allow for movement over long distances in pursuit of nutrients); and a cyst, which forms under adverse conditions. The amoeba grows best at higher temperatures up to 46 °C and is killed by freezing, drying, temperatures over 65 °C, and adequate levels of chlorination; it does not survive in salt water, although it is fairly tolerant of a wide range of temperatures and pH [34]. The trophozoite is the infective form. It enters hosts through the nasal mucosa, translocates across the cribriform plate, and travels via the olfactory neuroepithelia into the central nervous system. It then invades the olfactory bulbs and the base of the brain to cause hemorrhagic and necrotizing meningoencephalitis which results in cerebral edema, herniation, and eventually death [35].

Risk Factors and Incidence

Naegleria fowleri is a thermophilic organism; therefore it has been postulated that the epidemiology of PAM might be an indicator of climate change [36]. Cases have been noted worldwide and are also associated with warm waters, most notably in Pakistan [35]. Association with contaminated tap water has also been observed. Particularly noteworthy as a mode of exposure are practices in which the nasal passages are directly exposed to contaminated water, such as irrigation with a neti pot, or ritual nasal rinsing in Yogic, Ayurvedic, and Islamic traditions [36].

Between 1962 and 2019, 148 cases of PAM were reported to CDC [37, 38] (unpublished data). In the United States, PAM primarily occurs in previously healthy young men exposed to warm, recreational waters, predominantly in lakes, ponds, and reservoirs in southern-tier states during the summer months. Over half the cases occur in Texas and Florida. However, in recent years, cases associated with recreational water exposure in the United States have been seen farther north, including Kansas, Indiana, and even Minnesota [38]. It has been estimated that there may be 16 deaths annually in the United States due to *Naegleria fowleri*, with some cases being undiagnosed [39].

No cases of *Naegleria fowleri* infection have been documented in the transplant population to date, although there is some theoretical risk of transmission from donors [40]. See the *Naegleria fowleri* heading under the "Clinical Impact in Transplant" section below for current practice regarding infected donors.

Balamuthia

Ecology and Pathobiology

Balamuthia mandrillaris was first identified in 1986 from a mandrill (a type of primate) that died of encephalitis [41]. There have been a few reports of isolation from soil, dust, and water; the exact ecologic niche of *Balamuthia* remains unknown. In contrast to other FLAs, this infection is likely associated with exposure to contaminated soil. *Balamuthia* also has two stages to its life cycle, the trophozoite and the cyst stage. It is thought to enter the human body via the lungs by inhalation of cysts or direct inoculation of a break in the skin and then spreading via a hematogenous route.

Risk Factors and Incidence

CDC has identified 109 case reports of *Balamuthia* disease between 1974 and 2016, primarily in the western and southwestern regions of the United States [42]. Patient ages ranged from 4 months to 91 years, with a median age of 36 years, which is different from many etiologies of encephalitis which tend to affect the elderly. A predominance of males and Hispanic ethnicity is proposed to be due to occupations with heavy soil exposure such as landscaping and agriculture.

Approximately 40% of patients had an underlying immunocompromised state (which included diabetes, HIV, SOT, liver or renal failure, use of an immunosuppressive drug, or other hematologic or autoimmune condition). More specifically, 6% of cases were solid organ transplant recipients, and 5% were on an immunosuppressive drug. Alcohol use was reported as a comorbidity in 18% and drug use in 22% of cases.

Clinical Evaluation and Disease Diagnostics

Acanthamoeba

The clinical presentation of *Acanthamoeba* infection varies depending on the host immune system and the portal of entry. *Acanthamoeba* keratitis is characterized by "gray-dirty" epithelial changes, multifocal stromal infiltrates, ring infiltrates, and peripheral perineural infiltrates, with later complications including anterior uveitis, scleritis, synechiae, glaucoma, iris atrophy, cataract chorioretinitis, and retinal vasculitis [4].

Manifestations of disseminated disease include cutaneous lesions, rhinosinusitis, urethritis, and fatal GAE [3] (Figs. 1 and 2). At times the clinical course may be indolent. GAE may present over weeks and months as a progressive disease associated with headache, fever, and subtle neurologic changes [43]. Radiographic findings suggestive of GAE include solitary or multiple space occupying lesions, which may or may not be ring-enhancing. The cerebrospinal fluid (CSF) indices associated with GAE include hypoglycorrachia, elevated protein, and lymphocytic pleocytosis. Skin lesions may be associated with GAE, and biopsy can aid in the diagnosis if present.

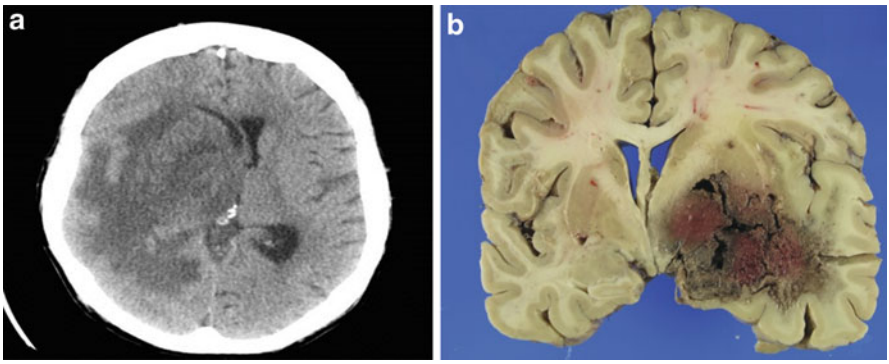


Fig. 1 A patient with a total artificial heart device with fatal *Acanthamoeba* encephalitis [68]. (**a**) CT of the head with and without contrast showing an ill-defined hypodensity at the right medial temporal lobe causing mass effect and uncal herniation with surrounding vasogenic edema. (**b**) Coronal section of cerebrum displaying a 6 × 6 × 5 cm area of gelatinous necrosis and hemorrhage. (Reproduced under Creative Commons Attribution-NonCommercial-NoDerivs license)

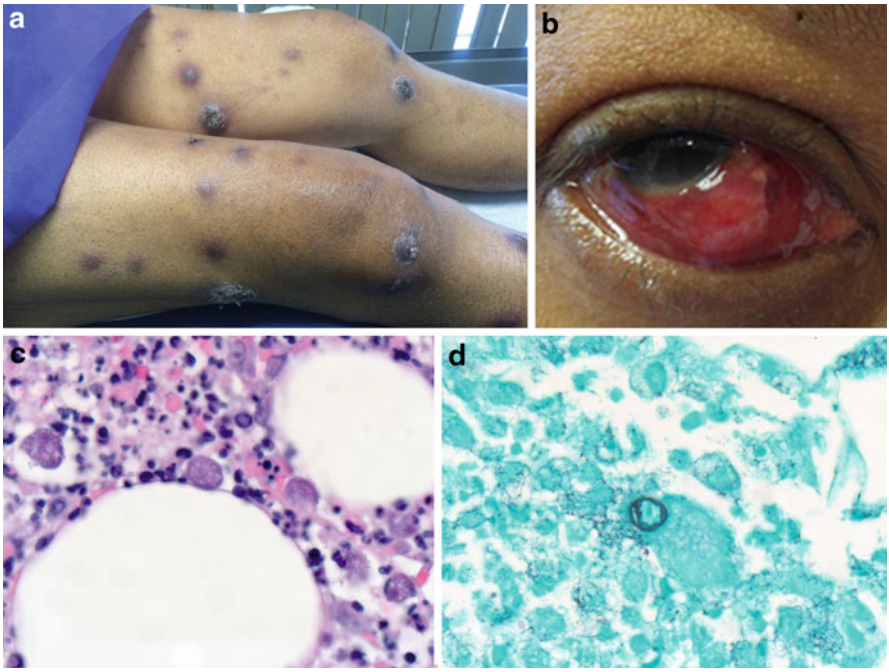


Fig. 2 A renal transplant recipient with disseminated *Acanthamoeba* infection [9] resulting in both (**a**) painful dermal and subcutaneous skin nodules and (**b**) endophthalmitis. Photomicrographs of the skin biopsy demonstrate (**c**) trophozoites stained by hematoxylin and eosin and (**d**) a cyst stained by Gomori methenamine silver. (Reproduced by permission of publisher, John Wiley & Sons, Inc.)

In almost all cases of *Acanthamoeba* infection, tissue diagnosis is required. The diagnosis of cutaneous lesions may be made with tissue biopsy stained for identification of trophozoites or cysts (Fig. 2). Second-level testing may then be pursued to include indirect immunofluorescent antibody (IFA) or immunohistochemical (IHC) staining of tissue and PCR testing (see Postscript).

It is more difficult to make the diagnosis of GAE as *Acanthamoeba* trophozoites are unlikely to be seen in CSF (wet mount or Wright-Giemsa stain), though it is recommended to perform lumbar puncture for CSF analysis and microscopy [1]. Brain biopsy with tissue diagnosis is the standard method to identify *Acanthamoeba* trophozoites and cysts. In many cases the diagnosis is made too late to affect clinical outcome [10, 22, 44] as the mortality associated with GAE is about 90%, with diagnosis often made at autopsy.

Naegleria fowleri

The clinical presentation of PAM due to *Naegleria fowleri* is best described in the most comprehensive review of cases from 1937 to 2013 [38]. In this case series, the median incubation period was 5 days, and the median time from onset of symptoms to death was also 5 days. The clinical presentation of PAM is often indistinguishable from bacterial meningitis with the most common presenting symptoms being headache (92%), fever (75%), and nausea/vomiting (65%). Late symptoms included nuchal rigidity, confusion, and other signs of progressive CNS disease. Only 27% of US cases were diagnosed before death.

CSF studies may be difficult to distinguish from bacterial meningitis. The median opening pressure is elevated at 385 mm H_2O, with a neutrophilic pleocytosis (median WBC count of 2400 with 83% neutrophils). The CSF fluid also has low glucose (median 23 mg/dL) and markedly elevated protein (365 mg/dL). Radiographic studies of the brain often demonstrate generalized edema but are not specific enough to distinguish from other etiologies, although the parasites may favor the frontal lobe and parietal lobes, and some cases have been associated with hydrocephalus [45] (Fig. 3).

A high index of suspicion is required to undertake CSF studies specific to *N. fowleri*. Microscopic examination should be conducted by an expert pathologist or microbiologist [36]. If it cannot be examined immediately, CSF should be stored at approximately 25 °C and handled aseptically before examination. To concentrate the specimen, it may be centrifuged at 5000 × *g* for 5 min and the supernatant removed to leave 200–300 μl. The slide may be incubated at 37 °C to facilitate movement of the amoebae. Wright and Giemsa stains may also be helpful in identifying the trophozoite. If the diagnosis is made in time to affect the clinical outcome, it is most likely to be made based on the presence of trophozoites in the CSF.

N. fowleri-specific PCR [46] can provide diagnosis from CSF or brain biopsy tissue. With proper notification of specimen shipment, the CDC Free-Living and Intestinal Amebas (FLIA) Laboratory can provide same-day PCR results from CSF for clinical decision-making; PCR is available at other reference labs in the United

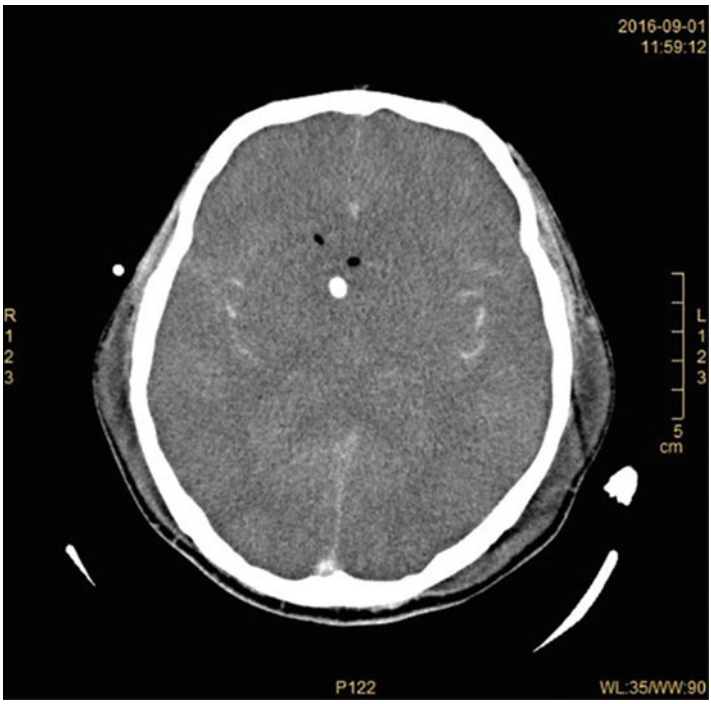


Fig. 3 Computed tomography scan of the brain of a patient with primary amoebic meningoencephalitis due to *Naegleria fowleri* demonstrating cerebral edema and almost absent ventricles [69]. (Reproduced under the Creative Commons Attribution 4.0 International License)

States as well (see Postscript). Contact the CDC Emergency Operations Center at 770-488-7100 for additional guidance. Culture and antigen detection are also employed but are unlikely to be used in a clinical setting.

Balamuthia

The clinical presentation of *Balamuthia* GAE is nonspecific [42]. When the skin is involved, lesions are often singular and located on the central face but occasionally on the extremities or trunk. They have been described as painless plaques that may progress to central ulceration. The most common general clinical features on presentation are fever (39%), headache (39%), vomiting (30%), and lethargy (28%). The most common neurologic features on presentation are altered mental status (30%), seizures (21%), and weakness (19%). Radiographic findings on brain imaging [42] have included enhancing lesions (29%), multifocal lesions (23%), and edema (27%) (Fig. 4) [45].

CSF studies typically demonstrate elevated protein, low or normal glucose, and a lymphocytic pleocytosis [42], with a median white blood cell count of 106 cells/μL and a median of 57% lymphocytes.

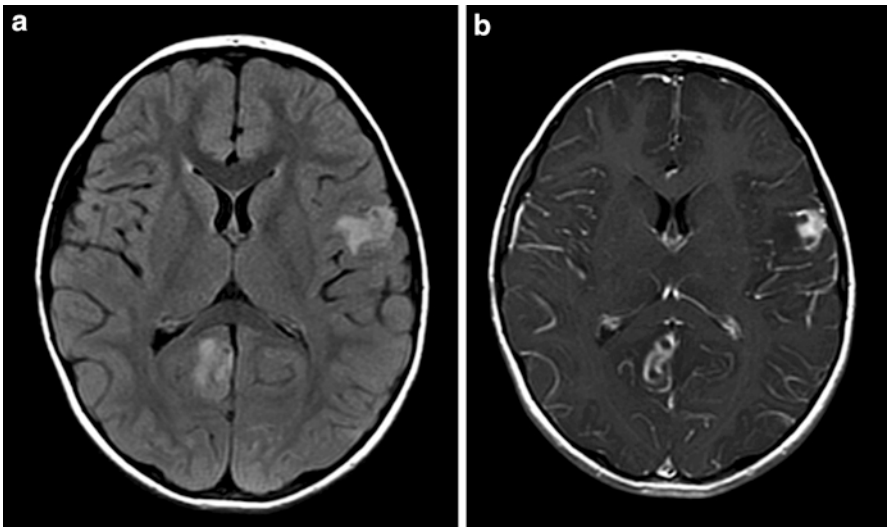


Fig. 4 Brain images showing contrast-enhanced lesions in the right occipital and left parietal lobes of a 4-year-old boy with encephalitis caused by infection with *Balamuthia mandrillaris* amebae [70]. (**a**) T2-weighted fluid-attenuated inversion recovery (FLAIR) image. (**b**) T1-weighted contrasted magnetic resonance image. (Reproduced courtesy of CDC)

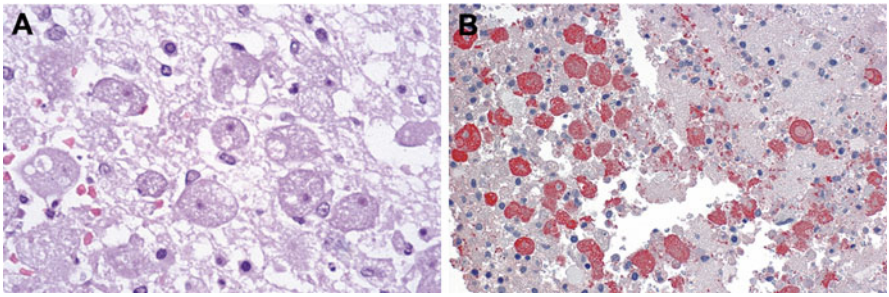


Fig. 5 Photomicrographs showing histopathologic features and immunolocalization of *Balamuthia mandrillaris* antigens in central nervous system tissue from a donor with *B. mandrillaris* infection [70]. (**a**) Typical amebic trophozoites with prominent karyosomes in central nervous system. Hematoxylin and eosin staining. Original magnification, ×158. (**b**) *B. mandrillaris* antigens in amebic trophozoites. Immunoalkaline phosphate staining, naphthol fast red substrate with light hematoxylin counterstain. Original magnification, ×100. (Reproduced courtesy of CDC)

Biopsy results (Fig. 5) may show the trophozoite stage when taken from the skin, while brain biopsy could show both cyst and trophozoite forms. Polymerase chain reaction (PCR) testing of CSF and/or brain tissue is also recommended [1] which is available at CDC and other reference labs (see Postscript).

Clinical Impact in Transplant

Acanthamoeba

While infection with *Acanthamoeba* spp. is rare in transplant patients, it has been associated with severe disease and fatal outcomes. As of the time of this publication, there have been no reports of donor-derived *Acanthamoeba* infection. The etiology of infection in transplant patients seems to be from exposure to contaminated water or soil in the setting of active immunosuppressive drug therapy. Of uncertain clinical significance in the transplant population, in particular, is the ability of *Acanthamoeba* spp. to carry other organisms, known as "endosymbionts," into the human host. Some of these endosymbiont viruses, bacteria, yeasts, and other protists are recognized human pathogens [3].

In a review of 22 cases of *Acanthamoeba* infection in heart, lung, liver, kidney, and multi-organ transplant recipients, the most common immunosuppression regimens included tacrolimus, mycophenolate, cyclosporine, and prednisone. The mean age of the patients identified was 50 years, with mean time to diagnosis after transplant of 18 months (range, 3 months to 6 years) [7]. In the majority of cases reviewed, there was a delay in diagnosis. Multiple case reports suggest that patients are often started on empiric antibacterial and antifungal therapy with little or no improvement, before the diagnosis is made with tissue specimen. The challenge for the frontline clinician is to consider this organism in these immunocompromised patients.

Because these infections are thought to be secondary to inoculation from cysts in the host's environment, there may be an opportunity to counsel the patient on risk reduction after transplant and before the patient is discharged home. The current recommendations from the American Society of Transplantation are "to exercise caution when engaging in occupations or recreational activities that expose them to untreated freshwater," [1] and similar recommendations exist for recipients of stem cell transplants [47]. While there have been studies to evaluate a risk assessment tool for infectious encephalitis in deceased organ donors that might be helpful in preventing *Balamuthia* infections [48], there is no indication that this would be helpful in preventing *Acanthamoeba* infections at this time as there have been no recognized donor-derived infections.

Naegleria fowleri

Organs have been successfully used for transplantation from 5 donors with known PAM due to *Naegleria fowleri* with no evidence of transmission to 21 recipients at follow-up of at least 6 months [40, 49, 50]. In one case [49], the donor had PAM established antemortem by visualization of motile amoebae in the CSF and confirmed by the state parasitology laboratory. Although multiple programs turned down the organ offers, recipients who gave informed consent received the kidneys, liver, right lung, and pancreas.

In four of the cases [40, 50], the diagnosis of PAM in the donor was only identified post-transplant. Five recipients had negative *N. fowleri* serologies 7 weeks to 2 years post-transplant, and one biopsy taken 1 month post-transplant from a donated heart tested negative for *N. fowleri* [40]. However, historical PAM case reports and animal experiments with *N. fowleri*, combined with postmortem findings from PAM patients, suggest that extra-CNS dissemination of *N. fowleri* can occur and might pose a risk for disease transmission via transplantation [40] (Fig. 6).

Given the lack of transmission from infected donors [40, 49, 50], current guidelines from the American Society of Transplantation suggest that "[i]f the donor has proven *N. fowleri*, the organs can be utilized with a low risk of transmission, as long as the recipients are informed of the risk and monitored closely" [2]. However, due to evidence for extra-CNS dissemination in mouse

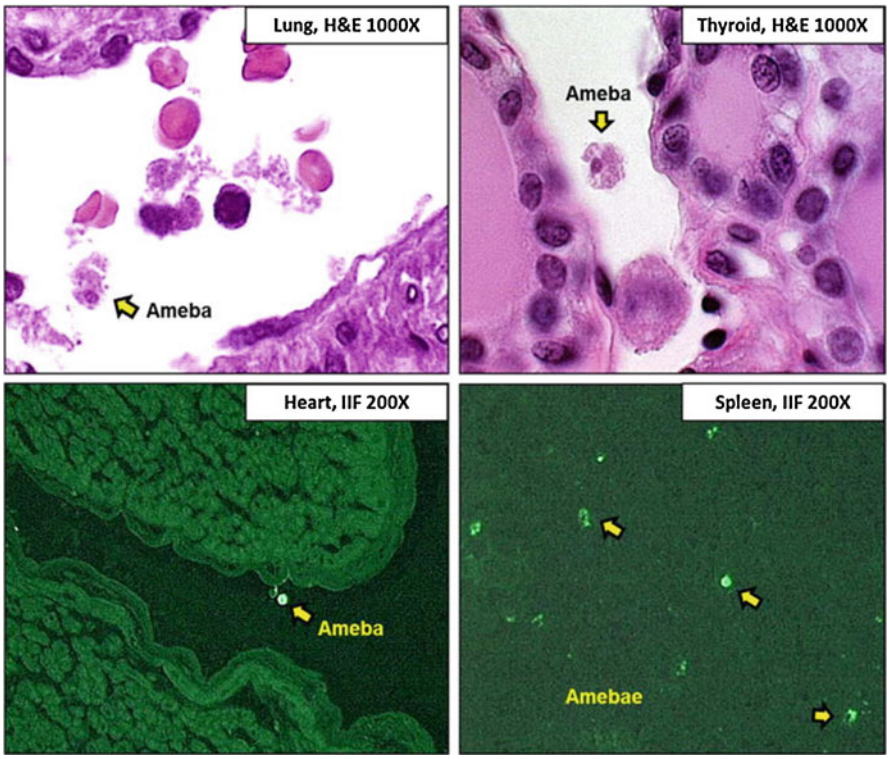


Fig. 6 Postmortem examination of lung, thyroid, heart, and spleen tissue from a 10-year-old male organ donor in Florida who died from primary amebic meningoencephalitis due to *Naegleria fowleri* [40]. *N. fowleri* trophozoites in lung (upper left) and thyroid (upper right) sections by hematoxylin and eosin (H&E) staining at 1000× magnification. *N. fowleri* trophozoites fluorescing in heart (lower left) and spleen (lower right) sections by indirect immunofluorescence (IIF) at 200× magnification. (Reproduced by permission of publisher, John Wiley & Sons, Inc.)

models and in occasional human cases, it has been proposed that a full autopsy of the donor should be performed in the case of a suspected or confirmed case of PAM, to determine the extent of dissemination [40]. If organ biopsies of the recipient are undertaken for other reasons (e.g., to monitor for rejection), it may be prudent to specifically look for *N. fowleri* in the tissue.

The guidelines for safe living after transplantation of solid organs [51] and stem cells [47] recommend that the transplant recipient is counseled to avoid contaminated freshwater, which from a practical perspective is essentially any water source not meant to be potable. This includes consumption or inadvertent swallowing of water from lakes, ponds, rivers, or untreated wells, especially those which may be contaminated (with sewage, human waste, or animal waste). This may also include water rides at amusement parks. The concern is primarily for *Cryptosporidium*, *Giardia*, and coliforms, but in warmer waters, this would also apply to *N. fowleri*.

Directing the recipient to avoid warm ponds altogether is not unreasonable. Water slide use has been associated with PAM as well [36]. Given the known inoculation by nasal irrigation [35, 36], it is reasonable to simply direct transplant recipients to avoid neti pots or nasal ablution entirely. Maintaining adequate levels of chlorine disinfectant in water distribution systems, particularly in those with elevated water temperatures, is thought to be an important strategy in preventing PAM cases related to tap water [36]. Transplant recipients aware of local water quality issues may consider boiled, filtered, or bottled water. Salt water is not known to be a risk factor for *N. fowleri*.

Balamuthia

Clusters of *Balamuthia* transmitted by solid organ transplant were described in 2009, 2010, and 2012 [52]. In the 2009 cluster [53], two kidney recipients were infected, and one died. The asymptomatic heart and liver recipients were treated preemptively, and the liver recipient developed a detectable serology. The donor infection was later confirmed on autopsy.

The 2010 cluster [54] resulted in the death of the recipients of the liver and the kidney-pancreas. The asymptomatic recipients of the heart and other kidney were also treated, with the kidney recipient developing a positive serology which later waned. In 2012, five recipients received *Balamuthia* infected organs, with the liver recipient developing encephalitis 18 days post-transplant and dying, while the remaining recipients had positive *Balamuthia* antibody titers, were treated preemptively, and survived [55].

The implications for screening donors for solid organ transplantation are that all donors with a potential diagnosis of encephalitis should be viewed with caution for infections with free-living amoebae, viruses, and other organisms. A risk calculator for infectious encephalitis has been constructed [48] which can be downloaded at https://chhs.gatech.edu/RiskCalculator.zip

Management

Drug Interactions and Toxicities

Transplant recipients are typically on immunosuppressive agents which are metabolized by the cytochrome P450 (CYP) 3A4 system, most notably the calcineurin inhibitors (CNIs), cyclosporine, and tacrolimus and the mammalian target of rapamycin (mTOR) inhibitors, sirolimus, and everolimus. Azole antifungals generally inhibit CYP 3A4, increasing the area under the curve (AUC) of these agents when coadministered, necessitating a decrease in the dose of CNI or mTOR inhibitors by one-half to two-thirds of the original dose [56]. They may also inhibit uridine diphosphate-glucuronosyltransferases, which may increase exposure to mycophenolate products.

Macrolides in general are CYP3A4 inhibitors. However, the macrolide most used for FLA infections is azithromycin, which has a relatively minor effect on immunosuppressive drug levels [56].

Conversely, the rifamycins are potent inducers of CYP3A4, and coadministration of rifampin may decrease the AUC, requiring an increase in the doses of immunosuppressive agents by two- to tenfold; they may also induce uridine diphosphate-glucuronosyltransferases and decrease mycophenolate levels as well [56]. To further complicate matters, the regimens used to treat FLAs may include both azoles and rifampin. Therefore, transplant patients undergoing therapy for FLAs require careful monitoring of levels of immunosuppressive agents.

In addition, the toxicities of the drugs may affect the organ transplanted, so the risks and benefits will have to be addressed on an individual basis. Since decreasing immunosuppression is a mainstay of the treatment of life-threatening infections in transplant recipients, the decrease in immunosuppression will have to be weighed against the risk of organ rejection.

Acanthamoeba

Therapy for *Acanthamoeba* infection is not standardized in the transplant population, and there are many therapeutic combinations that have been reported in the literature; however, combinations which include miltefosine may have a greater chance of eradicating infection [57, 58]. Miltefosine is an alkylphosphocholine analog and antiprotozoal medication that was recently approved by the US Food and Drug Administration (FDA) for use in treating leishmaniasis. It has also been found to have amebicidal properties, though the mechanism of action is not understood [59].

Prior to the availability of miltefosine, during the AIDS epidemic, there were reports of successful treatment of GAE with surgical resection of the CNS lesion in conjunction with sulfadiazine, pyrimethamine, and fluconazole [60]. Other patients with GAE have previously been treated with combinations of pentamidine, sulfadiazine, flucytosine, and either fluconazole or itraconazole [61]. A 2001 report described three immunocompetent children who developed chronic

Acanthamoeba meningitis, two of whom were successfully treated with a combination of oral trimethoprim/sulfamethoxazole, rifampin, and ketoconazole [62]. More recently, in 2011 a pediatric patient with acute lymphoblastic leukemia (ALL) developed GAE shortly after remission and had curative therapy with prolonged miltefosine, pentamidine, fluconazole, and trimethoprim/sulfamethoxazole in conjunction with hyperbaric oxygen [43]. (See the Postscript for directions on obtaining miltefosine on an urgent/emergent basis.)

Disseminated cutaneous infection in immunocompromised patients may require both systemic therapy and topical treatment. Various reports of successful therapy have included parenteral pentamidine, topical chlorhexidine, and 2% ketoconazole cream, followed by oral itraconazole [63] or voriconazole and amphotericin B lipid complex [16]. In a rare case of disseminated cutaneous disease with concomitant endophthalmitis in a renal transplant patient, bi-weekly intracameral and subconjunctival injections of voriconazole and dexamethasone were used in conjunction with systemic fluconazole, flucytosine, azithromycin, and miltefosine; sulfadiazine had been employed also in this case but was discontinued due to anemia [9].

Expert opinion suggests that a successful regimen involves at least miltefosine and an azole, with adjunctive therapy consisting of a sulfa drug, flucytosine, and/or azithromycin. Treating physicians have typically started with 3–4 drugs and, if the patient improves, scaled back to 2–3 oral drugs with close outpatient follow-up. Table 1 summarizes typical therapies and doses employed.

Table 1 Drugs and typical doses employed in the treatment of *Acanthamoeba* infections, including GAE [61]. When doses are not specified in the case reports, standard doses are recommended

Drug	Dose	Recommended adjustment in immunosuppressive dose for CNI or mTOR inhibitor when starting *Acanthamoeba* treatment
Pentamidine	3–4 mg/kg/day intravenously	–
Sulfadiazine	4–6 g/day orally divided into 4 doses	–
Trimethoprim/ sulfamethoxazole	15–20 mg/kg divided into 3–4 doses	–
Flucytosine	50–150 mg/kg/day oral divided into 4 doses	–
Fluconazole	9–12 mg/kg/day orally or intravenously	↓by 50% then monitor
Itraconazole	10 mg/kg/day orally divided into 2 doses, typically ≤400 mg/daily	↓by 50% then monitor
Voriconazole	8 mg/kg/day divided into 2 doses	↓by 70% then monitor
Azithromycin	10 mg/kg/day orally or intravenously	Monitor
Miltefosine	150 mg/day divided into 3 doses	–

While *Acanthamoeba* keratitis is not associated with the transplant or immunocompromised populations, it may be useful to note that when treatment can be achieved medically, recommended agents are typically a combination of a diamidine (such as propamidine isethionate or hexamidine diisethionate) plus a biguanide (such as polyhexamethylene biguanide or chlorhexidine), possibly with neomycin for both its amebicidal effects and to reduce the load of bacteria which may be a food source for the ameba [4]. Corneal transplant may be required if medical therapy fails.

Susceptibility testing of *Acanthamoeba* isolates have shown differences in drug sensitivity between species and even among strains of a single species, although antimicrobial susceptibility testing is not readily available and is only available in research settings [64].

Naegleria fowleri

In spite of the general approach for employing combination antimicrobial therapy, the case fatality rate for PAM remains high, with only 4 survivors out of 148 documented cases in the United States in the years 1937–2019 [38, 59]. None of these documented cases were transplant recipients, so treatment regimens would likely be extrapolated. Table 2 shows a list of the main therapies employed with typical doses.

Table 2 Drugs and typical doses employed in survivors of *Naegleria* PAM [59]. Posaconazole has been proposed as a replacement for fluconazole (see text) [66]. Note that the recommended formulation of amphotericin is not the liposomal or lipid complex, but conventional amphotericin B deoxycholate

Drug	Dose	Recommended adjustment in immunosuppressive dose for CNI or mTOR inhibitor when starting *Naegleria* treatment
Amphotericin B deoxycholate	1.0–1.5 mg/kg/day intravenously (in one case 0.1 mg intrathecally)	–
Rifampin	10–12 mg/kg/day orally or intravenously	↑twofold then monitor
Miltefosine	150 mg/day divided into 3 doses	–
Fluconazole	9–12 mg/kg/day orally or intravenously	↓by 50% then monitor
Posaconazole		↓by 70% then monitor
Azithromycin	10 mg/kg/day orally or intravenously	Monitor

Intravenous amphotericin B deoxycholate (conventional, not the lipid formulation) remains the backbone of therapy and has been used in all cases who survived; it has been occasionally administered intrathecally as well. Expert opinion suggests employing the deoxycholate formulation [38] for several reasons: all recent survivors received amphotericin B deoxycholate, none of the cases who received non-deoxycholate formulations survived, and in vitro data are conflicting on whether the lipid complex or liposomal formulations are as effective. The toxicities of the deoxycholate formulation (rigors, nephrotoxicity, gastrointestinal side effects) may be outweighed by the benefits in a desperately ill PAM patient.

Rifampin is the most commonly used adjunctive therapy to potentiate the effects of amphotericin B deoxycholate, the rationale based originally on in vitro data. It was also used in all documented survivors in the United States [38].

Miltefosine, discussed above in the treatment of *Acanthamoeba*, has demonstrated good activity in vitro against other amoebae and was used to treat both of the PAM survivors in 2013 as well [38, 59, 65]. Although not all recipients have survived, it is promising and should probably be employed in future cases [59]. (See the Postscript for directions on obtaining miltefosine on an urgent/emergent basis.)

Azoles also have in vitro activity, and miconazole, ketoconazole, and fluconazole have been employed intravenously, orally, and occasionally intrathecally. Posaconazole has been proposed as a superior azole on the basis of in vitro activity and animal models [66].

Azithromycin may be synergistic with amphotericin B and has activity in vitro and in mouse models. It has been employed in a number of cases, most notably in more recent survivors [38].

Aggressive management of elevated intracranial pressure was employed in most survivors [38, 59], including external ventricular or lumbar drain placement, therapeutic hypothermia, and dexamethasone.

Balamuthia

Based on their in vitro activity and successful use in survivor cases, the following drug combination is recommended for treatment of *Balamuthia* infection: pentamidine, sulfadiazine, flucytosine, fluconazole, azithromycin or clarithromycin, and miltefosine [42]. See Table 3 for doses.

Pentamidine may result in nephrotoxicity, hypotension, dysglycemias, pancreatitis, and hepatotoxicity. QT prolongation may be a concern with coadministration with other drugs with a similar effect, such as the azoles.

Sulfadiazine is contraindicated in the case of sulfa drug allergy and may result in blood dyscrasias.

Again, miltefosine may need to be obtained emergently from the manufacturer. See Postscript for directions on obtaining miltefosine on an urgent/emergent basis.

Table 3 Drugs and typical doses employed in survivors of *Balamuthia* GAE [42]

Drug	Dose	Recommended adjustment in immunosuppressive dose for CNI or mTOR inhibitor when starting *Balamuthia* treatment
Pentamidine	3–4 mg/kg/day intravenously	–
Sulfadiazine	4000–6000 g/day orally divided into 4 doses	–
Flucytosine	50–150 mg/kg/day oral divided into 4 doses	–
Fluconazole	9–12 mg/kg/day orally or intravenously	↓by 50% then monitor
Azithromycin	10 mg/kg/day orally or intravenously	Monitor
Miltefosine	150 mg/day divided into 3 doses	–

Nitroxoline, a quinolone antibiotic which has been used to treat urinary tract infections, has recently been identified to have potent in vitro amebicidal activity against *Balamuthia* [67].

Key Points

FLAs are environmental protists which rarely cause infection, but when they do, they can be devastating, particularly in the immunocompromised host. In addition, it may be difficult to determine whether a potential organ donor with encephalitis has an FLA infection, and the diagnosis may not be made until after transplantation.

Acanthamoeba spp. may present as keratitis, GAE, or a variety of disseminated infections in the immunocompromised host. The environmental exposure may be contaminated water or soil. It has not yet been recognized as a cause of donor-derived infection.

Naegleria fowleri is the etiology of PAM and is acquired by exposure of the nasal mucosa with contaminated warm water, from either nasal irrigation or recreational exposure in warm bodies of fresh water. The transplant recipient should avoid these environmental exposures to minimize the risk of PAM. Although transplantation of solid organs from infected donors has not resulted in transmission to recipients, there is evidence of dissemination outside of the central nervous system, so organs should be used with caution.

Balamuthia is another cause of GAE as well as cutaneous disease, likely acquired by inhalation or inoculation. Immunosuppression might be a particular risk factor for infection. Since many of the recognized donor-derived infections

have been fatal in spite of treatment, patients with suspected *Balamuthia* infection should not be considered as organ donors.

FLA infections may be difficult to diagnose and treat, and expert consultation should be sought immediately upon suspicion (see Postscript). Tissue and CSF are crucial in establishing the diagnosis but often require specialized diagnostic modalities, such as those available at CDC. Combination therapy often includes miltefosine, azoles, and macrolides and may include several other drugs, many of which have interactions with transplant medications. Due to the rarity and delayed diagnosis of FLA infections, outcomes are frequently poor.

Postscript: Obtaining Immediate Assistance

A reader in the United States who is considering the diagnosis of FLA infection in an ill patient should consider urgent or emergent consultation with experts at CDC who will facilitate the appropriate diagnostic testing and access the most appropriate therapies. CDC does take consultations from international physicians and will accept international specimens for diagnostic testing as well.

For 24/7 diagnostic assistance, specimen collection guidance, shipping instructions, and treatment recommendations, please contact the CDC Emergency Operations Center at 770-488-7100.

Directions for sending samples for testing for PCR, IFA, and/or IHC are available at https://www.cdc.gov/parasites/naegleria/pdf/Free-Living-Ameba-Testing-Factsheet-508c.pdf. The lab can be contacted by telephone at 404-718-4157, fax at 404-718-4197, and e-mail at CDCFLIALab@cdc.gov.

If submitting formalin-fixed paraffin-embedded specimens, please send your inquiry to pathology@cdc.gov and follow instructions for submission to the Infectious Diseases Pathology Branch.

PCR is also available at other reference labs in the United States:

Mayo Clinic Laboratories, Rochester, MN (800) 533-1710: https://www.mayocliniclabs.com/test-catalog/Overview/64718 (PAM and GAE); https://www.mayocliniclabs.com/test-catalog/Overview/64717 (ocular *Acanthamoeba* infection)

Department of Health, Wadsworth Center, Albany, NY (518) 474-4177: https://www.wadsworth.org/programs/id/idr

California Department of Public Health can also do FLA testing.

Updated information for health professionals is available online:

Acanthamoeba: https://www.cdc.gov/parasites/acanthamoeba/health_professionals/index.html

Naegleria fowleri: https://www.cdc.gov/parasites/naegleria/health_professionals.html

Balamuthia: https://www.cdc.gov/parasites/balamuthia/audience-hcp.html

Since the drug miltefosine plays a role in the treatment of FLAs and is now commercially available (http://www.impavido.com/amoebic-infections), after

discussions with CDC, contact the company directly at 908-635-2326 to expedite delivery of the drug.

Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

1. La Hoz RM, Morris MI, Infectious Diseases Community of Practice of the American Society of Transplantation. Tissue and blood protozoa including toxoplasmosis, Chagas disease, leishmaniasis, Babesia, *Acanthamoeba*, *Balamuthia*, & *Naegleria* in solid organ transplant recipients – guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation. Clin Transplant. 2019;33:e13546.
2. Wolfe CR, Ison MG, The ASTIDCoP. Donor-derived infections: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13547.
3. Siddiqui R, Khan NA. Biology and pathogenesis of *Acanthamoeba*. Parasit Vectors. 2012;5:6.
4. Szentmary N, Daas L, Shi L, Laurik KL, Lepper S, Milioti G, et al. *Acanthamoeba* keratitis – clinical signs, differential diagnosis and treatment. J Curr Ophthalmol. 2019;31(1):16–23.
5. Centers for Disease Control and Prevention. *Acanthamoeba* keratitis multiple states, 2005–2007. MMWR Morb Mortal Wkly Rep. 2007;56(21):532–4.
6. Barete S, Combes A, de Jonckheere JF, Datry A, Varnous S, Martinez V, et al. Fatal disseminated *Acanthamoeba lenticulata* infection in a heart transplant patient. Emerg Infect Dis. 2007;13(5):736–8.
7. Brondfield MN, Reid MJ, Rutishauser RL, Cope JR, Tang J, Ritter JM, et al. Disseminated *Acanthamoeba* infection in a heart transplant recipient treated successfully with a miltefosine-containing regimen: case report and review of the literature. Transpl Infect Dis. 2017;19(2): e12661. https://doi.org/10.1111/tid.12661.
8. Harrison WT, Lecky B, Hulette CM. Fatal granulomatous amebic encephalitis in a heart transplant patient: clinical, radiographic, and autopsy findings. J Neuropathol Exp Neurol. 2018;77(11):1001–4.
9. Kutner A, Aldrich M, Patel S, Kang JJ, Amin B, Mann R, et al. *Acanthamoeba* endophthalmitis during treatment for cutaneous disease in a renal transplant patient. Transpl Infect Dis. 2018;20(2):e12843.
10. Salameh A, Bello N, Becker J, Zangeneh T. Fatal granulomatous amoebic encephalitis caused by *Acanthamoeba* in a patient with kidney transplant: a case report. Open Forum Infect Dis. 2015;2(3):ofv104.
11. Breland M, Beckmann N. Amebic osteomyelitis in an immunocompromised patient. Radiol Case Rep. 2016;11(3):207–11.
12. Satlin MJ, Graham JK, Visvesvara GS, Mena H, Marks KM, Saal SD, et al. Fulminant and fatal encephalitis caused by *Acanthamoeba* in a kidney transplant recipient: case report and literature review. Transpl Infect Dis. 2013;15(6):619–26.
13. Mutreja D, Jalpota Y, Madan R, Tewari V. Disseminated *Acanthamoeba* infection in a renal transplant recipient: a case report. Indian J Pathol Microbiol. 2007;50(2):346–8.
14. Steinberg JP, Galindo RL, Kraus ES, Ghanem KG. Disseminated acanthamebiasis in a renal transplant recipient with osteomyelitis and cutaneous lesions: case report and literature review. Clin Infect Dis. 2002;35(5):e43–9.
15. Afshar K, Boydking A, Ganesh S, Herrington C, McFadden PM. Rapidly fatal disseminated acanthamoebiasis in a single lung transplant recipient. Ann Transplant. 2013;18:108–11.
16. Walia R, Montoya JG, Visvesvera GS, Booton GC, Doyle RL. A case of successful treatment of cutaneous *Acanthamoeba* infection in a lung transplant recipient. Transpl Infect Dis. 2007;9(1):51–4.

17. Duarte AG, Sattar F, Granwehr B, Aronson JF, Wang Z, Lick S. Disseminated acanthamoebiasis after lung transplantation. J Heart Lung Transplant. 2006;25(2):237–40.
18. Vernon SE, Acar BC, Pham SM, Fertel D. *Acanthamoeba* infection in lung transplantation: report of a case and review of the literature. Transpl Infect Dis. 2005;7(3–4):154–7.
19. Morrison AO, Morris R, Shannon A, Lauer SR, Guarner J, Kraft CS. Disseminated *Acanthamoeba* infection presenting with cutaneous lesions in an immunocompromised patient: a case report, review of histomorphologic findings, and potential diagnostic pitfalls. Am J Clin Pathol. 2016;145(2):266–70.
20. Voshtina E, Huang H, Raj R, Atallah E. Amebic encephalitis in a patient with chronic lymphocytic leukemia on Ibrutinib therapy. Case Rep Hematol. 2018;2018:6514604.
21. Nampoothiri RV, Malhotra P, Jain A, Batra N, Gupta K, Saj F, et al. An unusual cause of central nervous system infection during acute myeloid leukemia induction chemotherapy: *Acanthamoeba* brain abscess. Indian J Hematol Blood Transfus. 2018;34(1):153–5.
22. Coven SL, Song E, Steward S, Pierson CR, Cope JR, Ali IK, et al. *Acanthamoeba* granulomatous amoebic encephalitis after pediatric hematopoietic stem cell transplant. Pediatr Transplant. 2017;21(8):e13060. https://doi.org/10.1111/petr.13060.
23. Epperla N, Olteanu H, Hamadani M. Think outside the box: *Acanthamoeba* encephalitis following autologous haematopoietic stem cell transplantation. Br J Haematol. 2016;175(5):758.
24. Juan A, Alonso L, Olive T, Navarro A, Sulleiro E, Sanchez de Toledo J, et al. Successful treatment of sinusitis by *Acanthamoeba* in a pediatric patient after allogeneic stem cell transplantation. Pediatr Infect Dis J. 2016;35(12):1350–1.
25. Akpek G, Uslu A, Huebner T, Taner A, Rapoport AP, Gojo I, et al. Granulomatous amebic encephalitis: an under-recognized cause of infectious mortality after hematopoietic stem cell transplantation. Transpl Infect Dis. 2011;13(4):366–73.
26. Abd H, Saeed A, Jalal S, Bekassy AN, Sandstrom G. Ante mortem diagnosis of amoebic encephalitis in a haematopoietic stem cell transplanted patient. Scand J Infect Dis. 2009;41(8):619–22.
27. Kaul DR, Lowe L, Visvesvara GS, Farmen S, Khaled YA, Yanik GA. *Acanthamoeba* infection in a patient with chronic graft-versus-host disease occurring during treatment with voriconazole. Transpl Infect Dis. 2008;10(6):437–41.
28. Peman J, Jarque I, Frasquet J, Alberola C, Salavert M, Sanz J, et al. Unexpected postmortem diagnosis of *Acanthamoeba* meningoencephalitis following allogeneic peripheral blood stem cell transplantation. Am J Transplant. 2008;8(7):1562–6.
29. Castellano-Sanchez A, Popp AC, Nolte FS, Visvesvara GS, Thigpen M, Redei I, et al. *Acanthamoeba castellani* encephalitis following partially mismatched related donor peripheral stem cell transplantation. Transpl Infect Dis. 2003;5(4):191–4.
30. Feingold JM, Abraham J, Bilgrami S, Ngo N, Visvesara GS, Edwards RL, et al. *Acanthamoeba* meningoencephalitis following autologous peripheral stem cell transplantation. Bone Marrow Transplant. 1998;22(3):297–300.
31. Cope JR, Roy S, Ali I. Oral Abstract 865. *Acanthamoeba* Disease Associated with the Practice of Nasal Rinsing in Immunocompromised Patients. IDWeek 2018; October 4, 2018; San Francisco, CA2018.
32. Fowler M, Carter RF. Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. Br Med J. 1965;2(5464):740–2.
33. Butt CG. Primary amebic meningoencephalitis. N Engl J Med. 1966;274(26):1473–6.
34. Lam C, He L, Marciano-Cabral F. The effect of different environmental conditions on the viability of *Naegleria fowleri* amoebae. J Eukaryot Microbiol. 2019;66:752.
35. Siddiqui R, Ali IKM, Cope JR, Khan NA. Biology and pathogenesis of *Naegleria fowleri*. Acta Trop. 2016;164:375–94.
36. Cope JR, Ali IK. Primary amebic meningoencephalitis: what have we learned in the last 5 years? Curr Infect Dis Rep. 2016;18(10):31.

37. Yoder JS, Eddy BA, Visvesvara GS, Capewell L, Beach MJ. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962–2008. Epidemiol Infect. 2010;138(07):968–75.
38. Capewell LG, Harris AM, Yoder JS, Cope JR, Eddy BA, Roy SL, et al. Diagnosis, clinical course, and treatment of primary amoebic meningoencephalitis in the United States, 1937–2013. J Pediatric Infect Dis Soc. 2015;4(4):e68–75.
39. Matanock A, Mehal JM, Liu L, Blau DM, Cope JR. Estimation of undiagnosed *Naegleria fowleri* primary amebic meningoencephalitis, United States(1). Emerg Infect Dis. 2018;24(1):162–4.
40. Roy SL, Metzger R, Chen JG, Laham FR, Martin M, Kipper SW, et al. Risk for transmission of *Naegleria fowleri* from solid organ transplantation. Am J Transplant. 2014;14(1):163–71.
41. Visvesvara GS, Martinez AJ, Schuster FL, Leitch GJ, Wallace SV, Sawyer TK, et al. Leptomyxid ameba, a new agent of amebic meningoencephalitis in humans and animals. J Clin Microbiol. 1990;28(12):2750–6.
42. Cope JR, Landa J, Nethercut H, Collier SA, Glaser C, Moser M, et al. The epidemiology and clinical features of *Balamuthia mandrillaris* disease in the United States, 1974–2016. Clin Infect Dis. 2019 68(11):1815–1822.
43. Maritschnegg P, Sovinz P, Lackner H, Benesch M, Nebl A, Schwinger W, et al. Granulomatous amebic encephalitis in a child with acute lymphoblastic leukemia successfully treated with multimodal antimicrobial therapy and hyperbaric oxygen. J Clin Microbiol. 2011;49(1):446–8.
44. Epperla N, Olteanu H, Hamadani M. Think outside the box: *Acanthamoeba* encephalitis following autologous haematopoietic stem cell transplantation. Br J Haematol. 2016;175:758.
45. Ong TYY, Khan NA, Siddiqui R. Brain-eating amoebae: predilection sites in the brain and disease outcome. J Clin Microbiol. 2017;55(7):1989–97.
46. Qvarnstrom Y, Visvesvara GS, Sriram R, da Silva AJ. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. J Clin Microbiol. 2006;44(10):3589–95.
47. Yokoe D, Casper C, Dubberke E, Lee G, Munoz P, Palmore T, et al. Safe living after hematopoietic cell transplantation. Bone Marrow Transplant. 2009;44(8):509–19.
48. Smalley HK, Anand N, Buczek D, Buczek N, Lin T, Rajore T, et al. Assessment of risk for transplant-transmissible infectious encephalitis among deceased organ donors. Transpl Infect Dis. 2018;20(5):e12933.
49. Bennett WM, Nespral JF, Rosson MW, McEvoy KM. Use of organs for transplantation from a donor with primary meningoencephalitis due to *Naegleria fowleri*. Am J Transplant. 2008;8(6):1334–5.
50. Kramer MH, Lerner CJ, Visvesvara GS. Kidney and liver transplants from a donor infected with *Naegleria fowleri*. J Clin Microbiol. 1997;35(4):1032–3.
51. Avery RK, Michaels MG, Practice ASTIDCo. Strategies for safe living following solid organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13519.
52. Farnon EC, Kokko KE, Budge PJ, Mbaeyi C, Lutterloh EC, Qvarnstrom Y, et al. Transmission of *Balamuthia mandrillaris* by organ transplantation. Clin Infect Dis. 2016;63(7):878–88.
53. Centers for Disease Control and Prevention (CDC). *Balamuthia mandrillaris* transmitted through organ transplantation – Mississippi, 2009. MMWR Morb Mortal Wkly Rep. 2010;59(36):1165–70.
54. Centers for Disease Control and Prevention (CDC). Notes from the field: transplant-transmitted *Balamuthia mandrillaris* – Arizona, 2010. MMWR Morb Mortal Wkly Rep. 2010;59(36):1182.
55. Gupte AA, Hocevar SN, Lea AS, Kulkarni RD, Schain DC, Casey MJ, et al. Transmission of *Balamuthia mandrillaris* through solid organ transplantation: utility of organ recipient serology to guide clinical management. Am J Transplant. 2014;14(6):1417–24.

56. Sparkes T, Lemonovich TL, Practice ASTIDCo. Interactions between anti-infective agents and immunosuppressants – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13510.
57. Aichelburg AC, Walochnik J, Assadian O, Prosch H, Steuer A, Perneczky G, et al. Successful treatment of disseminated *Acanthamoeba* sp. infection with miltefosine. Emerg Infect Dis. 2008;14(11):1743–6.
58. Centers for Disease Control and Prevention. Investigational drug available directly from CDC for the treatment of infections with free-living amebae. MMWR Morb Mortal Wkly Rep. 2013;62(33):666.
59. Cope JR, Conrad DA, Cohen N, Cotilla M, DaSilva A, Jackson J, et al. Use of the novel therapeutic agent miltefosine for the treatment of primary amebic meningoencephalitis: report of 1 fatal and 1 surviving case. Clin Infect Dis. 2016;62(6):774–6.
60. Seijo Martinez M, Gonzalez-Mediero G, Santiago P, Rodriguez De Lope A, Diz J, Conde C, et al. Granulomatous amebic encephalitis in a patient with AIDS: isolation of *Acanthamoeba* sp. Group II from brain tissue and successful treatment with sulfadiazine and fluconazole. J Clin Microbiol. 2000;38(10):3892–5.
61. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol. 2007;50(1):1–26.
62. Singhal T, Bajpai A, Kalra V, Kabra SK, Samantaray JC, Satpathy G, et al. Successful treatment of *Acanthamoeba* meningitis with combination oral antimicrobials. Pediatr Infect Dis J. 2001;20(6):623–7.
63. Slater CA, Sickel JZ, Visvesvara GS, Pabico RC, Gaspari AA. Brief report: successful treatment of disseminated *Acanthamoeba* infection in an immunocompromised patient. N Engl J Med. 1994;331(2):85–7.
64. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int J Parasitol. 2004;34(9):1001–27.
65. Linam WM, Ahmed M, Cope JR, Chu C, Visvesvara GS, da Silva AJ, et al. Successful treatment of an adolescent with *Naegleria fowleri* primary amebic meningoencephalitis. Pediatrics. 2015;135(3):e744–8.
66. Colon BL, Rice CA, Guy RK, Kyle DE. Phenotypic screens reveal posaconazole as a rapidly acting amebicidal combination partner for treatment of primary amoebic meningoencephalitis. J Infect Dis. 2019;219(7):1095–103.
67. Laurie MT, White CV, Retallack H, Wu W, Moser MS, Sakanari JA, et al. Functional assessment of 2,177 U.S. and international drugs identifies the quinoline nitroxoline as a potent amoebicidal agent against the pathogen *Balamuthia mandrillaris*. MBio. 2018;9(5):e02051.
68. Tan SK, Gajurel K, Tung C, Albers G, Deresinski S, Montoya JG, et al. Fatal *Acanthamoeba* encephalitis in a patient with a total artificial heart (syncardia) device. Open Forum Infect Dis. 2014;1(2):ofu057.
69. Wang Q, Li J, Ji J, Yang L, Chen L, Zhou R, et al. A case of *Naegleria fowleri* related primary amoebic meningoencephalitis in China diagnosed by next-generation sequencing. BMC Infect Dis. 2018;18(1):349.
70. Basavaraju SV, Kuehnert MJ, Zaki SR, Sejvar JJ. Encephalitis caused by pathogens transmitted through organ transplants, United States, 2002–2013. Emerg Infect Dis. 2014;20(9):1443–51.

Part VI

Emerging Clinical Challenges

Ventricular Assist Devices, ECMO, and Cardiac Support Devices: Challenges in the Bridge to Transplant

59

Hao Tran and Saima Aslam

Contents

Introduction 1475
Types of Mechanical Circulatory Support 1476
Current Changes to UNOS Listing Criteria in the United States [14] 1478
Definitions of VAD Infections 1479
Epidemiology of VAD Infections 1480
Microbiology 1481
Pathogenesis 1482
Risk Factors for Infections 1482
Strategies for Prevention 1484
Clinical Outcomes 1485
Management of Infection 1486
Significant Circumstances 1488
Post-heart Transplant Outcomes in VAD Infection 1489
Cerebrovascular Risk in VAD Infection 1489
Conclusions 1489
Key Points 1490
References 1490

Introduction

Over the last decade and a half, left ventricular assist device (LVAD) implantation has emerged as steadfast therapy for patients inflicted with advanced stages of heart failure. Although the continuum of care is more complex than simple dichotomous

H. Tran
Division of Cardiovascular Medicine, University of California San Diego, La Jolla, CA, USA
e-mail: hatran@health.ucsd.edu

S. Aslam (✉)
Division of Infectious Diseases and Global Public Health, University of California San Diego, La Jolla, CA, USA
e-mail: saslam@health.ucsd.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_59

designations, currently these mechanical circulatory support (MCS) devices can act as temporary support while a patient awaits heart transplantation (bridge-to-transplant, or BTT), or definitive lifelong therapy (destination therapy, or DT). Large multicenter MCS registries report a progressively increased volume of patients implanted. The International Society for Heart and Lung Transplantation (ISHLT) Registry for Mechanically Assisted Circulatory Support (IMACS) recently reported outcomes from over 14,000 patients from 35 countries between 2013 and 2016 [1]. A recent paper from the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) published outcomes of more than 22,000 LVAD recipients between 2006 and 2016 [2].

The widespread success and generalized acceptance of this therapy have been impacted by the high rates of device-related complications. Although the second and third generations of continuous-flow LVADs (CF-LVAD) have significantly evolved to mitigate complications related to the device, infections continue to be prevalent in this patient population [3, 4].

In this chapter, we will review the patient risk factors, timing and management of infection, and improvements in device design for both durable LVADs (including the HeartMate II, HeartWare HVAD, HeartMate 3, and Jarvik) and temporary LVADs (including the intra-aortic balloon pump, Impella, and extracorporeal membrane oxygenation, ECMO). The latter group has become important in the management of patients with advanced heart failure particularly with the new 2018 United Network for Organ Sharing (UNOS) changes to listing criteria for heart transplant in the United States, in which use of temporary MCS devices is designated with a higher listing status and potentially shorter waitlist time [5].

Types of Mechanical Circulatory Support

Cardiogenic shock affects nearly 50,000 patients annually in the United States and carries more than 40% mortality despite the use of vasopressors, inotropes, and MCS [6, 7]. Temporary cardiac and circulatory support devices are employed to bridge the failing heart toward heart transplantation, durable LVADs, or cardiac recovery. Figure 1 illustrates the different types of temporary (**1a**) and durable (**1b**) MCS.

The intra-aortic balloon pump (IABP) is a catheter-mounted pneumatic pump that can be rapidly inserted at the bedside. Balloon inflation during the diastolic phase displaces blood in the descending aorta thereby proximally augmenting coronary perfusion and distally increasing tissue perfusion. Balloon deflation during systole promotes forward flow and reduces left ventricle (LV) afterload thereby increasing LV stroke volume [8].

The Impella (Abiomed Inc, Danvers, MA) is composed of a set of catheter-mounted axial flow pumps positioned across the aortic valve that continuously displace blood from the LV to the ascending aorta. This family of pumps include the Impella 2.5, CP, and 5.0, roughly providing circulatory support of 2.5 L/min, 3.0–3.5 L/min, and 5.0 L/min, respectively.

The venoarterial extracorporeal membrane oxygenator (VA-ECMO) system consists of a centrifugal pump, a heat exchanger, and a membrane oxygenator. A

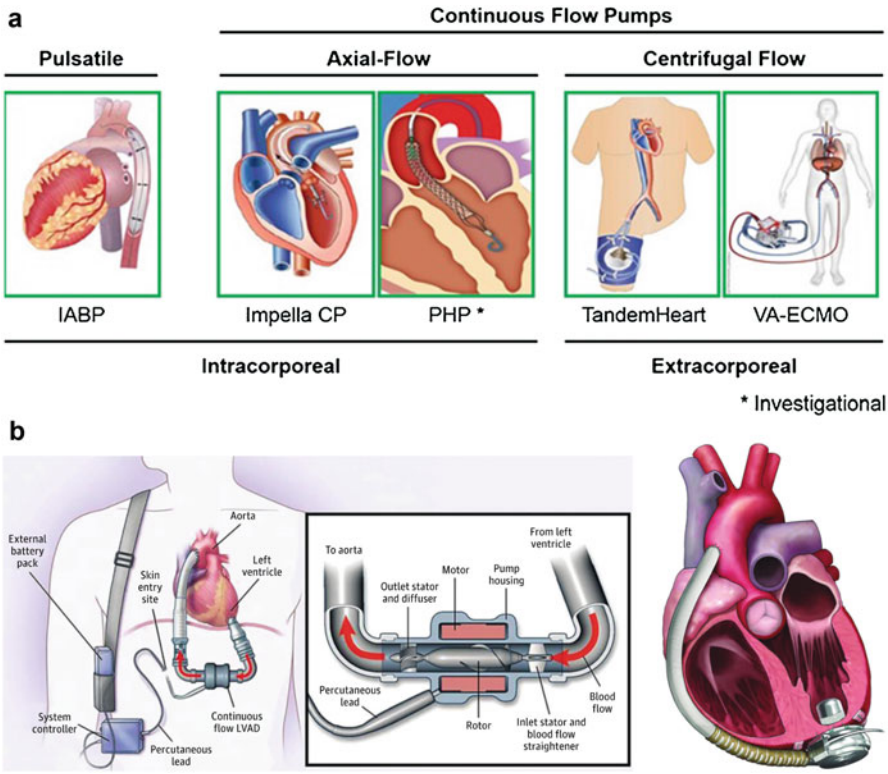


Fig. 1 (**a**) **Types of temporary mechanical circulatory support.** Intra-aortic balloon pump (IABP), Impella axial flow catheter, percutaneous heart pump (investigational axial flow catheter), TandemHeart centrifugal flow pump, and venoarterial extracorporeal membrane oxygenation (VA-ECMO). (Figure adapted with permission from Morine et al. [12]) (**b**) **Types of durable mechanical circulatory support.** (Left) Abbott (Thoratec) HeartMate II axial continuous-flow pump and (right) HeartWare HVAD centrifugal continuous-flow pump. Not pictured, HeartMate XVE and HeartMate 3 devices from Abbott. (Figure adapted with permission from Miller et al. [13])

percutaneous catheter placed in the right atrium or superior vena cava delivers venous blood to the arterial system with a catheter in the descending aorta, using an extracorporeal centrifugal pump. This versatile system provides biventricular circulatory support and provides gas exchange with oxygenation.

The TandemHeart (Cardiac Assist, Inc., Pittsburgh, PA) is an extracorporeal centrifugal pump with inflow cannula placed in the left atrium utilizing a transseptal puncture and an outflow cannula positioned in the femoral artery. This pump reduces LV preload and provides between 3.0 and 5.0 L/min of support.

Durable MCS devices, consisting of ventricular assist devices (VADs), comprise of an inflow cannula surgically implanted in the apex of the LV and pump blood through a motor, either an axial or a centrifugal pump, to the outflow cannula anastomosed to the ascending aorta. There are five main components of a VAD:

the inflow cannula, a pump, an outflow cannula, a percutaneous driveline, and an electrical controller. The pumping chamber is placed within the abdominal wall or intrapericardial space [9, 10]. The percutaneous driveline carries the electrical cable and air vent to the battery packs and electronic control unit, worn either on a shoulder holster or belt. Both second- and third-generation pumps are used today, offering continuous flow for the patient; these have supplanted the first-generation bulky pulsatile pumps (Fig. 1b).

VADs are classified as durable devices because patients can be discharged home on support and can be maintained on these devices for months to years while awaiting transplantation. The second-generation pump, the HeartMate II (Abbott, Pleasanton, CA), has been implanted in over 26,600 heart failure patients [11]. The mechanism of propulsion of blood is based on an axial rotor with a casing unit settling in a surgical pocket underneath the diaphragm. The HeartWare HVAD (Medtronic, Framingham, MA) is the first available third-generation LVAD. This system is directly inserted in the left ventricular apex. The HVAD mechanism of flow is based on a centrifugal system pulling blood from the ventricle and delivering the blood up the outflow cannula. As the newest third-generation device, the HeartMate 3 (Abbott, Pleasanton, CA) has a very similar centrifugal mechanism to the HeartWare HVAD; however HeartMate 3 does have an artificial pulse aimed at washing the housing unit to prevent clot formation.

Current Changes to UNOS Listing Criteria in the United States [14]

The major goal behind the recent changes to the US UNOS listing criteria was to decrease mortality rates for transplant-eligible patients on the waitlist. The previous three-tier system was too narrow to sufficiently prioritize medically urgent cases. The new heart transplant allocation guidelines, introduced October 2018, implemented six tiers with more specific descriptions for each tier. Significantly, LVADs are now seen as viable, long-term alternatives to cardiac transplantation. Advances in LVAD surgical technique, medical management, and patient selection have improved overall patient outcomes [15, 16]. Therefore, stable LVAD patients are listed in Tier 4.

Meanwhile patients on ECMO receive the highest priority, Tier 1. These patients are the sickest and represent the highest mortality on the waitlist. This first tier is followed by patients with a non-dischargeable, surgically implanted device, or the IABP. Consequently, the transplant community is eager to monitor the incidence of usage of the aforementioned temporary MCS devices [5].

There are concerns with the implementation of the new cardiac allograft allocation system, as many believe that prioritizing ECMO patients will result in worse post-transplant outcomes or are concerned with the possibility that hearts will be given to patients who will experience a smaller marginal benefit when compared to other patients. In particular, infectious complications are a concern that potentially could impact post-transplant as well as waitlist mortality. Therefore, centers are urged to be highly selective in listing patients with ECMO or temporary MCS for transplant, even though these patients have the highest priority in the allocation process [5].

Definitions of VAD Infections

With growth in the emerging MCS field, standardization of infection definitions and diagnostic criteria has become paramount. In 2011, the ISHLT commissioned a consensus paper detailing standard definitions to improve clinical communication and facilitate comparison between practices and centers [17]. These definitions – based on the pathophysiology of equivalent infectious processes (e.g., indwelling prosthetic devices and catheter-related infections) – have been divided into three categories as noted in Fig. 2:

VAD-specific infections are directly related to the device hardware such as pump and/or cannula infections, pocket infections, and percutaneous driveline infections (superficial or deep).

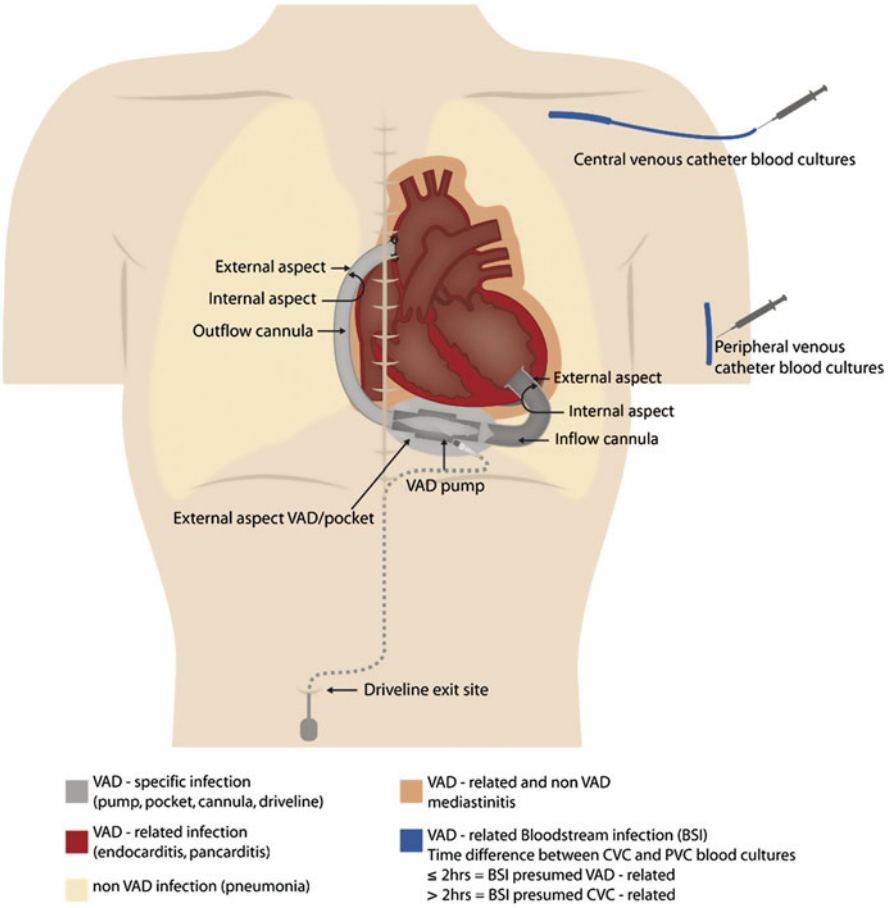


Fig. 2 Categories of LVAD infections. (Figure adapted with permission from Hannan et al. [17])

VAD-related infections warrant special considerations due to the presence of the LVAD, such as infective endocarditis, bloodstream infections, and mediastinitis.

Non-VAD infections are unrelated to the indwelling device and consist of lower respiratory tract infections, cholecystitis, *Clostridiodes difficile* infection, urinary tract infections, and others.

Epidemiology of VAD Infections

The incidence of VAD infections varies depending on the device used and subsequent management. Of the >15,000 patients in the most recent seventh INTERMACS report, infection was the fourth most common cause of death within 1 year after implantation (8.8%), following multisystem organ failure (MSOF, 15.6%), neurologic complications (15.6%), and withdrawal of support (10.4%) [18]. As the incidence of MSOF or withdrawal of support caused by severe infection cannot be delineated in the report, the true incidence of infection leading to death may be underestimated. In the European Registry for Patients with Mechanical Circulatory Support (EUROMACS) inaugural report, infection was the most common major adverse event occurring in 153 of 741 patients (35.3%) and accounted for 23% of the total reported cases of mortality [19].

Of potential device-related complications, infection is a common reason for readmission [20, 21]. Table 1 lists several of the recent studies that report the incidence of infection after VAD implantation. The true incidence of VAD-associated infection is difficult to ascertain, in part due to a relative lack of infectious disease-related variables in larger databases.

As detailed later in this chapter, the transition from pulsatile-flow to continuous-flow devices was associated with a decline in infection rates.

In a global analysis between 2013 and 2015, non-VAD infections were the leading category of infection and the most common within 3 months of implantation [28]. Of 10,171 patients, 4,501 individuals had a non-VAD infection, while 1,756 and 501 patients had VAD-specific infections and VAD-related infections, respectively. Pneumonia, non-VAD-related bloodstream infection, and urinary tract infection were the most common non-VAD infections. It appears the more invasive the pump and power mechanism, the higher the risk of adverse events overall. A meta-analysis comparing IABP to percutaneous VAD (pVAD) noted that although survival benefit was similar with either device strategy, pVADs were associated with more adverse events including infection, acute kidney injury, major bleeding, and vascular injury [29]. ECMO-supported patients harbor the greatest risk of bleeding, cerebrovascular events, and infection. In a Korean study, 23% of ECMO patients were recorded to have nosocomial infections including respiratory tract and bloodstream infections. Independent predictors of infection during ECMO were preoperative creatinine and duration of ECMO support [30]. Similarly, a Chinese study reported a nosocomial infection rate of 25.7% in their cohort of ECMO patients. The majority of cases involved Gram-negative bacterial infections (74.1%) [31]. Finally, in a recent meta-analysis of 2,986 post-cardiotomy ECMO patients, 14.7% of patients were recorded to have deep

Table 1 Recent studies involving LVAD patients with incidence of infection

Study	Number of patients (timeframe)	Device(s) used (no of patients)	Patients in the study with infection (%)
Schaffer et al. [22]	133 patients (Jun 2000–May 2009)	HeartMate XVE (47) HeartMate II [85]	DLI: XVE 19 patients (40%), HMII 28 patients (30%)
Nienaber et al. [22]	247 patients (Jan 2005–Dec 2011)	HeartMate II HeartWare	Total: 78 patients (32.3%) DLI: 37 patients (14.9%)
Hasin et al. [23]	115 patients (Jan 2008–July 2011)	HeartMate II	Total: 25 patients (21.7%)
Slaughter et al. [4]	332 patients (Aug 2008–Dec 2011)	HeartWare	DLI: 56 patients (16.9%)
Simeon et al. [24]	159 patients (2007–2012)	HeartMate II [33], HeartWare [2], Ventracor [1]	Total: 36 patients (22.6%) DLI: 17 patients (10.6%)
Mehra et al. [3]	366 patients (Sep 2014–Nov 2015)	HeartMate II (176), HeartMate 3 (190)	DLI: 79 patients (21.8%) Non-LVAD: 130 patients (36%)
Gustafsson et al. [25]	482 patients (Jan 2015–current)	HeartMate 3	Total: 163 patients (35.2%) DLI: 54 patients (11.7%)
Hanke et al. [26]	27 patients (Jun 2014–Apr 2017)	HeartMate 3	Total 13 patients (48.1%); VAD related 6 patients (24%)
Olmsted et al. [27]	437 patients (May 2009–Mar 2016)	HeartMate II (314), HeartWare (123)	Total: 244 patients (55.8%) DLI: 69 patients (15.8%)

Abbreviations: *DLI* driveline infections

sternal wound infection/mediastinitis [32]. The studies concluded that in order to reduce the incidence of ECMO-associated infections, preventative strategies should be employed to shorten the duration of ECMO support.

Microbiology

Despite the evolution of VAD management and device design, pathogens implicated in infections have not changed over time. The percutaneous driveline is a common portal of entry for these organisms. The most common organisms, *Staphylococcus aureus* and *Staphylococcus epidermidis*, are responsible for more than 50% of MCS infections. These pathogens are present on the skin and adhere to indwelling devices creating a biofilm [33–35]. *Enterococcus* species are the next most common Gram-positive organism [23]. The most common Gram-negative bacteria are *Pseudomonas aeruginosa* (22–28%), followed by *Enterobacteriaceae* such as *Klebsiella* species (2–4%) and *Enterobacter* species (2%) [23].

While the prevalence of fungal infection is low, high morbidity and mortality are associated with this type of organisms due to the difficulty in achieving eradication. Candidemia has been reported in 1.3–9.7% of VAD recipients; fungal VAD infections carry an overall mortality of up to 90% [36, 37]. The most common fungal pathogen is *Candida albicans* (70%), followed by *C. glabrata* (10%) [23].

Pathogenesis

Bacterial and fungal organisms infecting cardiac devices rapidly form biofilms which are difficult to eradicate with antimicrobial therapy alone. A biofilm consists of microbial cells that are adherent to the underlying device surface and encased within an extracellular polysaccharide matrix [38, 39]. This protective mechanism of growth allows survival in otherwise hostile environments; thereby organisms within a biofilm behave in a fundamentally different manner than their free-floating (planktonic) counterparts [34]. In fact, gene expression profiling and proteomic techniques, used to compare organisms in a biofilm and planktonic organisms, have revealed significant differences in gene and protein expression [40, 41]. The extracellular polysaccharide matrix offers a distinct advantage by preventing the penetration of opsonizing antibodies and mitigating the phagocytic ability of macrophages. Additionally, organisms in a mature biofilm diminish the efficacy of antibiotics due to (1) slower rate of division, (2) centralized density of pathogens within the biofilm, (3) changes in bacterial metabolism, and (4) antimicrobial resistance due to proximity-facilitated bacterial conjugation [42, 43]. These mechanisms lead to increased difficulty in organism eradication, often necessitating prolonged courses of antibiotic administration or, ultimately, device removal. Moreover, the minimal inhibitory concentrations for organisms within a biofilm can be approximately 1,000-fold higher than the planktonic counterparts [34]. A recent study evaluated biofilm formation and migration using a drip-flow reactor assay. This study revealed that *P. aeruginosa* and *C. albicans* have the greatest biomass compared to other organisms, highlighting that different pathogens appear to have a propensity to different components of the driveline. Finally, as these protective properties of mature biofilm deem these organisms extremely difficult to eradicate, patients with infected devices may experience prolonged bridge-to-transplant VAD waiting period and worse post-transplant outcomes [44].

Risk Factors for Infections

The body of literature describing risk factors for VAD-related infections is based on limited retrospective analyses. A recent systematic review and meta-analysis of studies revealed younger age and higher body mass index (BMI) were associated with higher rates of VAD infections [45, 46]. Diabetes mellitus (DM) is often a comorbidity with advanced heart failure. Sepsis was found to be higher in diabetics based on retrospective studies of patients undergoing LVAD implantation [46, 47]. The Ventricular Assist Device Infection Study Group prospectively evaluated preoperative indices and noted both depression and elevated baseline serum creatinine were independent predictors of VAD infection [48].

Malnutrition is a significant comorbidity in advanced heart failure patients. Comprehensive preoperative evaluation of nutritional status, including measurements of albumin and BMI, has been shown to be essential in managing heart failure patients [49, 50]. The immune system is attenuated in patients on VAD support and

Table 2 Risk factors for infection in patients with VAD

Extremes of age	Intravascular lines	Organisms capable of biofilm formation
Diabetes mellitus	Delayed sternal closure	T-cell dysfunction associated with the device
Renal failure, hemodialysis	Longer ICU stay	Hypogammaglobulinemia
Malnutrition	Prolonged duration of device support	Trauma to the driveline
Obesity	Concomitant surgery at the time of VAD placement	Severity of heart failure (i.e., cardiogenic shock)
Depression	Frailty	

therefore plays an even more important role in infection risk. This occurs primarily due to cytokine downregulation and emergence of suppressive T-regulatory cells regardless of the age of the patient [51]. Quantitative immunoglobulin G (IgG) levels were evaluated in a bridge-to-transplant cohort, and it was found that hypogammaglobulinemia (HGG) is associated with an increased risk of infection [52]. Table 2 summarizes risk factors for infection in patients with VAD.

Perioperative technique has a significant influence on the risk of infection. Registry analysis was performed comparing outcomes in patients with the HeartMate II device placed utilizing a surgical tunneling technique to keep the entire driveline (DL) velour portion in the subcutaneous tunnel, resulting in a silicone-skin interface (SSI) at the exit site, as opposed to patients with device placement via the velour-to-skin method used in the HMII DT trial [53]. The results demonstrated that leaving the entire DL velour portion below the skin was associated with at least a 50% reduction in DL infection compared to the standard procedure. In a related study, patients implanted with HMII at a single institution were divided between two eras: the externalized velour era (2008–2009) and the externalized silicone era (2010–2011) [54]. Externalization of only the silicone portion of the DL led to a significant reduction in infections, 44% in the externalized velour group vs 8.8% in the externalized silicone group ($p<0.05$). Delayed sternal closure in patients with coagulopathy, hemodynamic instability, or isolated right ventricular dysfunction has been associated with longer ICU stays but does not significantly increase the risk of infection or death [55]. Other studies have shown prolonged ICU stay after implantation, and longer duration of VAD support is associated with an increased risk of infection [56, 57].

Evolutions in design and device selection play a role in the risk of infection as well. The early-generation pulsatile-flow LVAD (PF-LVAD), HeartMate XVE pump, was crippled by infection among other complications. In retrospective analyses of the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial and HeartMate XVE registry, sepsis was the leading cause of death in patients with PF-LVAD, accounting for 29.5–41% of deaths [58, 59]. Although the study was based on patients not eligible for transplant, the data reveal how infections plagued the earlier devices.

New-generation continuous-flow LVADs (the axial pump HeartMate II and the continuous-flow pumps HeartWare HVAD and HeartMate 3) have been the most widely available devices in the last decade owing to the enhanced design, smaller device pocket, and lower complication profiles. In the HeartMate II BTT study, sepsis accounted for 20% of deaths at 6 months, while retrospective analyses illustrate lower incidences of infection in both newer-generation devices compared to the predecessor device [60–62].

As noted earlier, the percutaneous driveline is a common portal of entry for microbial pathogens. Many VAD-related infections occur early after implantation before the driveline site has fully healed and integrated into the host tissue. Factors that impair healing, such as excessive movement or trauma at the exit site, poor nutritional or overall status of the patient at the time of implantation, and diabetes mellitus, can all increase the likelihood of driveline infections.

Finally, the daily care provided by patients and their caregivers is crucial in attenuating infection risk. Disruption of the seal between the driveline and adjacent skin, primarily through pulling of the driveline, has been reported as a common cause of infection [63].

Strategies for Prevention

The prevention strategies involved in curbing VAD infection rates are multifaceted but can be divided into four targeted categories [64]:

1. *Preoperative risk factor modification*: Management of modifiable risk factors include weight loss in obese patients, nutritional optimization in malnourished patients (e.g., enteral feeding tube), euglycemia in patients with diabetes mellitus, complete treatment of open wounds, minimizing the use of urinary and vascular catheters, and using tunneled catheters when vascular access is needed for more than 14 days. A full screening of active infections along with a full dental evaluation in those with poor dentition is also recommended.
2. *Antibiotic prophylaxis at time of surgery*: Although no randomized trials exist to guide the use of antibiotics and antifungals, in VAD recipients, an ISHLT consensus recommendation included anti-staphylococcal agents (e.g., vancomycin or cefazolin) as prophylaxis for a maximum period of 48 h postoperatively [64]. This was based on studies of prophylaxis in cardiac surgery patients in general.
3. *Surgical techniques*: Driveline tunneling technique has evolved with subsequent LVAD generations. The new standard of care involves tunneling of the velour portion of the DL into the subcutaneous tissue. As noted earlier, this technique is associated with a substantial reduction in DL infection [54]. A minimally invasive, lateral thoracotomy approach to spare the sternum has shown

to decrease infection risks when compared to the median sternotomy, albeit without significant impact on survival, though this remains investigational at present [65, 66].

4. *Long-term driveline management strategies*: Current recommendations include extensive patient and caregiver education to prevent VAD driveline-related trauma (e.g., dropping the battery), DL immobilization using a binder or anchoring device immediately postoperatively, aseptic dressing change techniques (in accordance with institutional policies) using sterile gloves and mask, and the use of chlorhexidine as a cleaning agent at the DL exit site [64].

Clinical Outcomes

Survival after heart transplant is similar for patients with or without VADs leading up to the transplant [67]. Other studies note that patients with VAD infection can be transplanted successfully as well [46, 56, 68].

Infection is the fourth most common cause of death in patients with durable VADs within 1 year and steadily rises over a 4-year follow-up period [18]. Device infection often presents as a spectrum of disease progression. Superficial DLI may evolve over time to become deep tissue infection, and those with deep tissue infection are more likely to develop bacteremia [69, 70]. DLIs are the most common VAD-specific infection. Superficial DLIs may be treated with a 2-week course of antibiotics. However, deep DLIs generally require prolonged intravenous antibiotics as well as suppressive therapy as they are difficult to eradicate. Suppressive therapy is generally continued until transplantation. Intraoperative cultures at the time of transplant surgery may be used to guide the selection and duration of post-transplant antibiotics. Bloodstream infections are less frequent but are associated with increased incidence of cerebrovascular events and mortality [71]. Deep DLIs can be associated with BSIs as well.

The timing of infection after LVAD implantation influences the type of infection. Initial infections within the first 3 months of device implant tend to be nosocomial infections that are common in a critical care setting [28]. Among nosocomial infections, pneumonia was the leading cause, followed by bloodstream infection (BSI), urinary tract infection (UTI), and gastrointestinal (GI) infection. Early DL infection within 3 months of implant may be directly related to the implant surgery. Generally, outside of this time period, device-specific and related infections tend to be more prevalent. The risk of infection increases as a function of time on device support, and this is particularly true of DL infection [28].

The infecting organism also may differ depending on duration of time elapsed after LVAD implantation [72]. In one study, patients were divided into two groups: early ($<$180 days) and late ($\geq$180 days). Superficial driveline-related infection was the most common type of infection in both groups, whereas driveline drainage

was more prevalent in the late group. *S. aureus* was the most commonly isolated organism in early VAD infection (69% vs 33%, p = 0.049), while *P. aeruginosa* was the most prevalent bacteria in late VAD infection (25% vs 57%, p = 0.045). And as expected, deeper infections were associated with worse outcomes, but the overall mortality in this study was extremely high (13% vs 46%, p 0.046).

In a Taiwanese study of all infected ECMO patients in the national hospital system, pneumonia accounted for 50%, while primary bloodstream infection accounted for 15%. The primary pathogens were evenly attributed to Gram-negative bacteria, *Enterobacteriaceae*, and Gram-positive cocci [73].

Management of Infection

Without randomized, controlled studies, the principles governing guideline-based therapies are limited to observational data and expert opinion. In a consensus document, a formal infectious disease consultation is recommended in VAD patients with proven or suspected infection. The overall management goals should include evaluation of the extent of infection as well as microbiologic identification of the causative organism [65]. The general approach to infection management is detailed in Table 3.

Basic laboratory testing, microbiologic cultures, and imaging studies are recommended. Computed tomography (CT) and ultrasonography (US) of the chest and abdomen, although lacking in specificity, are endorsed as initial imaging modalities to assist in identifying deep DLI as well as abscess. Single-photon emission computed tomography scans (SPECT) with radiolabeled leukocytes can improve both sensitivity and specificity and therefore can be considered as additional diagnostic modalities [74–77]. A recent paper demonstrated the feasibility of [18] F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) for the diagnosis of left ventricular assist device (LVAD) infection as well [78].

Once an infection is suspected, empiric antimicrobial therapy against *Staphylococcus* species and *P. aeruginosa* should be started promptly. As illustrated in Table 3, in patients with infection limited to the superficial plane without systemic signs of sepsis, cultures of the DL should be collected followed by antibiotic therapy for 2 weeks. Patients with deeper infection (e.g., driveline, pocket infection, pump, cannula infection, systemic illness, or sepsis) should be hospitalized and prompt a procedure of surgical drainage with consideration for wound vacuum-assisted closure (VAC) system [79, 80]. In patients with pump and cannula infections, persistent bloodstream infection, septic emboli, or sepsis despite adequate antimicrobial or drainage therapy, device explant may be considered. Device removal/heart transplant remains the definitive cure of a VAD-specific infection [81–83].

Procalcitonin (PCT) has been widely used to assist in antibiotic stewardship in bacterial infection. However, a study in VAD patients noted that PCT levels in this population were not correlated with infection but serial changes may have been related to postoperative inflammation [84].

Table 3 Management of VAD-specific and VAD-related bacterial infections [64]

Infection types	Medical management	Surgical management
VAD-specific		
Superficial DLI	Treat with IV or PO antibiotics for a minimum of 2 weeks or until infection has resolved (drainage, redness, tenderness, etc.)	None
Deep DLI/pocket infection	Treat with IV antibiotics until clinical stabilization and improvement of infection (usually 6 to 8 weeks), followed by long-term PO suppression therapy	Surgical debridement and/or abscess drainage and wound VAC as necessary. New drive-line exit site from previous infected tract may be required
VAD pump and/or cannula	Treat with IV antibiotics for at least 6–8 weeks. If bacteremia resolves, recommend PO suppression until transplantation in BTT patients. Check intraoperative cultures: If positive, recommend 4–6 weeks of IV antibiotics following transplantation If negative, recommend 2 weeks of PO antibiotics following transplantation	Surgical drainage and debridement may be required to control infection. Source control: BTT: explant the device for HT DT: explant/exchange device for control of infection
Persistent bacteremia, relapsing infection, septic emboli, sepsis despite adequate antimicrobial/ surgical therapy	BTT: IV antibiotics should continue until after HT. Base duration and route of antibiotics on intraoperative cultures DT: IV antibiotic treatment is followed by long-term PO antibiotic suppression; if at times PO options are not available, IV therapy is continued beyond 8 weeks. Duration of antibiotic treatment after device exchange depends on the clinical course and pathogen. A longer course (4–6 weeks) should be offered with positive intraoperative cultures or recent preoperative bacteremia, and a shorter course (14 days) may be offered in the absence of such conditions	Strong consideration for device explant/exchange. One could consider bridging patients with a percutaneous device to achieve infection control prior to VAD exchange

(continued)

Table 3 (continued)

Infection types	Medical management	Surgical management
VAD-related		
Bacteremia	Duration of antibiotics depends on the source, organism, and clearance of bacteremia *S. aureus*: treat for 4–6 weeks and remove catheter *S. aureus* negative, blood cultures negative within 24–48 hrs without signs of metastatic infection: 2 weeks from first negative blood culture may be adequate (e.g., urinary tract source) No identifiable source: treatment may be considered as with VAD pump and cannula infection	
Bacterial mediastinitis	Duration of antibacterial therapy is at least 6 to 8 weeks after last surgical debridement	Surgical debridement is often indicated. Open chest and VAC wound closure may be required
Infective endocarditis	Duration of antibacterial therapy is the same as for MCS pump and cannula infection	Surgical intervention may be required

Adapted with permission from Kusne et al. *Abbreviations: BTT* bridge to transplant, *DL* driveline, *DT* destination therapy, *HT* heart transplant, *IV* intravenous, *PO* oral, *VAC* vacuum-assisted closure

Significant Circumstances

Multidrug resistant (MDR) bacteria. MDR bacterial infections lead to longer intensive care unit stay ($p < 0.0001$) and longer cumulative hospital stay ($p = 0.04$) in patients with VADs. Colonization with MDR bacteria leads to a higher risk of infection-associated death as well [85].

Fungal infection. Although fungal infection such as fungemia, necrotizing pulmonary aspergillosis, and *Aspergillus* device infection are rare, the potential devastating and fatal complications after VAD implantation warrant some attention. A retrospective review of 292 patients receiving VAD support between 1996 and 2009 revealed only seven cases of VAD-associated fungemia: five patients with *Candida* species and two with *Aspergillus* species [86]. The latter two succumbed to multiorgan failure and sepsis, while all five patients with *Candida* infections were successfully treated with systemic antifungal therapy leading to transplantation in four of the five. The last patient remained on DT support at the publication of the review. In a separate case report, a patient with necrotizing pulmonary aspergillosis and device involvement was successfully treated by segmentectomy and prolonged antifungal treatment without device exchange or removal [87].

Post-heart Transplant Outcomes in VAD Infection

Patients with a history of device infection are at increased risk of infectious complications following heart transplantation. Studies have shown patients with pre-transplant DLI predict post-transplant infection and increased overall post-transplantation hospital length of stay; however, post-transplant survival risks may vary [70, 73, 74, 88].

Two large recent analyses report a decrease in survival in VAD patients with pre-transplant infections. A retrospective analysis of UNOS database involving 15,253 patients listed as status 1A (the highest status in the previous listing algorithm) noted that patients with pre-transplant device infection had increased mortality compared to those without complications [89]. A recent meta-analysis including 6,067 LVAD patients demonstrated increased post-transplant mortality in patients with a VAD infection (hazard ratio [HR] 1.30, 95% CI: 1.16–1.46, $p < 0.001$) [90].

Cerebrovascular Risk in VAD Infection

Cerebrovascular accident (CVA) remains one of the most devastating complications in VAD patients, with high associated morbidity and mortality. Bloodstream infections (BSI) have been shown to be associated with CVA in recent reports. This was first described in the continuous axial pump population (HeartMate II) in which patients with VAD and bacteremia were found to have a much greater incidence of CVA compared to those without BSI (43.3% vs 10.0%, $p < 0.0001$), carrying an eightfold increase in risk (OR 7.9, [CI] = 2.4–25.5; p 0.001], compared to VAD patients without BSI [91]. In particular, *P. aeruginosa* BSI are associated with hemorrhagic CVA [92–94]. A recent INTERMACS analysis of 16,597 CF-LVAD recipients reported that infected VAD patients had a significantly higher prevalence of CVA compared to patients without an infection (18% vs 11%, $p < 0.001$). Of the three ISHLT categories of infection, VAD-related infections were associated with the highest risk for CVA, followed by non-VAD related and then VAD-specific infections.

Conclusions

We reviewed current MCS devices used as a bridge to transplant in patients with heart failure including VADs, percutaneous cardiac support devices, and ECMO. Most of the current literature regarding infection in MCS is in patients with VADs; definitions, microbiology, prevention, and management strategies specific to VADs are discussed in this chapter. Given an increase in the use of percutaneous devices and ECMO as a bridge to transplant, there is a need to study infections in this population and in particular a need to study post-transplantation outcomes.

Key Points

1. Infections in patients with ventricular assist devices (VADs) are categorized as VAD-specific (driveline, pump, pocket), VAD-related (bacteremia, mediastinitis, infective endocarditis), and non-VAD–related infections (urinary tract infections, *Clostridioides difficile*, among others).
2. Patients with temporary mechanical circulatory support devices, such as intra-aortic balloon pump, percutaneous VAD, and extra-corporeal membrane oxygenation (ECMO), have high rates of infection.
3. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are key pathogens that cause VAD infections, and initial empiric therapy should include agents active against both.
4. Strategies to prevent VAD infections are modification of pre-operative risk factors, antibiotic prophylaxis at the time of surgery, change in surgical practices (such as externalization of the silicone portion of the driveline, modification in thoracotomy approaches), and driveline exit site management.
5. Management of deep driveline, pump, pocket, and other complicated VAD-related and VAD-specific infections consists of prolonged courses of intravenous antibiotics followed by suppressive therapy till transplant.
6. Bloodstream infections in VAD recipients are associated with cerebrovascular complications.

References

1. Kirklin JK, Xie R, Cowger J, et al. Second annual report from the ISHLT mechanically assisted circulatory support registry. J Heart Lung Transplant. 2018;37(6):685–91.
2. Kirklin JK, Pagani FD, Kormos RL, et al. Eighth annual INTERMACS report: special focus on framing the impact of adverse events. J Heart Lung Transplant. 2017;36(10):1080–6.
3. Mehra M, Goldstein M, Uriel N, et al. Two-year outcomes with a magnetically levitated cardiac pump in heart failure. N Engl J Med. 2018;378(15):1386–95.
4. Slaughter M, Pagani F, McGee E. HeartWare ventricular assist system for bridge to transplant: combined results of the bridge to transplant and continued access protocol trial. J Heart Lung Transplant. 2013 Jul;32(7):675–83.
5. Yin MY, Wever-Pinzon O, Mehra M, et al. Post-transplant outcome in patients bridged to transplant with temporary mechanical circulatory support devices. J Heart Lung Transplant. 2019. [Epub ahead of print];38:858.
6. Hochman JS, Sleeper LA, Webb JG, et al. Early revascularization in acute myocardial infarction complicated by cardiogenic shock. SHOCK investigators. Should we emergently revascularize occluded coronaries for cardiogenic shock. N Engl J Med. 1999;341:625–34.
7. Kolte D, Khera S, Aronow WS, et al. Trends in incidence, management, and outcomes of cardiogenic shock complicating ST-elevation myocardial infarction in the United States. J Am Heart Assoc. 2014;3:e000590.
8. DeSilva K, Lumley M, Kailey B, et al. Coronary and microvascular physiology during intra-aortic balloon counterpulsation. J Am Coll Cardiol Intv. 2014;7(6):631–40.
9. Miller LW, Pagani FD, Russell SD, et al. HeartMate II clinical investigators. Use of a continuous-flow device in patients awaiting heart transplantation. N Engl J Med. 2007;357(9):885–96.

10. Slaughter MS, Pagani FD, Rogers JG, et al. Clinical management of continuous-flow left ventricular assist devices in advanced heart failure. J Heart Lung Transplant. 2010; 29(4 Suppl):S1–S38.
11. Abbott. Data as of May 10, 2018. SJM-HM-1016-0032(3).
12. Morine KJ, Kapur NK. Percutaneous mechanical circulatory support for cardiogenic shock. Curr Treat Options Cardio Med. 2016;18:6.
13. Miller LW, Rogers JG, et al. Evolution of left ventricular assist device therapy for advanced heart failure: a review. JAMA Cardiol. 2018;3(7):650–8.
14. United Network for Organ Sharing, Organ Procurement and Transplantation Network. Adult heart allocation changes: 2018. https://optn.tranpslant.hrsa.gov/media/2413/adult_heart_criteria.pdf.
15. Dardas T, Mokadam NA, Pagani F, et al. Transplant registrants with implanted left ventricular assist devices have insufficient risk to justify elective organ procurement and transplantation network status 1A time. J Am Coll Cardiol. 2012;60:36–43.
16. Wever-Pinzon O, Drakos SG, Kfoury AG, et al. Morbidity and mortality in heart transplant candidates supported with mechanical circulatory support: is reappraisal of the current united network for organ sharing thoracic organ allocation policy justified? Circulation. 2013;127:452–62.
17. Hannan M, Husain S, Mattner F, et al. Working formulation for the standardization of definitions of infections in patients using ventricular assist devices. J Heart Lung Transplant. 2011; 30(4):375–84.
18. Kirklin J, Naftel D, Pagani F, et al. Seventh INTERMACS annual report: 15,000 patients and counting. J Heart Lung Transplant. 2015;34:1495–504.
19. de By T, Mohacsi P, Gummert J, et al. The European registry for patients with mechanical circulatory support (EUROMACS): first annual report. Eur J Cardiothorac Surg. 2015 May; 47(5):770–6.
20. Agrawal S, Garg L, Shah M, et al. Thirty-day readmissions after left ventricular assist device implantation in the United States: insights from the nationwide readmissions database. Circ Heart Fail. 2018;11(3):e004628.
21. Vidula H, Kutyifa V, Johnson B, et al. Readmission patterns during Long-term follow-up after left ventricular assist device implantation. Am J Cardiol. 2018;122(6):1021–7.
22. Nienaber J, Kusne S, Riaz T, et al. Clinical manifestations and management of left ventricular assist device-associated infections. Clin Infect Dis. 2013;57(10):1438–48.
23. Hasin T, Marmor Y, Kremers W, et al. Readmissions after implantation of axial flow left ventricular assist device. J Am Coll Cardiol. 2013;61(2):153–63.
24. Simeon S, Flecher E, Revest M, et al. Left ventricular assist device-related infections: a multicentric study. Clin Microbiol Infection. 2017;23(10):748–51.
25. Gustafsson F, Shaw S, Lavee J, et al. Six-month outcomes after treatment of advanced heart failure with a full magnetically levitated continuous flow left ventricular assist device: report from the ELEVATE registry. Eur Heart J. 2018;39(37):3454–60.
26. Hanke J, Dogan G, Zoch A, et al. One-year outcomes with the HeartMate 3 left ventricular assist device. J Thorac Cardiovasc Surg. 2018;156(2):662–9.
27. Olmsted R, Cristinelis A, Kurihara C, et al. Severe LVAD-related infections requiring surgical treatment: incidence, predictors, effect on survival and impact of device selection. J Card Surg. 2019;34(2):82–91.
28. Hannan MM, Xie R, Cowger J, et al. Epidemiology of infection in mechanical circulatory support: a global analysis from the ISHLT mechanically assisted circulatory support registry. J Heart Lung Transplant. 2019;38(4):364–73.
29. Rios SA, Bravo CA, Weinreich M, et al. Meta-analysis and trial sequential analysis comparing percutaneous ventricular assist devices versus intra-aortic balloon pump during high-risk percutaneous coronary intervention or cardiogenic shock. Am J Cardiol. 2018;122:1330–8.
30. Kim GS, Lee KS, Park CK, et al. Nosocomial infection in adult patients undergoing veno-arterial extracorporeal membrane oxygenation. J Korean Med Sci. 2017;32(4):593–8.

31. Sun G, Li B, Lan H, et al. Risk factors for nosocomial infections in patients receiving extracorporeal membrane oxygenation supportive therapy. Med Clin (Barc). 2017;149(10):423–8.
32. Biancari F, Perrotti A, Dalen M, et al. Meta-analysis of the outcome after postcardiotomy venoarterial extracorporeal membrane oxygenation in adult patients. J Cardiothorac Vasc Anesth. 2018;32(3):1175–82.
33. Padera RF. Infection in ventricular assist devices: role of biofilm. Cardiovasc Pathol. 2006; 15(5):264–70.
34. Aslam S, Darouiche R. Role of antibiofilm-antimicrobial agents in controlling device-related infections. Int J Artif Organs. 2011;34(9):752–8.
35. Toba F, Akashi H, Arrecubieta C, et al. Role of biofilm in Staphylococcus aureus and Streptococcus epidermidis ventricular assist device driveline infections. J Thorac Cardiovasc Surg. 2011;141:1259–64.
36. Aslam S, Hernandez M, Thornby J, et al. Risk factors and outcomes of fungal ventricular-assist device infections. Clin Infect Dis. 2010;50(5):664–71.
37. Maly J, Szarszoi O, Netuka I, et al. Fungal infections associated with long-term mechanical circulatory support-diagnosis and management. J Card Surg. 2014;29(1):95–100.
38. Qu Y, et al. Biofilm formation and migration on ventricular assist device drivelines. J Thorac Cardiovasc Surg. 2019. [Epub adhead of print].
39. Donlan RM, et al. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002;8(9):881–90.
40. Sauer K, Camper A, Ehrlich G, et al. Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. J Bacteriol. 2002;184(4):1140–54.
41. Lazazzera B. Lessons from DNA microarray analysis: the gene expression profile of biofilms. Curr Opin Microbiol. 2005;8(2):222–7.
42. Donlan R, Costerton J. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167–93.
43. Aslam S. Ventricular assist device infections. Cardiol Clin. 2018;36(4):507–17.
44. Herrmann M, Weyand M, Greshake B, et al. Left ventricular assist device infection is associated with increased mortality but is not a contraindication to transplantation. Circulation. 1997; 95(4):814–7.
45. O'Horo JC, Abu Saleh OM, Stulak JM, et al. Left ventricular assist device infections: a systemic review. ASAIO J. 2018;64(3):287–94.
46. John R, Aaronson KD, Pae WE, et al. Drive-line infections and sepsis in patients receiving the HVAD system as a left ventricular assist device. J Heart Lung Transplant. 2014;33(10): 1066–73.
47. Topkara VK, Dang NC, Martens TP, et al. Effect of diabetes on short- and long-term outcomes after left ventricular assist device implantation. J Heart Lung Transplant. 2005;24(12):2048–53.
48. Gordon RJ, Weinberg AD, Pagani FD, et al. Prospective, multicenter study of ventricular assist device infections. Circulation. 2013;127:691–702.
49. Imamura T, Kinugawa K, Nitta D, et al. Readmission due to driveline infection can be predicted by new score by using serum albumin and body mass index during long-term left ventricular assist device support. J Artif Organs. 2015;18(2):120–7.
50. Holdy K, Dembitsky W, Eaton LL, et al. Nutrition assessment and management of left ventricular assist device patients. J Heart Lung Transplant. 2005;24:1690–6.
51. Schaenman JM, Rossetti M, Korin Y, et al. T cell dysfunction and patient age are associated with poor outcomes after mechanical circulatory support device implantation. Hum Immunol. 2018;79(4):203–12.
52. Yamani MH, Chuang HH, Ozduran V, et al. The impact of hypogammaglobulinemia on infection outcome in patients undergoing ventricular assist device implantation. J Heart Lung Transplant. 2006;25:820–4.
53. Dean D, Kallel F, Ewald GA, et al. Reduction in driveline infection rates: results from the HeartMate II multicenter driveline silicone skin interface (SSI) registry. J Heart Lung Transplant. 2015;34(6):781–9.

54. Singh A, Russo MJ, Valeroso TB, et al. Modified HeartMate II driveline externalization technique significantly decreases incidence of infection and improves long-term survival. ASAIO J. 2014;60(6):613–6.
55. Stulak JM, Romans T, Cowger J, et al. Delayed sternal closure does not increase late infection risk in patients undergoing left ventricular assist device implantation. J Heart Lung Transplant. 2012;31:1115–9.
56. Poston RS, Husain S, Sorce D, et al. LVAD bloodstream infections: therapeutic rationale for transplantation after LVAD infection. J Heart Lung Transplant. 2003;22:914–21.
57. Zierer A, Melby SJ, Voeller RK, et al. Late-onset driveline infections: the Achilles' heel of prolonged left ventricular assist device support. Ann Thorac Surg. 2007;84:515–20.
58. Holman W, Park S, Long J, et al. Infection in permanent circulatory support: experience from the REMATCH trial. J Heart Lung Transplant. 2004;23:1359–65.
59. Lietz K, Long J, Kfoury A, et al. Outcomes of left ventricular assist device implantation as destination therapy in the post-REMATCH era: implications for patient selection. Circulation. 2007;116:497–505.
60. Miller LW, Pagani FD, Russell SD, et al. Use of a continuous-flow device in patients awaiting heart transplantation. N Engl J Med. 2007;357:885–96.
61. Stulak JM, Davis ME, Haglund N, et al. Adverse events in contemporary continuous-flow left ventricular assist devices: a multi-institutional comparison shows significant differences. J Thorac Cardiovasc Surg. 2016;151(1):177–89.
62. Olmsted R, Critsinelis A, Kurihara C, et al. Severe LVAD-related infections requiring surgical treatment: incidence, predictors, effect on survival, and impact of device selection. J Card Surg. 2019;34(2):82–91.
63. Zierer A, Melby SJ, Voeller RK, et al. Late-onset driveline infections: the Achilles' hell of prolonged left ventricular assist device support. Ann Thorac Sug. 2007;84:515–20.
64. Kusne S, Mooney M, Danziger-Isakov L, et al. An ISHLT consensus document for prevention and management strategies for mechanical circulatory support infection. J Heart Lung Transplant. 2017;36(10):1137–53.
65. Maltais S, Anwer LA, Tchantchaleishvilli V, et al. Left lateral thoracotomy for centrifugal continuous-flow left ventricular assist device placement: an analysis from the mechanical circulatory support research network. ASAIO J. 2018;64(6):715–20.
66. McGee E, Danter M, Strueber M, et al. Evaluation of a lateral thoracotomy implant approach for a centrifugal-flow left ventricular assist device: the LATERAL clinical trial. J Heart Lung Transplant. 2019;38(4):344–51.
67. Urban M, Pirk J, Dorazilova Z, et al. How does successful bridging with ventricular assist device affect cardiac transplantation outcome? Interact Cardiovasc Thorac Surg. 2011;13:405–9.
68. Quader MA, Wolfe LG, Kasirajan V. Heart transplantation outcomes in patients with continuous-flow left ventricular assist device-related complications. J Heart Lung Transplant. 2015;34:75–81.
69. Koval C, Thuita L, Moazami N, et al. Evolution and impact of drive-line infection in a large cohort of continuous-flow ventricular assist device recipients. J Heart Lung Transplant. 2014;33:1164–72.
70. Schulman A, Martens T, Russo M, et al. Effect of left ventricular assist device infection on post-transplant outcomes. J Heart Lung Transplant. 2009;28(3):237–42.
71. O'Horo JC, et al. Left ventricular assist device infections: systemic review. ASAIO J. 2018; 64(3):287–94.
72. Lehnert AL, et al. Left ventricular assist device-related infection: does the time of onset matter? J Artif Organs. 2018. [Epub ahead of print].
73. Cheng A, Sun HY, Tsai MS, et al. Predictors of survival in adults undergoing extracorporeal membrane oxygenation with severe infections. J Thorac Cardiovasc Surg. 2016;152(6):1526–36.
74. Bar-Shalom R, Yefremov N, Guralnik L, et al. SPECR/CT using 67Ga and 111In-labled leukocyte scintigraphy for diagnosis of infection. J Nucl Med. 2006;47:587–94.

75. Litzler P, Manrique A, Etienne M, et al. Leukocyte SPECT/CT for detecting infection of left ventricular assist devices: preliminary results. J Nucl Med. 2010;51:1044–8.
76. Tilli G, Picard F, Pinaquy J, et al. The usefulness of FDG PET/CT imaging in suspicion of LVAD infection. J Nucl Cardiol. 2014;21:845–8.
77. Dell'Aquila AM, Mastrobuoni S, Alles S, et al. Contributory role of fluorine 18-fluorodeoxyglucose positron emission tomography/computed tomography in the diagnosis and clinical management of infections in patients supported with a continuous-flow left ventricular assist device. Ann Thorac Surg. 2016;101:87–94.
78. Kim J, et al. FDG PET/CT for early detection and localization of left ventricular assist device infection: impact on patient management and outcome. JACC Cardiovasc Imaging. 2018. [Epub ahead of print].
79. Baradarian S, Stahovich M, Krause S, et al. Case series: clinical management of persistent mechanical assist device driveline drainage using vacuum-assisted closure therapy. ASAIO J. 2006;52:354–6.
80. Kouretas P, Burch P, Kaza A, et al. Management of deep wound complications with vacuum-assisted therapy after Berlin Heart EXCOR ventricular assist device placement in the pediatric population. Artif Organs. 2009;33:922–5.
81. Chamogeorgakis T, Koval C, Smedira N, et al. Outcomes associated with surgical management of infections related to the HeartMate II left ventricular assist device: implications for destination therapy patients. J Heart Lung Transplant. 2012;31:904–6.
82. Levy D, Guo Y, Simkins J, et al. Left ventricular assist device exchange for persistent infection: a case series and review of the literature. Transpl Infect Dis. 2014;16:453–60.
83. Abicht T, Gordon R, Meehan K, et al. Complex HeartMate II infection treated with pump exchange to HeartWare HVAD. ASAIO J. 2013;59:188–92.
84. Kettner J, Holek M, Franekova J, et al. Procalcitonin dynamics after long-term ventricular assist device implantation. Heart Lung Circ. 2017;26(6):599–603.
85. Papathanasiou M, et al. Colonization with multiresistant bacteria: impact on ventricular assist device patients. Ann Thorac Surg. 2018;105(2):557–63.
86. Bagadasarian N, Malani AN, Pagani FD, et al. Fungemia associated with left ventricular assist device support. J Card Surg. 2009;24(6):763–5.
87. Komberger A, Walter V, Jaeger F, et al. Necrotizing pulmonary aspergillosis and ventricular assist device infection: case report and review of literature. Transpl Infect Dis. 2015;17(5):737–43.
88. Lerman T, et al. The impact of infection among left ventricular assist device recipients on post-transplantation outcomes: A retrospective review. Transpl Infect Dis. 2018. [Epub ahead of print].
89. Healy A, Baird B, Drakos S, et al. Impact of ventricular assist device complications on post-transplant survival: an analysis of the united network of organ sharing database. Ann Thorac Surg. 2013;95(3):870–5.
90. Chahal D, et al. The impact of Left ventricular assist device infections on postcardiac transplant outcomes: a systemic review and meta-analysis. ASAIO J. 2019. [Epub ahead of print].
91. Aggarwal A, Gupta A, Kumar S, et al. Are blood stream infections associated with an increased risk of hemorrhagic stroke in patients with a left ventricular assist device? ASAIO J. 2012; 58(5):509–13.
92. Kanjanahattij N, et al. Blood stream infection is associated with cerebrovascular accident in patients with left ventricular assist device: a systemic review and analysis. J Artif Organs. 2018;21(3):271–7.
93. Yosihioka D, Sakaniwa R, Toda K, et al. Relationship between bacteremia and hemorrhagic stroke in patients with continuous-flow left ventricular assist device. Circ J. 2018;82(2):448–56.
94. Cho S-M, et al. Stroke risk following infection in patients with continuous-flow left ventricular assist device. Neurocrit Care. 2019. [Epub ahead of print].

Emerging Transplant Infections: Cystic Fibrosis

60

Omar H. Mohamedaly and Carrie A. Johnson

Contents

Introduction 1496
MDR/XDR Organisms 1496
Burkholderia 1497
Epidemiology 1497
Clinical Impact in Transplant 1498
Diagnostics and Clinical Evaluation 1498
Management 1498
Scedosporium 1499
Epidemiology 1499
Clinical Impact in Transplant 1499
Diagnostics and Clinical Evaluation 1500
Management 1501
Scedosporium Apiospermum 1501
Lomentospora (formerly Scedosporium) Prolificans 1502
Mycobacterium Abscessus 1502
Bacteriophage Therapy 1504
CFTR Modulators 1505
Emerging Considerations in the Patient with Cystic Fibrosis 1507
Conclusion 1507
Key Points 1508
References 1509

O. H. Mohamedaly (✉) · C. A. Johnson
Pulmonary, Allergy and Critical Care Medicine, Duke University, Durham, NC, USA
e-mail: omar.mohamedaly@duke.edu; Carrie.a.johnson@duke.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_60

Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disorder of salt and water transport stemming from a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. It is a multisystem disease due to the near-ubiquitous expression of the *cftr* gene, though the pulmonary manifestations tend to be the main driver of survival. As of 2018, the predicted median survival for individuals with CF was 47.4 years [1].

Lung transplantation remains an integral therapy in the spectrum of CF disease management with about 16% of all lung transplants being performed for CF[2]. CF-related liver disease, while prevalent, progresses to end-stage liver disease requiring liver transplantation at lower rates compared to the lung disease. Only 52 simultaneous lung-liver transplantations were performed in the United States between 2005 and 2016; internationally that number was 108 in a similar time period (2004 to June 2017) [2, 3].

The changes in airway surface liquid hydration, defective mucociliary clearance, and altered airway inflammatory responses in CF result in airway colonization with a plethora of microorganisms, most characteristic of which is *Pseudomonas aeruginosa*, especially of the mucoid phenotype [4]. Other bacterial pathogens such as *Staphylococcus aureus*, *Burkholderia* spp., and Stenotrophomonas, fungal pathogens such as *Aspergillus* spp. and *Scedosporium* spp., as well as non-tuberculous mycobacteria comprise the rich microbiome of CF airways [5]. When combined with the immunosuppression needed for management of transplant allograft survival, unique infectious challenges arise in for CF patients. Furthermore, these issues require thoughtful, multidisciplinary approaches to transplant candidate selection, pretransplant CF disease management, perioperative antimicrobial selection, and posttransplant surveillance.

While the full spectrum of infections in CF transplant recipients is beyond the scope of this chapter, we will focus on particularly challenging infections and their management from a transplant perspective, novel approaches to widely drug-resistant species, and CF-specific considerations.

For a more detailed discussion on antimicrobial resistance, please refer to ► Chap. 14, "Multidrug-Resistant Organisms: Posttransplant Management for Extended-Spectrum Beta-Lactamase Producers and Carbapenem-Resistant Enterobacterales."

MDR/XDR Organisms

Before we discuss specific pathogens of interest in the CF transplant population, it is worth noting that while often considered a relative contraindication to lung transplantation, the presence of multidrug-resistant bacterial pathogens is generally not deemed a contraindication at larger or more experienced transplant centers, as long as a suitable antimicrobial plan can be devised that is both active against the pathogen in question and tolerable by the patient.

There is a lot of heterogeneity in how terms relating to degree of resistance are used. However, we suggest adhering to standardized nomenclature [6, 7]: Multidrug-

resistant (MDR) pathogens are thus those with non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) pathogens are those with non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and pan drug-resistant (PDR) pathogens are those with non-susceptibility to at least one antimicrobial in all categories.

With this distinction in mind, several studies have failed to show a significant deleterious impact of pan-resistant organisms on long-term survival after lung transplantation. For instance, while CF lung transplant recipients with PDR *Pseudomonas aeruginosa* had worse overall survival compared to those with sensitive organisms in a two-center study, the survival of the PDR group was no different from that of non-CF lung transplant recipients in the United Network of Organ Sharing (UNOS) registry [9]. Death was attributable to the PDR Pseudomonas only in 3 out of 45 patients in the study group in that study. A large single-center study assessing the same question found similar outcomes between CF patients with PDR Pseudomonas and those with sensitive pathogens [8]. Using data from the CFF and UNOS registries, Pseudomonas infection was not found to be an independent predictor of mortality post-lung transplant [10]. Similar results were found when analyzing outcomes in CF patients infected with *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia*; their outcomes were similar to non-cepacia CF lung transplant recipients [11].

This is not to say that non-cepacia PDR organisms are necessarily safe. Growing evidence suggests that chronic lung allograft dysfunction (CLAD), an umbrella term describing chronic rejection and graft loss post-lung transplant, is linked to airway colonization with Pseudomonas posttransplant, especially in patients with CF [12].

Burkholderia

Epidemiology

Burkholderia cepacia complex (Bcc) was named after a plant pathologist at Cornell University, Burkholder, who discovered the organism in 1949 [12]. It was first described as pathogenic in humans in the 1950s. Bcc is a ubiquitous aerobic Gram-negative bacillus commonly found in water, soil, and moist environments and is composed of up to 20 closely related bacterial subspecies also referred to by genomovars [13–15]. Bcc is a common colonizer of the upper and lower respiratory tract in CF patients and frequently carries multidrug resistance. The most common causes of Bcc colonization and infection in CF patients are *B. cenocepacia* (genomovar III) and *B. multivorans* (genomovar II) [14].

For a more detailed discussion on Burkholderia, please refer to ▶ Chap. 17, "*Burkholderia* in Transplant: Important to Speciate and Important to Treat."

It is important to recognize that horizontal transmission of Bcc can occur directly via person to person contact or indirectly through droplet or fomite transmission. Several Bcc outbreaks have been described due to contaminated medications,

medical products, and equipment [15]. However, due to stringent contact precautions and infection prevention strategies the Cystic Fibrosis Foundation has actually reported a decrease in the prevalence of Bcc respiratory infections in CF patients in the United States from 3.6% in 2006 to 2.6% in 2018, suggesting that local level controls can be impactful [16, 17].

Clinical Impact in Transplant

Early studies of CF patients colonized with Bcc demonstrated increased morbidity and mortality in the posttransplant period. However, additional studies and identification of specific Burkholderia genomovars suggest that the increased posttransplant mortality is associated primarily with *B. cenocepacia* (genomovar II) and not *B. multivorans* (genomovar II) or other subspecies [18]. *B. cenocepacia* is associated with accelerated decline in lung function in CF patients prior to transplant, early recurrence in the allograft leading to declining lung function, as well as significant antimicrobial resistance patterns all contributing to increased morbidity and mortality [15]. In one study, *B. cenocepacia* colonization in the pretransplant period led to 1-year and 5-year survival of 68% and 48%, respectively, versus 94 and 74%, respectively, for *B. cepacia* negative CF patients [19].

Patients with CF who are colonized with Bukholderia other than subspecies cenocepacia have rates of survival, rejection, and bronchiolitis obliterans syndrome (BOS) similar to the general CF population. As a result of better understanding and identifying the *B. cepacia* genomovars, only *B. cenocepacia* continues to be regarded as a relative contraindication to lung transplant due to the associated early mortality [14].

Diagnostics and Clinical Evaluation

All CF patients who are undergoing lung transplant evaluation should have sputum samples obtained in the pretransplant period to identify colonization and guide antimicrobial therapy in the perioperative period. As with all organisms with the potential for multidrug resistance, antibiotic susceptibility testing should be completed ahead of transplant to guide perioperative antibiotics.

Management

Once Bcc is established in the airways of a patient with CF for more than several months, the organisms persist despite aggressive attempts at eradication [20]. Removal of any indwelling intravenous catheters as is common in the CF population is generally recommended at the time of transplant. If a patient has concomitant sinus disease with known colonization of the lower respiratory tract, consideration for early sinus surgery and intervention is recommended, in order to minimize the

burden of bacteria before the introduction of immunosuppression. Although this is our specific practice, granular data to support the advantage of sinus debridement ahead of transplant for Burkholderia specifically is lacking.

In general, parenteral trimethoprim-sulfamethoxazole has been the drug of choice against Bcc in combination with additional intravenous therapy [21]. Synergistic effects as well as the potential for emerging resistance with monotherapy suggest that treatment should include at least two parenteral antimicrobial agents. Two-drug combinations of parenteral trimethoprim-sulfamethoxazole plus a β-lactam (ceftazidime, piperacillin, meropenem) or a fluoroquinolone should be considered [21].

Early review of susceptibility testing as well as drug allergies in the pretransplant evaluation process should be completed in order to devise a patient-specific treatment plan prior to listing for transplantation. The optimal duration of therapy is not known; however, extended therapy for 4–6 weeks in the perioperative period depending on additional patient-specific factors is generally recommended.

It is for these reasons that CF patients who are found to be colonized with Bcc prior to transplant should be evaluated at transplant centers with experience in managing such patients with a multidisciplinary approach including a specialized transplant infectious disease physician.

Scedosporium

Epidemiology

Scedosporium species are ubiquitous, filamentous fungi found in soil and water sources with a global distribution and are the second most common cause of fungal infection in organ transplant recipients; Aspergillus species being the most common [22]. Infections in humans are primarily acquired by direct inhalation to the sinuses and respiratory tract or direct inoculation by skin puncture. The majority of infections are due to two species: *Scedosporium apiospermum* and *Lomentospora prolificans* formerly *Scedosporium prolificans* [23, 24]. For the purpose of this chapter we will consider both together. *S. apiospermum* complex has greater response to azole therapy as compared to *L. prolificans* which has significant resistance to antifungal therapies.

The role and pathogenicity of Scedosporium are controversial. Immunocompetent patients colonized with Scedosporium may not manifest true infection, although allergic bronchopulmonary disease and rare infections have been described [22]. However, patients with underlying sinus disease, bronchiectasis, viscous secretions, and impaired mucociliary clearance as is common in cystic fibrosis (CF) are especially at risk for colonization and infection.

Clinical Impact in Transplant

Scedosporium in immunocompromised patients after lung transplantation has a variety of clinical presentations ranging from asymptomatic colonization of the

respiratory tract to life threatening infections if unrecognized and without appropriate timely therapy. The presence of Scedosporium species found in bronchoalveolar lavage has been associated with bronchiolitis obliterans and airway stenosis, especially if anatomical issues support its growth. A bronchial anastomosis that becomes telescoped in on itself, for example, is at high risk for the development of fungal infection due to the poor delivery of systemic antifungal agents [25]. Other infections associated with Scedosporium include pneumonia, disseminated blood stream infection, endophthalmitis, keratitis, central nervous system infections, skin and soft tissue infections, as well as osteomyelitis [26].

For a more detailed discussion on skin manifestations in transplant patients, please refer to ▶ Chap. 63, "Rashes to Recognize in the Immunocompromised Transplant Patient: Focus on the Solid Organ Transplant Recipient."

Due to the association with increased morbidity and mortality, some centers consider Scedosporium colonization a contraindication to lung transplant, even though patients have been transplanted with reported successful outcomes. This is especially true for *L. prolificans* where 31% of respondents to a multicenter survey said this would be a contraindication at their center [27]. Despite being recognized as an emerging opportunistic pathogen, the reported data on management and outcomes of scedosporial disease in the CF population after lung transplantation remain scarce.

In a single-center 15-year experience published in 2017 [22], the clinical outcomes of 14 patients with CF colonized by Scedosporium species following lung transplantation were described. Nine patients were colonized prior to transplantation, five were colonized in the posttransplant period, and only two were colonized with *L. prolificans*. The patients were all treated with an azole, either voriconazole or posaconazole. Only one patient who was colonized prior to transplantation developed significant scedosporial disease. Among the remaining eight patients colonized prior to transplantation, the median survival was 1.92 years (range 0.21–12.5 years) and death was due to unrelated causes. All but one became free of fungal colonization with treatment. For the five patients colonized in the posttransplant period, the median survival was 1.75 years (range 0.1–13 years).

This highlights the importance of early recognition of Scedosporium as well as initiation of azole therapy in the pre- and post-lung transplantation period, thereby decreasing the progression from colonization to Scedosporial disease and achieving fungal clearance. It is important to observe that pretransplant colonization was not a risk factor for increased morbidity and mortality in this series.

Diagnostics and Clinical Evaluation

The diagnosis and clinical evaluation for Scedosporium species is patient specific. Proven invasive mold infection is defined as histopathology consistent with tissue invasion and a culture result positive for mold or clinical or radiographic findings consistent with infection and a positive culture for mold obtained using sterile technique from a normally sterile site [28]. Additional pieces of softer supportive evidence can be obtained by looking at whether KOH staining is positive

on bronchoscopic sampling of the lower respiratory tree, and whether the fungal culture represents a single isolate or multiple isolates found repeatedly on multiple days.

Specimens including transbronchial biopsies and bronchoalveolar lavage as well as radiographic evidence for fungal disease should be routinely obtained whenever a clinical suspicion for infection exists. They are also variably obtained in centers where scheduled bronchoscopies occur post lung transplant, in which case preemptive therapy can sometimes be initiated. It is important to note that positive cultures may represent colonization rather than true disease, though both may warrant initiation of therapy in the context of enhanced immunosuppression. Additionally, in patients with identified Scedosporium in the lower respiratory tract, a thorough investigation of the sinuses and upper respiratory tract is again warranted to identify the extent of involvement. In the CF population, radiographic evidence of sinus disease combined with a positive Scedosporium sinus culture is diagnostic.

Fungal blood cultures are used in identification of disseminated disease in the appropriate clinical context post-transplant. Noninvasive tests include beta-D-glucan, which is a cell wall constituent of many fungi including Scedosporium. However, the diagnostic accuracy of this test for Scedosporium infections is not yet known, nor is it validated in CF or solid organ transplant recipients.

Management

Susceptibility testing from isolates should be used in guiding therapy in addition to regular therapeutic drug monitoring. The optimal choice and duration of therapy is unknown. In patients who are receiving immunosuppressive therapy, we recommend reducing the immunosuppressive therapy when feasible and clinically appropriate.

Scedosporium Apiospermum

Voriconazole is a broad spectrum triazole agent that is fungicidal for the majority of *Scedosporium apiospermum* isolates. Posaconazole also shows activity against *Scedosporium apiospermum*; however, voriconazole has demonstrated greater in vitro activity [29]. Originally, the food-dependent absorption of posaconazole, particularly in CF patients with pancreatic insufficiency, limited its activity, with drug levels demonstrating unreliable bioavailability [26]. However, newer formulations of posaconazole have made this less of an issue, and for now, voriconazole remains an ideal first-line agent in the CF population due to lower MIC's. It should be noted that isavuconazole has shown in vitro activity against some isolates of *S. apiospermum* and could also be considered in the treatment based on susceptibility testing [30]. Echinocandins have variable activity depending on the species and are generally not used as first-line treatments [31]. Perhaps most importantly, *S. apiospermum* is essentially always resistant to amphotericin B products.

Lomentospora (formerly Scedosporium) Prolificans

L. prolificans presents greater challenges to treatment, as it is resistant to most antifungal agents. As a result, combination antifungal therapy is often used. Although studies have shown poor in vitro potency of terbinafine monotherapy against Scedosporium species, the combination of terbinafine with voriconazole or itraconazole when used against *L. prolificans* in vitro has demonstrated synergistic activity [26]. Synergy has also been described with the combination of voriconazole plus terbinafine with the addition of micafungin although all these approaches should be considered salvage [32].

Given the potential for increased risk and complications associated with delayed identification and treatment of Scedosporium, the rarity of these infections, as well as the resistance associated with *L. prolificans*, a skilled multidisciplinary team approach involving an infectious disease specialist is recommended. Therapy should be tailored to the individual patient including surgical debridement when indicated as well as optimization of the patient's immunosuppression in order to achieve the best clinical response to treatment. Antifungal sensitivity patterns should also be sought, although these will often require subspecialty mycology laboratory services, and may not be available to all centers.

Mycobacterium Abscessus

Non-tuberculous mycobacteria (NTM) are particularly challenging in the CF population, especially in the context of immunosuppression posttransplant [33]. They appear to be on the rise nationally, with regional variation. Almost 20% of all CF patients with end-stage lung disease referred for transplant evaluation will have NTM-positive sputum cultures [34]. The combination of difficult-to-eradicate NTM from advanced bronchiectatic airways, high toxicity and side effect burden of available antimicrobial agents, and potentially serious morbidity and mortality of untreated NTM requires a thoughtful, multidisciplinary approach to NTM. Here we will focus on *M. abscessus* infections, as they remain controversial in the management of patients with CF peri-transplant. This is perhaps best highlighted by the fact that the reported prevalence of *M. abscessus* among lung transplant recipients tends to hover around only 1–2%.

Historically, *M. abscessus* infections have been considered to be relative contraindications to lung transplantation due to the risk of dissemination and poor outcomes posttransplant [35]. Indeed, several instances of fatal and nonfatal disease disseminations following lung transplantation have been reported in the older literature. In an international survey of 31 transplant centers, for instance, 17 patients with *M. abscessus* infections were identified. Seven mortalities were reported, only two of which were attributable to *M. abscessus* [36].

More recent literature, however, shines a more optimistic light on transplantation outcomes with *M. abscessus* infections. It should be noted that most of the available data are in the form of single-center retrospective analyses. In a review of 13 lung

transplant recipients with at least one positive respiratory sample for *M. abscessus* at one center, there were no deaths in the 20-year period analyzed and no statistically significant difference in survival when compared to contemporaneous CF recipients without *M. abscessus* [37]. A similar experience was reported more recently at another lung transplant center at which seven cases were identified in an 18-year period whose survival and time to development of chronic lung allograft dysfunction (CLAD) were similar to the comparator group of CF lung transplant recipients [38]. The single mortality in that study was unrelated to *M. abscessus*. Of note, all seven patients were smear-negative at the time of listing, while five remained culture-positive.

Not all transplant centers reported a neutral effect of *M. abscessus* on survival after lung transplantation, though. Twenty-four cases of *M. abscessus* infection were identified in what is likely the biggest series to date [39]; 22 of which were pulmonary isolates. Mortality among the subset with pulmonary disease was significantly higher than those merely colonized based on the American Thoracic Society/Infectious Diseases Society of America guidelines: 1-year all-cause mortality was reported at 50% versus 0% for the pulmonary disease versus colonization groups, respectively. Differences in methodology and definitions notwithstanding, this center's finding that 73% of patients required changes or interruptions in therapy due to toxicity and/or intolerance is noteworthy [40].

It is therefore not surprising that despite the increasing experience with transplantation in the setting of *M. abscessus* colonization and infection, there remains a great deal of controversy regarding the optimal approach. In a survey of 37 transplant centers in Europe, North America, and Australia, only 29% of centers were found to have a clear written policy on NTM [41]. While only four centers considered NTM isolation to be an absolute contraindication, the majority of respondents (76%) continued to count it as a relative contraindication to listing. While the requirement for treatment pretransplant is near-universal, practices differ regarding listing when patients are smear-negative only or culture-negative as well. In one case, a favorable outcome was reported in a CF patient who was smear- and culture-positive for *M abscessus* at the time of transplant, though thankfully such reports are insufficient to call into question the prevailing strategy of suppression pretransplant [42]. Our strategy is to evaluate the extent of disease and at a minimum, commence 3–4 drug therapy ahead of transplant. Optimally the patient would convert their AFB stain from positive to negative, or even become culture negative. However, bronchiectatic patients pretransplant often have cavitary disease making airway sterilization impossible. In that case, 4–6 weeks of pretransplant *M. abscessus* therapy is used principally to test tolerance, recognizing the drugs may well be needed for more than 6 months posttransplant.

As far as treatment is concerned, surgical source control is crucial in the cases of surgical site infections, empyema, or other discrete skin and soft tissue infections. Antimicrobial susceptibility testing remains the cornerstone of guiding medical therapy, which is almost always parenteral combination therapy. Macrolide resistance can be assumed to be less likely in patients infected with the *M. abscessus ss massiliense* subspecies compared to those with the *M. abscessus ss bolletii* and

abscessus subspecies, as the latter two demonstrate inducible macrolide resistance as a result of the presence of the erm(41) gene [43, 44]. Up to 80% of *M. abscessus* isolates may be macrolide-resistant, especially since chronic azithromycin use for its anti-inflammatory effects in those CF patients colonized with Pseudomonas is common practice [45].

As such, common antimicrobial regimens tend to be centered on a combination of intravenous, inhaled, and oral agents. Common intravenous antimicrobials include imipenem and cefoxitin. The aminoglycoside amikacin is preferentially administered in inhaled form in cases of pulmonary infection without extrapulmonary involvement, especially in light of the frequent changes in renal function in the early posttransplant phase. Macrolides such as azithromycin may or may not be included in the regimen depending on subspecies and susceptibility; clarithromycin tends to be avoided given its potential for drug–drug interactions with immunosuppressants, for example, via inhibition of P-glycoprotein. Oral clofazamine and bedaquiline are becoming more widely used as we encounter more patients with unfavorable *M. abscessus* susceptibility patterns. Frequent monitoring and adjustments in the *M. abscessus* regimen based on intolerances and side effects are necessary, especially given the protracted duration that comes with NTM treatment.

The CF Foundation guidelines suggest that for patients with CF, treatment of *M. abscessus* should be 3–12 weeks of IV amikacin with at least one additional drug, guided by sensitivity profile. In our experience, once a patient approaches their transplant window, treatment should be longer, often at least a month pretransplant, and 3–6 months after transplant at a minimum, sometimes even a whole year posttransplant depending on institutional practices. When the patient's lung disease has progressed closer to their transplant window, we advocate for multidisciplinary management by transplant pulmonologists and transplant infectious disease physicians with an eye on consistency and suitability of the peri-transplant regimen.

For a more detailed discussion on *M. abscessus* in transplant patients, please refer to ▶ Chap. 19, "Rapidly Growing Mycobacterial Infections in Transplant: Evolving Epidemiology and Treatment Options."

Bacteriophage Therapy

Given the complexity and pitfalls inherent in treating drug-resistant infections and given the increasing development of resistance by the time a patient with cystic fibrosis typically gets referred for transplantation, alternative options are needed. Specifically, there is a growing interest in the transplant community for alternative, or at least adjunctive, therapies to antibiotics. Bacteriophages are naturally occurring viruses with bacterium-specific cell killing capabilities. They are in their nascent stages of development as potential therapeutic agents in select cases.

Bacteriophages are particularly attractive in the CF patient population due to their ability to break down biofilms, a common adaptive defense mechanism of pathogens colonizing CF airways or recolonizing lung allograft airways posttransplant from their sinus reservoir. In addition, while data are limited, there is some evidence that

treatment with bacteriophages may overcome antibiotic resistance patterns and confer susceptibility to antibiotics previously inactive against the target bacterium in question [46].

Currently, data are limited to case reports of successful eradication of difficult-to-treat bacterial and mycobacterial infections, but even in the limited time since the availability of bacteriophage libraries at select institutions in the United States came to light, clinicians are increasingly attempting to pursue bacteriophage therapy for their patients with untreatable infections pre- or posttransplant. Typically, the process of acquiring a phage involves an effort to match a particular bacterial strain of interest with a specific phage from an institutional library. This is laborious and slow, often taking weeks. An early example in the literature demonstrated safety and tolerability of a *Pseudomonas aeruginosa* bacteriophage in conjunction with systemic antibiotics for a woman with advanced CF awaiting lung transplant [47].

Of course, the excitement should be tempered given the limitations of bacteriophage therapy. Notably, there are no well-designed randomized trials supporting their use, and existing literature is scarce. In addition, beyond the labor and time-intensive bioengineering often required to target an individual patient's specific pathogens, the concern for lysogenic insertion and possibility of resultant mutations, including those conferring more antimicrobial resistance, should give us pause. Of note, the significance of bacterial evolution against the phage and host immune responses against the virus are two open questions that remain unanswered. Whether the patient's immune response against the phage has any subsequent impact on lung allograft function is unknown as well. Finally, dosing and route of administration remain unresolved questions. Studies so far have combined both inhaled and intravenous routes.

CFTR Modulators

The advent of CFTR modulators in the last decade has been an exciting expansion to the CF therapeutic armamentarium. Having an understanding of the way they interact with multiple other medications, including anti-infectives and immunosuppressives, is crucial. Ivacaftor (trade name Kalydeco), a CFTR potentiator that targets the so-called 'gating' mutation G551D, showed multiple benefits in terms of improvement in lung function, reduction in pulmonary exacerbations, and weight gain [48]. Subsequent studies based on several years of ivacaftor use have also shown a reduction in the rate of lung function decline by about 50% [49]. Ivacaftor use has been expanded to 37 additional mutations since its initial approval and now affords access to modulator therapy to about 5% of all individuals with CF.

Lumacaftor/ivacaftor (trade name Orkambi) is a combination modulator that was introduced for patients homozygous for F508del, the most common CFTR mutation and accounting for about 45% of all individuals with CF. It is a combination of a folding corrector (lumacaftor) addressing the main folding defect of

F508del with ivacaftor addressing the pleiotropic gating defect seen in F508del CFTR once folding is corrected. Its effect on lung function improvement was more modest than that of ivacaftor in its eligible patients and was limited by airway side effects [49]. Tezacaftor/ivacaftor (trade name Symdeko) is a corrector-potentiator combination that exhibits benefit in patients homozygous for F508del and those heterozygous with a second residual-function mutation and does not have the same limiting side effects as Orkambi [50, 51]. More recent studies combining Symdeko with an additional second-generation corrector called elexacaftor have been concluded and show great promise with a bigger impact on lung function and a potential to cover over 90% of all individuals with CF [52, 53]. They have led to FDA approval of the combination elexacaftor/tezacaftor/ivacaftor (trade name Trikafta).

The use of CFTR modulators is limited in the transplant setting owing to the multitude of drug–drug interactions, especially with calcineurin inhibitors, a mainstay of immunosuppression, and azoles, which are often needed for prophylaxis or treatment of invasive fungal infections. In particular, ivacaftor is a weak inhibitor of CYP3A4 and P-glycoprotein (P-gp), the latter interaction would be of additional interest when concomitantly used with tacrolimus. Lumacaftor, on the other hand, is a potent CYP3A4 inducer, and the net effect of lumacaftor/ivacaftor use is strong CYP3A4 induction. Tezacaftor is a CYP3A4 substrate, and as such dose reduction is necessary when used concomitantly with strong CYP3A4 inhibitors such as voriconazole and posaconazole or moderate inhibitors such as fluconazole.

In the lung transplant community, ongoing CFTR modulation following transplantation is generally considered unnecessary thanks to the replacement of the disease- and prognosis-defining organ. An argument can be made that the quality of life and weight gain benefits of CFTR modulators are now provided by the lung transplant and the resolution of chronic airway inflammation. In addition, the risks of subtherapeutic calcineurin inhibitor levels and the potential for higher rates of both acute cellular rejection and chronic lung allograft dysfunction do not justify continuing CFTR modulator therapy.

However, there are other extrapulmonary benefits to modulators, for example, improvements in exocrine pancreatic function, glycemic control, and sinus disease, which have emerged from clinical use. Depending on the disease phenotype of each particular patient, those may tip the risk-benefit balance toward continuing modulators post-lung transplant. As modulators gain widespread use earlier in the course of the disease, it is conceivable that more patients would maintain lung function longer and potentially need other organ transplants prior to the progression of their lung disease. In such instances, the need for lung function-preserving modulator therapy will need to be thoughtfully weighed against the drug–drug interactions between modulators and immunosuppressants or azoles. Recent case reports of CFTR modulators being continued after liver transplantation have highlighted exactly that conundrum and provide an early blueprint for dose adjustment and monitoring to allow for concomitant use of all necessary medications [54, 55]. What impact modulator therapy will have on the heretofore progressive colonization of airways with multidrug-resistant microorganisms remains to be seen.

Emerging Considerations in the Patient with Cystic Fibrosis

Many other considerations play into a patient with cystic fibrosis being an acceptable candidate for lung transplant. Many vary according to center preference and center experience. Anatomical considerations should be considered. Patients with considerable bronchiectatic change or bullous disease often have such significant burden of bacteria and/or fungi such that sterilization of the airway becomes impossible. Furthermore, bullae near the surface of the lung, or especially those that have burst resulting in a pneumothorax or pleurodesis, require special consideration. Our approach is to still take those patients to transplant, but to have frank discussions with surgical colleagues ahead of time, with regard to cautious extensive debridement to dissect the lung away from the thoracic wall, followed by aggressive washing out of the chest cavity, with either bacitracin or even Dakin's solution if frank empyema is noted.

The other reason to consider the anatomical differences in patients with CF is the impact on drug absorption. Generally speaking, the more bronchiectasis noted on chest imaging, the less likely sterilization is possible, and the more drug penetrance becomes variable.

When considering the CF patient with known liver disease, multidisciplinary management is often easiest. Combined liver/lung transplant is done at a limited number of centers and is felt best when a patient has underlying moderate to severe fibrotic function. Understanding how antibiotics and other medications are metabolized through the cirrhotic pretransplant liver is important. For example, to avoid decompensated liver failure, clinicians at our center tend to try an IV echinocandin instead of a triazole to reduce the fungal burden associated with pulmonary Aspergillus colonization ahead of transplant.

In very rare situations, pretransplant control of infection can be achieved by complete double pneumonectomy, using extracorporeal membrane oxygenation (ECMO) as a bridge to buy time for antibiotics to clear bacteremia where the lungs are felt to be the source, thereby facilitating transplant [56, 57]. An example (Fig. 1) was used to clear recurrent episodic *Burkholderia* bacteremia in a patient with terminal cystic fibrosis. This should be considered largely experimental at this time, although as advancing surgical techniques and ECMO support continue to grow, more significant surgical solutions to complicated pretransplant cystic infections may become possible [58].

Conclusion

Cystic fibrosis transplant recipients tend to enjoy better survival outcomes compared to non-CF recipients. While likely due to their younger age and fewer comorbidities on average, advances in CF care, availability of effect CFTR modulators to attenuate disease progression, and the resultant changes in airway microbiota are likely to change the landscape of transplant in CF toward older patients and those with more

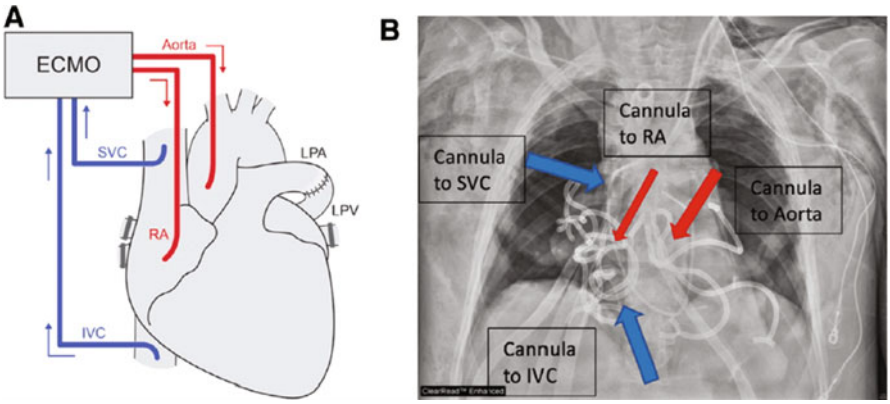


Fig. 1 (**a**) Schematic diagram of the VAC ECMO support configuration after bilateral pneumonectomy. The LPA is connected to the LPV (this is a side-to-side, functional end-to-end anastomosis). This allows for blood flow from the right heart to the left heart. In blue are the venous limb cannulas of the ECMO circuit and in red are the arterial limbs. (**b**) Post-operative chest X-ray demonstrating the support ECMO apparatus and the absence of lungs. ECMO, extracorporeal membrane oxygenation; IVC, inferior vena cava; LPA, left pulmonary artery; LPV, left superior pulmonary vein; RA, right atrium; SVC, superior vena cava; VAV, veno-arteriovenous. (Used with permission from Barac YD, Bryner B, Bonadonna D, et al. Bilateral pneumonectomy with veno-arterial extracorporeal membrane oxygenation as a bridge to lung transplant. *J Heart Lung Transplant*. 2019;38(11):1231–1232)

complex microbial pathogens. Novel therapeutic approaches and more elaborate multidisciplinary management will thus become increasingly crucial for the effective management of future challenges.

Key Points

- Cystic fibrosis care has improved significantly pretransplant, leading to a much slower progression to end-stage pulmonary failure. Transplant remains the only option for those with end-stage disease.
- CFTR modulators have shown a lot of promise in further controlling the progression of CF, although they have significant drug interactions.
- Protracted antibiotic courses can be necessary for those patients with multi-drug resistant organisms. This is especially true for *Burkholderia* species and non-tuberculous mycobacteria.
- Bacteriophage therapy provides a potentially novel and targeted treatment that can be used in conjunction with an optimized antibiotic regimen. Currently, it should be reserved to use in more critical situations.
- Multidisciplinary discussions between pulmonologists, infectious disease physicians, and thoracic surgeons are crucial, especially where bullous disease has ruptured into the thoracic cavity or pleurodesis has been previously performed.

References

1. Cystic Fibrosis Foundation Patient Registry, 2018 Annual Data Report, 2019.
2. Chambers DC, Cherikh WS, Goldfarb SB, Hayes D Jr, Kucheryavaya AY, Toll AE, Khush KK, Levvey BJ, Meiser B, Rossano JW, Stehlik J. International Society for Heart and Lung Transplantation; The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-fifth adult lung and heart-lung transplant report-2018; Focus theme: Multiorgan Transplantation. J Heart Lung Transplant. 2018; 37(10):1169–83.
3. Freischlag KW, Messina J, Ezekian B, Mulvihill MS, Barbas A, Berg C, Sudan D, Reynolds J, Hartwig M, Knechtle S. Single-center long-term analysis of combined liver-lung transplant outcomes. Transplant Direct. 2018;4(5):e349.
4. De Boeck K, Amaral MD. Progress in therapies for cystic fibrosis. Lancet Respir Med. 2016; 4(8):662–74.
5. Gibson RL, et al. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med. 2003;168:918.
6. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268–81.
7. Morrell MR, Kiel SC, Pilewski JM. Organ transplantation for cystic fibrosis. Semin Respir Crit Care Med. 2019;40:842–56.
8. Hadjiliadis D, Steele MP, Chaparro C, et al. Survival of lung transplant patients with cystic fibrosis harboring panresistant bacteria other than *Burkholderia cepacian* compared with patients harboring sensitive bacteria. J Heart Lung Transplant. 2007;26:834–8.
9. Dobbin C, Maley M, Harkness J, et al. The impact of pan-resistant bacterial pathogens on survival after lung transplantation in cystic fibrosis: results from a single large referral centre. J Hosp Infect. 2004;56:277–82.
10. Liou TG, Adler FR, Huang D. Use of lung transplantation survival models to refine patient selection in cystic fibrosis. Am J Respir Crit Care Med. 2005;171:1053–9.
11. Lobo JL, Tulu Z, Aris RM, et al. Pan-resistant Achromobacter xylosoxidans and Stenotrophomonas maltophilia infection in cystic fibrosis does not reduce survival after lung transplantation. Transplantation. 2015;99:2196–202.
12. Vos V, Vanaudenaerde BM, Geudens N, et al. Pseudomonal airway colonization: risk factor for bronchiolitis obliterans after lung transplantation? Eur Respir J. 2008;31:1037–45.
13. Burkholder WH. Sour skin, a bacterial rot of onion bulbs. Phytopathology. 1950;40(1):115–7.
14. Dupont. Lung transplantation in cystic fibrosis patients with difficult to treat lung infections. Curr Opin Pulm Med. 2017;23:574–9.
15. Sfeir M. *Burkholderia cepacia* complex infections: more complex than the bacterium name suggest. J Inf Secur. 2018;77:166–70.
16. https://www.cff.org/Research/Researcher-Resources/Patient-Registry/2018-Patient-Registry-Annual-Data-Report.pdf
17. Salsgiver EL, et al. Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis. Chest. 2016;149(2):390–400.
18. Alexander BD, et al. Survival after lung transplantation of cystic fibrosis patients infected with B cepacia complex. Am J Transplant. 2008;8(5):1025.
19. Murray S, et al. Impact of Burkholderia infection on lung transplantation in cystic fibrosis. Am J Respir Crit Care Med. 2008;178:363–71.
20. Regan KH. Eradication therapy for *Burkholderia cepacia* complex in people with cystic fibrosis. Cochrane Database Syst Rev. 2019;4:CD009876.
21. Avgeri SG, et al. Therapeutic options for *Burkholderia Cepacia* infections beyond co-trimoxazole: a systematic review of the clinical evidence. Int J Antimicrob Agents. 2009; 33(5):394–404.

22. Parize P, et al. Clinical outcome of cystic fibrosis patients colonized by Scedosporium species following lung transplantation: a single center 15-year experience. Transpl Infect Dis. 2017;19:e12738.
23. Simarro E, et al. Fungemia due to Scedosporium prolificans: a description of two cases with fatal outcome. Clin Microbiol Infect. 2001;7(11):645–7.
24. Abela IA, Murer C, Schuurmans MM, et al. A cluster of scedosporiosis in lung transplant candidates and recipients: the Zurich experience and review of the literature. Transpl Infect Dis. 2018;20:e12792.
25. Rolfe NE, et al. Managemnet of Scedosporium apiospermum in a pre and post lung transplant patient with cystic fibrosis. Med Mycol Case Rep. 2013;2:37–9.
26. Castiglioni B, et al. Pseudallescheria boydii (Anamorph Scedosporium apiospermum). Infection in solid organ transplant recipients in a tertiary medical center and review of the literature. Medicine (Baltimore). 2002;81(5):333–48.
27. Rammaert B, Puyade M, Cornely OA, Seidel D, Grossi P, Husain S, Picard C, Lass-Flörl C, Manuel O, Le Pavec J, Lortholary O, SCEDO-LUNG Collaborative Group. Perspectives on Scedosporium species and Lomentospora prolificans in lung transplantation: results of an international practice survey from ESCMID fungal infection study group and study group for infections in compromised hosts, and European Confederation of Medical Mycology. Transpl Infect Dis. 2019;21(5):e13141. https://doi.org/10.1111/tid.13141. Epub 2019 July 22
28. De Pauw B, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813–2.
29. Meletiadis J, et al. Invitro activities of new and conventional antifungal agents against clinical Scedosporium isolates. Antimicrob Agents Chemother. 2002;46(1):62–8.
30. Guinea J, et al. In vitro antifungal activities of isavuconazole and fluconazole against 1007 isolates of zygomycete, Candida, Aspergillus, Fusarium, and Scedosporium species. Antimicrob Agents Chemother. 2008;52(4):1396–400.
31. Lackner M, et al. Species-specific antifungal susceptibility patterns of Scedosporium and Pseudallescheria species. Antimicrob Agents Chemother. 2012;56(5):2635.
32. Meletiadis J, et al. In vitro interaction of terbinafine with itraconazole against clinical isolates of Scedosporium prolificans. Antimicrob Agents Chemother. 2000;44(2):470–2.
33. Osmani M, Sotello S, Alvarez S, et al. Mycobacterium abscessus infections in lung transplant recipients: 15-year experience from a single institution. Transpl Infect Dis. 2018;20:e12835.
34. Chalermskulrat W, Sood N, Neuringer IP, et al. Non-tuberculous mycobacteria in end stage cystic fibrosis: implications for lung transplantation. Thorax. 2006;61:507–13.
35. Orens JB, Estenne M, Arcasoy S, et al. International guidelines for the selection of lung transplant candidates: 2006 update – a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2006;25:745.
36. Chernenko SM, et al. Mycobacterium abscessus infections in lung transplant recipients: the international experience. J Heart Lung Transplant. 2006;25(12):1447–55.
37. Lobo LJ, Chang LC, Esther CR Jr, et al. Lung transplant outcomes in cystic fibrosis patients with pre-operative Mycobacterium abscessus respiratory infections. Clin Transpl. 2013;27:523–9.
38. Perez AA, Singer JP, Schwartz BS, et al. Management and clinical outcomes after lung transplantation in patients with pre-transplant Mycobacterium abscessus infection: a single center experience. Transpl Infect Dis. 2019;21:e13084.
39. Hamad Y, Pilewski JM, Morrell M, et al. Outcomes in lung transplant recipients with Mycobacterium abscessus infection: a 15-year experience from a large tertiary center. Transplant Proc. 2019;15:2035–42.

40. Griffith D, Aksamit T, Brown-Elliott B, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial disease. Am J Respir Crit Care Med. 2007;175:367–416.
41. Tissot A, Thomas MF, Corris PA, et al. Nontuberculous mycobacteria infection and lung transplantation in cystic fibrosis: a worldwide survey of clinical practice. BMC Pulm Med. 2018;18:86.
42. Raats D, Lorent N, Saegeman V, et al. Successful lung transplantation for chronic Mycobacterium abscessus infection in advanced cystic fibrosis, a case series. Transpl Infect Dis. 2019;21:e13046.
43. Griffith DE, Brown-Elliott BA, Benwill JL, et al. Mycobacterium abscessus "pleased to meet you, hope you guess my name. . .". Ann Am Thorac Soc. 2015;12:436–9.
44. Benwill JL, Wallace RJ Jr. Mycobacterium abscessus: challenges in diagnosis and treatment. Curr Opin Infect Dis. 2014;27:506–10.
45. Saiman L, Marshall BC, Mayer-Hamblett N, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. JAMA. 2003;290:1749–56.
46. Trend S, Fonceca AM, Ditcham WG, Kicic A, Arest CF. The potential of phage therapy in cystic fibrosis: essential human-bacterial-phage interactions and delivery considerations for use in *Pseudomonas aeruginosa*-infected airways. J Cyst Fibros. 2017;16(6):663–70. https://doi.org/10.1016/j.jcf.2017.06.012. Epub 2017 July 15.
47. Law N, Logan C, Yung G, Furr CL, Lehman SM, Morales S, Rosas F, Gaidamaka A, Bilinsky I, Grint P, Schooley RT, Aslam S. Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant *Pseudomonas aeruginosa* infection in a cystic fibrosis patient. Infection. 2019;47(4):665–8. https://doi.org/10.1007/s15010-019-01319-0. Epub 2019 May 17
48. Ramsey, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med. 2011;365:1663–72.
49. Sawicki GS, et al. Sustained benefit from ivacaftor demonstrated by combining clinical trial and cystic fibrosis patient registry data. Am J Respir Crit Care Med. 2015;192(7):836–42.
50. Taylor-Cousar, et al. Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del. N Engl J Med. 2017;377:2013–23.
51. Rowe, et al. Tezacaftor–Ivacaftor in Residual-Function Heterozygotes with Cystic Fibrosis. N Engl J Med. 2017;377:2024–35.
52. Davies JC, et al. VX-659–Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. N Engl J Med. 2018;379:1599–611.
53. Keating D, et al. VX-445–Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. N Engl J Med. 2018;379:1612–20.
54. Choucane I, et al. Lumacaftor/ivacaftor initiation in two liver transplantation patients under tacrolimus and antifungal azoles. Clin Case Rep. 2019;7:616–8.
55. Mithcell MM, et al. CFTR modulator therapy in patients with cystic fibrosis and an organ transplant. Ped Respir Rev. 2018;27:6–8.
56. Barac YD, Bryner B, Bonadonna D, et al. Bilateral pneumonectomy with veno-arterial extracorporeal membrane oxygenation as a bridge to lung transplant. J Heart Lung Transplant. 2019;38(11):1231–2.
57. Cypel M, Waddell T, Singer LG, et al. Bilateral pneumonectomy to treat uncontrolled sepsis in a patient awaiting lung transplantation. J Thorac Cardiovasc Surg. 2017;153(4):e67–9.
58. Todd EM, Biswas Roy S, Hashimi AS, et al. Extracorporeal membrane oxygenation as a bridge to lung transplantation: a single-center experience in the present era. J Thorac Cardiovasc Surg. 2017;154(5):1798–809.

Vascular Composite Allografts (VCA): New Transplants, New Infections

61

Julie M. Steinbrink, Timothy L. Pruett, and Linda C. Cendales

Contents

Background 1514
Introduction 1514
Introduction to Immunosuppression and Infection Risk 1514
Tissue-Specific Risk of Infection 1516
Mucosa 1516
Skin 1519
Muscle 1520
Bone 1521
Nerve 1521
Lymphoid Tissue 1522
Blood Vessel 1522
Salivary Glands 1522
Organ-Specific Infections Particular to VCA 1522
General History 1523
Systemic Infections that Can Be Predicted from Donor Testing 1525
Cytomegalovirus 1525
Epstein-Barr Virus 1525
Toxoplasma 1525
Syphilis 1526
Additional Considerations 1526
Vaccinations 1526
Safe Living Practices Posttransplant 1527

J. M. Steinbrink
Division of Infectious Diseases, Duke University Medical Center, Durham, NC, USA
e-mail: julie.steinbrink@duke.edu

T. L. Pruett
Department of Surgery, University of Minnesota, Minneapolis, MN, USA
e-mail: tlpruett@umn.edu

L. C. Cendales (✉)
Department of Surgery, Duke University Medical Center, Durham, NC, USA
e-mail: linda.cendales@duke.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_61

Conclusion 1527
Key Points 1527
References 1528

Background

Introduction

A vascularized composite allograft (VCA) is a quality of life transplant that encompasses the transplantation of multiple tissues as a functional unit [1]. Indications for transplantation vary based on the tissue defect and the type of transplant being performed. Due to their unique nature and wide variability, VCAs come with their own distinctive challenges and infectious threats. As a field in development compared to most other solid organ transplants (SOT), much of what is known about overall outcomes and infectious complications is based on small-number reviews and case reports or extrapolated from SOT data. This chapter will specifically address abdominal wall graft, face, hand, trachea/larynx, penile, and uterine transplantation. However, based on the definition of VCA, other types can also be included, and the same considerations of infection risks in different tissues can be therefore applied to any theoretical allograft.

Introduction to Immunosuppression and Infection Risk

Induction Immunosuppression

A large contributor to the infectious risk of any transplant population is the duration and intensity of immunosuppression the patient receives. A unique aspect and a challenge of VCA is that different tissue layers have varying levels of immunogenicity, with skin often considered the most immunogenic [2, 3]. Nonetheless, much of the information that guides VCA immunosuppression recommendations comes from the SOT literature.

Current experience shows that VCA recipients require similar immunosuppression regimens and doses to those used for other SOTs [4–9]. Specifically, hand and face transplants most commonly receive induction immunosuppression with monoclonal or polyclonal antibodies [10–12] such as antithymocyte globulin (ATG) or basiliximab [13], often along with mycophenolic mofetil [14] and steroids.

Maintenance Immunosuppression

Following induction, maintenance regimens vary from monotherapy to triple or quadruple drug regimens [11, 14–16]. For the most part, these include a combination of calcineurin inhibitors, mycophenolic mofetil, and steroids, with target drug levels akin to regimens used for renal transplantation; thus systemic infection risk with VCA appears consistent with renal transplantation. Treatment duration may be shorter for uterine transplant than for other grafts due to the decreased planned time of organ retention – currently about 5 years, up to two pregnancies [17]. Most

other VCA transplants will require indefinite immunosuppression for the life of the allograft. The average graft survival for other VCAs has not been defined and is likely different for each VCA type. However, if similar to all other organs, the average graft survival would be 15 years [1].

Rejection

One of the benefits of skin-containing VCAs is that initial evidence of rejection may be seen on external exam and can be readily biopsied [18]. Initial acute rejection is usually treated with intravenous steroid burst and oral taper [11, 14]. Though topical agents including steroids and tacrolimus have been used in the past [10], it is not recommended to use these agents in the absence of augmented systemic immunosuppression, as skin manifestations are often an indication of deeper rejection, which topical agents cannot penetrate [19]. However, it is important to remember that increased doses of systemic corticosteroids and other immunosuppressive agents can have widespread effects on the immune system and significantly increase the risk for infection, as discussed in other chapters [20].

Perioperative Antibiotics

Perioperative antibiotic regimens have increased in spectrum of coverage over time. Initial face transplants used antibacterial agents such as third-generation cephalosporins with metronidazole or amoxicillin-clavulanate monotherapy, with gram-negative, anaerobic activity, and streptococcal coverage specifically targeting bacterial flora of the oral cavity and sinuses, which were felt likely to cross-contaminate the graft site [12, 21, 22]. Presently, agents with a broader antibacterial spectrum, including MRSA coverage, are now being utilized – primarily vancomycin and a third- or fourth-generation cephalosporin, with antibiotics tailored to donor and recipient culture results as well as taking into consideration the microbiome of the transplanting hospital [14, 23–25]. The specific VCA type also impacts choice of perioperative antibiotics – while face transplants require a focus on upper respiratory tract flora, uterine and potentially penile transplants need consideration of genitourinary flora, which includes more anaerobes, *Enterobacteriaceae*, and *Candida*.

Typically antiviral prophylaxis with ganciclovir or valganciclovir is added for cytomegalovirus (CMV) prevention, along with trimethoprim-sulfamethoxazole for *Pneumocystis* (PCP) prophylaxis and dosing is the same as SOT. For a detailed discussion of the preventative and treatment strategies for CMV and PCP, please refer to the corresponding chapters in this book.

Furthermore, some centers add antifungal prophylaxis, particularly if there are concerns for *Candida* colonization [14, 26]. It should be noted that there is a lack of data to support this approach. In comparison to the SOT population, antifungal prophylaxis would not normally be required in most abdominal organ transplantation – such as the kidney or liver – based solely on the level of immunosuppression.

Graft Preparation

Preparation of VCA grafts is not a standardized process. Typical methods arose out of traditional plastic surgery techniques and include flushing the graft

post-procurement with University of Wisconsin (UW) solution, followed by wrapping in moist gauze and transferring on ice [27, 28]. This does carry the risk of exposing the graft to potential UW contamination issues; however, there is a lack of data describing alternative methods of graft preparation. Furthermore, prospective data tracking potential for skin and tissue contamination using this approach is lacking. Thus, this will require continued research and investigation to consider its potential implications and complications.

Tissue-Specific Risk of Infection

VCAs differ significantly by the type of tissue transplanted. Therefore, this chapter will approach the infectious risks in this population per tissue and structures involved. As a result, a clinician faced with a VCA need only understand the type of tissues involved in order to better understand the infectious implications for the recipient. Examples of the kinds of tissues transplanted during face, hand, uterine, and abdominal wall transplants are included; see Figs. 1, 2, 3, and 4. Other systemic infections that are not tissue specific will be covered elsewhere in their respective chapters.

Mucosa

1. **Mouth, nose, sinus, upper airways**
   This is often the first portal of entry for many inhaled environmental pathogens and can carry a number of colonizing pathogens that are predisposing factors for

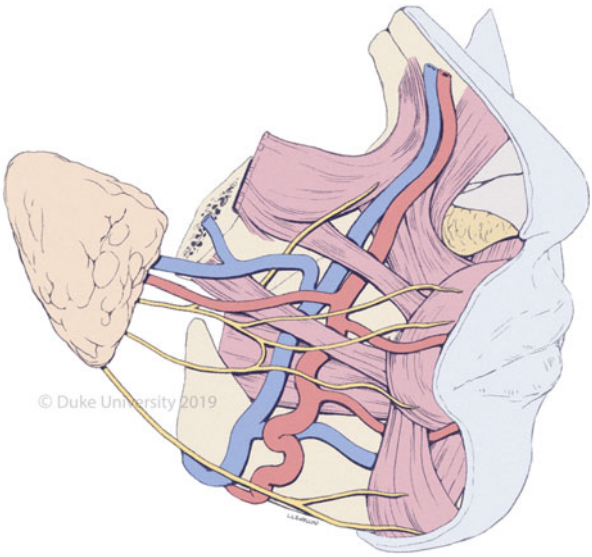


Fig. 1 Face transplant allograft

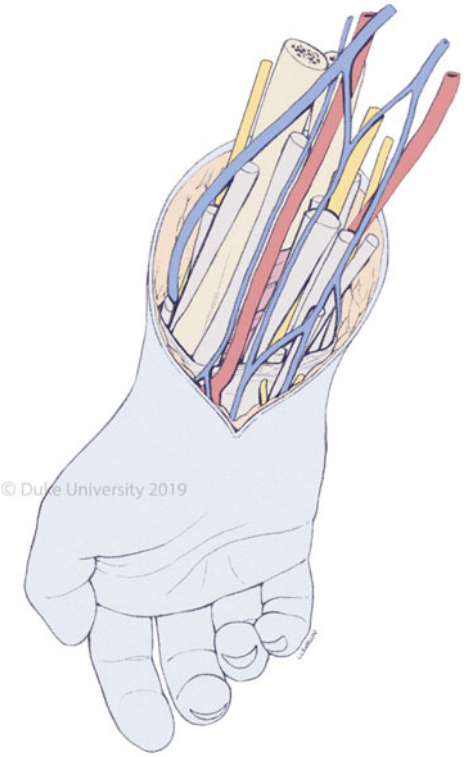


Fig. 2 Hand transplant allograft

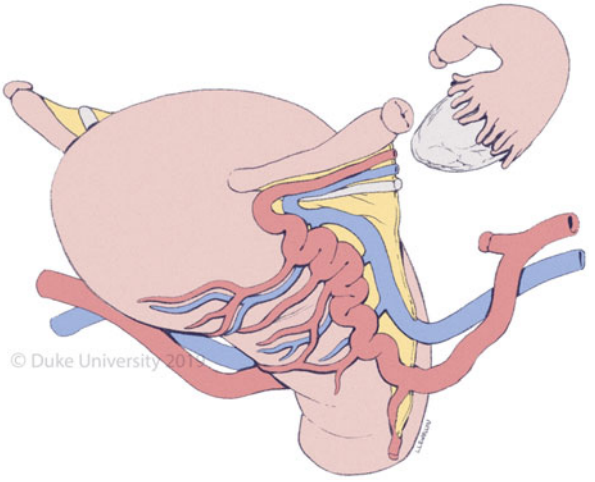


Fig. 3 Uterine transplant allograft

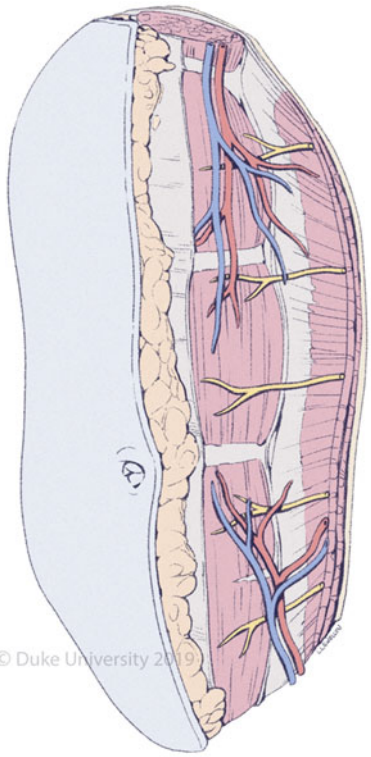


Fig. 4 Abdominal wall transplant allograft

future infection [25]. Thus, it is prudent to obtain mucosal bacterial, mycobacterial, and fungal cultures of the recipient pretransplant and of the donor during transplant evaluation. Recipient cultures are best done weeks in advance, to facilitate preparation. For the donor, in the United States, many of the bacterial lower respiratory tract cultures are routinely collected by the organ procurement organization (OPO), in accordance with national policy. However, sinus or oropharyngeal cultures are not typically collected, so close working relationships – often in real time during donor evaluations – with the OPO are imperative to ensure appropriate tissue cultures are obtained. Similarly, given the length of time taken to finalize fungal and mycobacterial cultures, care must be taken to ensure ongoing post-procurement communication to ensure timely communication of results. Empiric antimicrobial coverage may wish to cover *streptococcal* species, *Staphylococcus aureus*, *Candida*, and anaerobes but should be tailored based on culture results.

2. **Vaginal**
   Candida colonization or infection may be present in the genitourinary tract, which can pose a significant risk factor for infection. The first uterine transplant in the United States succumbed to a severe *Candida* infection ultimately requiring organ removal [29]. Additionally, it is important to consider screening for other

genitourinary infections including sexually transmitted infections. Donor and recipient screening tests should include vaginal wet mount to assess for various causes of vaginitis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* PCR, herpes simplex virus (HSV), human papillomavirus (HPV), and syphilis serologies. Other colonizing bacteria vary by the age of the donor patient (affected by major life episodes such as puberty, pregnancy, and menopause) and include *Lactobacillus* and anaerobes [30].

Skin

The skin carries its own unique reasons for potential complications. While its external location makes it easy to visualize any areas of concern for rejection and readily biopsy, this same location also can intensify its infectious risk. This site is in continuous exposure to the surrounding environment, which can increase the risk of surgical site infections and mechanical trauma [25, 31]. Skin is also colonized by normal flora such as *Streptococcus*, coagulase-negative *Staphylococcus*, and *Staphylococcus aureus*, which by itself may not be pathogenic but when introduced into a surgical site, can cause both local and invasive diseases. Additionally, many potential donors during their terminal hospitalization will have peripheral and central intravenous lines that cause further breaks in the skin and portals for future infection [32]. Minimizing such interventions once the move toward VCA transplant is recognized becomes crucially important to maintain healthy skin viability and reduce infection risk. Other systemic bacterial infections such as *Nocardia* and non-tuberculous mycobacteria can cause cutaneous lesions in the donor graft, especially if the donor cause of death involved trauma and skin penetration. Such atypical bacteria have a high predilection to disseminate in the immunosuppressed transplanted recipient; however, generally they are rare in the donor pre-procurement [33, 34].

Nonbacterial infections should also be taken into consideration. Topical viral infections such as HPV have been reported on bilateral hand transplants, either latent in the donor at the time of procurement or acquired posttransplant, and have been treated with topical cidofovir [35, 36]. Topical cidofovir has also proven to be useful in cutaneous HSV cases in VCA transplant patients that, under the influence of immunosuppression and repeated treatment, have become drug-resistant or particularly refractory to treatment [37, 38].

Fungal skin infections can also be present, as in the reported case of *Alternaria alternata* in a forearm transplant that was treated with amphotericin and itraconazole [36, 39] or *Candida* surgical site infection in a face transplant recipient, requiring echinocandin therapy [16]. Additional fungi have also been documented in the skin leading to superficial infections of SOTs. For example, in addition to *Alternaria*, other dematiaceous molds can cause nail or skin lesions, particularly in areas that have had minor trauma [40]. These are often treated with surgical removal and azole therapy. The dermatophytic fungus *Trichophyton rubrum* was also identified in the feet of a face transplant recipient and treated with 3 months of terbinafine [14].

Although the cutaneous manifestations of fungal infections usually represent complications of primary direct inoculation, care should be taken to consider the risk of disseminated disease in any VCA recipient. For example, the cutaneous nodules frequently seen with *Cryptococcus*, *Coccidioides*, or *Mucorales* may reflect cutaneous presentation of widely disseminated disease. With the exception of *Candida,* we recommend evaluating any recipient for other sites of infection with targeted history, exam, and chest imaging at a minimum.

It is also important to note that some skin infections or common skin conditions can appear clinically similar to acute rejection on visual examination. One example is a bilateral hand transplant patient who developed an erythematous maculopapular rash along the allografts over 16 years after transplantation. While initial presentation was felt to be most consistent with acute rejection, the rash also seemed to involve additional non-transplanted areas of the skin, including the abdomen. Skin biopsy was obtained and was ultimately consistent with scabies infection [41]. This case highlights the importance of having a low threshold to obtain skin biopsy for abnormal cutaneous lesions to ensure appropriate treatment is pursued [42]. Other noninfectious inflammatory skin conditions such as rosacea or coloring changes due to prolonged sun exposure can also manifest similarly and be difficult to diagnose both clinically and even on histopathology, particularly in face transplants [43].

Muscle

Particular pathogens prefer to primarily affect muscle tissue. *Toxoplasma* tachyzoites tend to target muscle and the central nervous system and cause both myositis and myocarditis [44, 45]. Thus, *Toxoplasma* has already been shown to cause significant morbidity in the transplant population, particularly in heart transplants [46, 47]. Although there has not yet been a case reported of *Toxoplasma* transmission from skeletal muscle transplantation alone, we recommend *Toxoplasma* serology be performed in both donor and recipient pretransplant. Transplant recipients whose serology does not demonstrate prior *Toxoplasma* exposure (IgG negative) should be advised to avoid possible risk factors for acquisition, including undercooked meat and close proximity to feline feces. Fortunately, trimethoprim-sulfamethoxazole (TMP-SMX), typically administered in posttransplant cases for *Pneumocystis* prophylaxis, is also effective against *Toxoplasma* [48]. Consideration should be given for extended prophylaxis in transplants where skeletal muscle is used and the donor antibodies are positive but recipient antibodies are negative, although precise duration is unknown for non-cardiac transplantation. Typically, in non-heart SOT populations, we recommend TMP-SMX or atovaquone prophylaxis for 3–6 months. Notably dapsone or inhaled pentamidine has no activity against toxoplasmosis.

In the setting of immunosuppression and recent surgery after transplantation, there can also be a risk of bacterial pyomyositis. Often this is caused by hematogenous spread, with a partiality to the muscle due to minor muscle injury and inflammation from surgery or trauma. In community-acquired cases, the most common pathogen is *Staphylococcus aureus* [49]. However other pathogens

including *Streptococcus* and gram-negative organisms have also been reported, including polymicrobial infections [50]. There have been multiple reports of bacterial infections transmitted from tissue allografts of deceased donors [51–54]. This risk can be augmented if appropriate donor screening is not performed, or if sterility is not maintained during tissue processing and transplantation.

Bone

Some of the risks of bone transplantation in the face or arms can be extrapolated from known history of bone graft transplantation [54, 55]. Certain infections can be transmitted via bone specifically. Up to 40 possible cases of bacteria transmission of various species have been reported in bone graft transplantation, including tuberculosis [54, 56]. These also include both gram-negative and gram-positive pathogens such as *Citrobacter*, *Streptococcus*, *Staphylococcus aureus*, and *Pseudomonas*.

Other types of infections reflect blood-borne transmission of systemic illnesses due to the vascular nature of the bone marrow. However, advancements in laboratory screening for many communicable diseases have significantly decreased the infection transmission rate. The rate of HIV transmission dropped significantly after nucleic acid testing (NAT) and p24 antigen testing of the donor were initiated, provided this testing is done within close timing to organ donation, preferably within 7 days of organ recovery [1, 57–61]. Hepatitis C and HTLV transmission events have been reported, although again, with appropriate donor screening, the risk for a VCA recipient should be no different than any other SOT [62–65]. (It is important to keep in mind however that the overall rise of hepatitis C donors is increasing in contemporary periods, particularly with the rise of fatalities due to the opioid epidemic. Transplantation of these organs has been demonstrated to be safe, and the virus can be cured with direct-acting antiviral agents within an appropriate time window without sacrificing patient welfare or graft function. At the time of publication, there have been no documented cases of intentional D+/R- HCV transplants within the VCA field) [66–68].

Nerve

Herpesviruses such as HSV and varicella zoster virus (VZV) have been known to lie latent in the nerve root ganglia and can reactivate under circumstances of significant stress or immunosuppression [31, 69]. For HSV, this typically causes painful oral or genital blisters [70]. However it can progress to disseminated disease with multi-organ involvement [71]. Donors and recipients should be tested for HSV IgG prior to transplant. It is important to recognize that HSV testing is not presently routine for solid organ donors, and this would need to be requested at the time of procurement. Acyclovir can be used for prophylaxis if the patient also does not have risk factors for CMV. Ganciclovir and valganciclovir, used for CMV prophylaxis, also have activity against HSV and are protective. Topical cidofovir is also available for reactivated or primary cutaneous cases that become drug-resistant or particularly refractory to treatment [37, 38].

VZV also remains latent in nerves and can be reactive years later as herpes zoster in both immunocompetent and immunocompromised patients, particularly in times of physiological stress. This typically manifests with a painful blistering rash but also can lead to disseminated infection. Varicella serology should be tested in all recipients pretransplant and appropriate pretransplant vaccination can be pursued [72, 73]. The treatment for both disseminated HSV and VZV is high-dose intravenous acyclovir. For a more detailed discussion of herpesvirus in transplant, please refer to ▶ Chap. 26, "Herpes Simplex and Varicella Zoster in Transplant Candidates and Recipients."

Lymphoid Tissue

Because many VCA transplants involve the transplantation of multiple lymph nodes, special consideration should be given to risks associated with these tissues. It should be noted that documented cases of lymph node associated transmission events are not present in the literature, but the passenger lymphocyte syndrome and graft versus host disease (GVHD), as described in ▶ Chap. 68, "Transplant Infectious Disease Evaluation of Cytopenias," may be more likely than in other transplant recipients.

Blood Vessel

There have been reports of HCV transmission by saphenous vein transplant [62] as well as rabies by iliac artery segment [74]. Additionally bacterial and fungal anastomotic mycotic aneurysms have been commonly associated with allografts, especially in the first few months after transplant. VCA transplants would be expected to carry a similar risk to SOT, proportional to the number of vessels used, and anastomoses formed.

Salivary Glands

Multiple episodes of infectious parotitis have been reported in one patient after face transplantation, all treated with cephalosporins [14]. The patient ultimately underwent dilation of a stenotic left parotid duct and ductoplasty 3 years after transplantation with resolution of symptoms. An additional patient with an sialocele infected with *C. albicans* and *Peptostreptococcus saccharolyticus* within the first month after face transplantation was treated with fluconazole and amoxicillin-clavulanate [16].

Organ-Specific Infections Particular to VCA

For systemic bacterial, fungal, mycobacterial, and viral pathogens transmissible by SOT, refer to the chapter on donor-derived infections.

General History

It is important to take a thorough medical and social/occupational history of the donor prior to transplantation. Some potential exposures that can lead to recipient infection have been discussed in other sections of this chapter but include HSV or HPV (verruca vulgaris risk) and occupational history (particularly fungal exposures). It is also important to be aware of health maintenance and preventative measures of the donor. Particularly for genitourinary transplantation, this can include including papanicolaou smear history, vaccine history (including HPV vaccination), previous multi-parity/uniparity, miscarriages, and sexually transmitted infection history.

Abdominal Wall Graft

Normal skin flora of the donor can potentially lead to surgical site or other wound infection. Recognizing that the abdominal wall flap typically includes fat, muscle, and neuronal tissue as well as the skin, apart from standard skin flora, little additional risk is acquired through abdominal wall transplants.

Face

Empiric antibiotic coverage should be based on pretransplant donor and recipient antimicrobial colonization. These include organisms found in the sinuses, nasal passages, and oropharynx. Face transplant recipients have had documented eye infections including palpebral conjunctivitis and bacterial blepharitis – treated with topical antibacterial ointment [16].

Hand

Fungal infections, particularly tinea infections, have been reported in hand transplantation, including the nail [70]:

1. **Occupational/activity-related or mortality-related infections** [75]. Thorough social history of donors and recipients should be obtained to assess for potential risk factors based on occupation/hobbies/endemic region or the circumstances leading up to the acute period before donor death:
   (i) *Donor who was a farmer/gardener or with motor vehicle accident as the cause of death*: Consider soil exposure pathogens, including *Nocardia*, endemic fungi*, Aspergillus, Mycobacterium*, and *Sporotrichoid*.
   (ii) *Donor with aquatic exposure during life or with drowning as the cause of death*: Differences in pathogens to consider can depend on whether the donor was exposed to salt water, fresh water, brackish water, or aquarium water. These organisms can include *Vibrio* species, *Aeromonas* species, and *Mycobacterium marinum*.
   (iii) *Donor who was an animal farmer, sheep handler*: Consider potential infections that can be transmitted from animals to humans, such as Orf virus, poxvirus, brucellosis, and *Coxiella*.

Larynx

Routine bacterial infections should be considered. One of the first laryngeal transplants (laryngopharyngeal complex, including thyroid, parathyroid, and five rings of trachea) in 1998 developed three separate episodes of tracheobronchitis within the first 15 months, one with *Pneumocystis* [76, 77]. Additionally, it would be important to also consider respiratory viruses, which can primarily affect this anatomic region and are not typically screened for in organ donation.

Penile

Screening for sexually transmitted infections should be done prior to transplantation. These include HSV, HPV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas*, and syphilis. Special preparatory discussion should occur between penile VCA programs and their associated organ procurement organizations, in order to facilitate such testing. There have also been documented fungal infections, such as one report of cutaneous infection with *Alternaria alternata*, treated with topical antifungals [78–80].

Uterine

Screening for sexually transmitted infections (as discussed in the above "Penile" section, but also including vaginal wet mount) should be done prior to transplantation. Genital HSV has been documented in a uterine transplant recipient within the first month posttransplant who subsequently developed postinfectious cervical stenosis [81]. Donors should also be tested for HPV, including but not limited to high-risk types (such as 16 and 18) associated with cervical cancer [82]. Additionally, common organisms found in the genitourinary tract can cause severe infection if they become more widespread. For example, the first uterine transplant in the United States succumbed to a severe *Candida* infection resulting in loss of the graft [29].

Furthermore, uterine transplants carry a similar ischemia-reperfusion risk to other SOT recipients. This can be of particular concern during transplantation when the uterus is exposed to vaginal flora, increasing the risk of graft infection. One uterine transplantation in a Swedish cohort of nine patients resulted in graft loss and ultimately graft removal due to *E. faecalis* abscess formation that was not responsive to antibiotics and surgical evacuation [81, 83].

With uterine transplant, it is also important to consider not only infectious risks associated with the graft itself but also potential risks that can arise for any future fetuses that may develop from that uterus. Specific considerations should be taken for cytomegalovirus, syphilis, and *toxoplasma*, specially D+/R- cases where control of infections may be more difficult because of immunosuppression. Active maternal infections during pregnancy can cause congenital infections with high morbidity in a fetus and are discussed later in this chapter, as are unique considerations for pretransplant vaccination in the uterine transplant population.

Systemic Infections that Can Be Predicted from Donor Testing

Cytomegalovirus

Cytomegalovirus (CMV) is a very common infectious complication of all types of transplantation, including VCA. CMV seroprevalence in the general population (including both adult and pediatric patients) has been widely variable in reports, referenced between 30% and 97% [84–86]. As with other SOTs, the greatest risk of disease occurs when a recipient is seronegative and is transplanted from a donor who is CMV IgG positive [26, 31, 36, 70, 87–96]. However, this discordance of serologies is of even higher concern when considering VCA transplantation, particularly uterine transplantation. In this situation, primary CMV in utero could have devastating congenital effects on the fetus. Therefore, uterine transplant programs should strongly consider CMV seromatching their donors and recipients or at the very least continuing antiviral prophylaxis for the duration of the graft until pregnancy is complete. CMV infection is typically treated with intravenous ganciclovir or oral valganciclovir. For a more extensive discussion on CMV disease and management, refer to ▶ Chap. 24, "Cytomegalovirus in Solid Organ Transplant Recipients: Prevention, Diagnosis, and Treatment."

Epstein-Barr Virus

Epstein-Barr virus (EBV) is another frequently seen complication in posttransplant patients, particularly in transplant recipients with negative EBV serology but a donor with positive serology. This can lead to a spectrum of both infection and malignancy including primary viremia and posttransplant lymphoproliferative disorder (PTLD). There are no dedicated antiviral agents for EBV infection, and treatment is typically focused on reducing immunosuppression. Ganciclovir, foscarnet, or cidofovir can be used for treatment of severe infection. For a more extensive discussion on EBV and PTLD, refer to ▶ Chap. 27, "Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorders."

Toxoplasma

As with CMV, the serologies of *Toxoplasma* are particularly important in the situation of uterine transplantation. A donor positive/recipient negative serology could potentially lead to primary infection in utero, which would carry high infant morbidity and mortality. *Toxoplasma* mismatch, where a recipient is seronegative and a donor is seropositive, should probably be avoided altogether in the setting of uterine transplant, although to date, *Toxoplasma* in a subsequent fetus has not been documented. Clinicians should consider prophylaxis with TMP-SMX for at least the first 3–6 months posttransplant if a mismatch occurs.

Syphilis

Syphilis is a sexually transmitted infection that can cause both a local chancre and disseminated infection including neurologic and cardiac involvement. Care should be taken to perform syphilis serologies on all recipients and donors prior to receiving a VCA transplant, particularly those that involve genitourinary organs. Detecting and treating syphilis infection is particularly a concern in the setting of uterine transplantation, as congenital syphilis can lead to significant fetal impairment.

Additional Considerations

Vaccinations

VCA transplants should follow the same vaccination schedule and recommendations as those for SOT, at a minimum [97]. Every effort should be made to administer all vaccinations pretransplant, particularly live vaccinations, as investigations are still ongoing as to live vaccination safety posttransplant (e.g., measles-mumps-rubella, varicella). As with other SOT, live vaccines should be administered at least 4 weeks prior to transplantation to allow for appropriate host response before starting systemic immunosuppression. If vaccinations are administered posttransplant, care should be taken to wait at least three to six months after transplantation, ensure the recipient is on stable immunosuppression, and that it is an inactivated vaccine.

Additionally, given many VCA transplants involve skin, HPV vaccination should be considered ahead of transplant for all recipients under the FDA-approved age of 45 years. Data is not available for older recipients, although vaccination is likely to be safe. HPV infection appears to have an accelerated course with increased severity and complications in the transplant population. It is unknown how vaccination impacts cases where the donor already has documented HPV, though it is possible it may still have a partial protective effect.

There are some unique immunization recommendations for VCA cases, predominantly for the pre-uterine transplant population, where the ultimate goal of transplantation is to produce a viable pregnancy. In this group, specific recommended live pre-pregnancy vaccinations (particularly measles-mumps-rubella and varicella) should be administered pretransplantation [98]. There are high maternal and fetal morbidity concerns for measles, rubella, and varicella infections during pregnancy. Serologies should be tested in the recipient pretransplant, and if they are found to not have documented evidence of immunity, then vaccination should be pursued according to routine guidelines. All live vaccines should be completed at least 4 weeks prior to transplantation. Additional routine inactivated prenatal vaccinations should also be considered pretransplant, including tetanus-diphtheria-pertussis (TDAP) vaccines and seasonal flu vaccines, as well as additional routine pre-transplant vaccines such as pneumococcal and hepatitis A and B.

Safe Living Practices Posttransplant

The majority of safe living practices that are recommended after solid organ transplantation are also recommended after VCA transplantation [99]. For more extensive discussion on safe living after tranpslant, refer to ▶ Chap. 11, "Preventative Health in Solid Organ Transplant: Safe Living Post-transplant."

Environmental Exposures

After VCA transplant, patients should avoid sick contacts and encourage appropriate hand hygiene, both for themselves and those with whom they are in contact. Care should be taken to use facemask protection if they may be exposed to any sick contacts or if they are working outdoors to reduce the risk of respiratory inhalation of pathogens. Particular care should be taken to avoid trauma to the VCA skin. Other recommendations vary based on the potential posttransplant exposures of the recipient based on their home or work environment and hobbies.

Future Directions

Surgical advances and transplant techniques are continuing to evolve, and throughout this process, SOT and VCA transplantation practices continue to influence each other. Currently, different VCA types include vascularized, upper and lower limb, head and neck, abdominal wall, genitourinary organs, adrenal gland, spleen, and musculoskeletal composite graft segments. In addition, current and future considerations include progression toward a VCA type concomitant with various organ transplantations, akin to abdominal wall transplantation currently pursued with intestinal transplant. However, even in this ever-evolving field, if infection risk is considered in terms of the tissue layers transplanted, as described throughout this chapter, potential complications can be predicted and managed accordingly.

Conclusion

VCA transplantation is a novel, exciting, and continuously evolving field. Due to similarities in immunosuppression, much of the guidance designed to prevent and treat infections can be extrapolated from solid organ transplant experience. However, VCAs also have their own unique risks and considerations that must be taken into consideration. This is a new field without significant historical context and a body of literature that remains somewhat sparse. Consequently, a systematic approach to infection management is most beneficial. As multidisciplinary transplant providers, we need to publish and share information together to continue to grow this field.

Key Points

- Many different types of vascularized composite allografts (VCA) exist, although the most common are hands, face, and uterine transplants.

- The goals of VCA transplant differ depending on the tissue type but are typically quality of life affirming, rather than necessarily life-saving. Donor-recipient matching factors in many unique features such as size, gender, skin tone, etc.
- Immunosuppressive regimens are generally on par with deceased donor kidney transplants in terms of intensity and therefore infection risk.
- By better understanding the individual risk associated with any one tissue, infectious disease physicians can learn to appreciate the risk that any unique VCA may pose to the recipient.
- Advice regarding safe living, and strategies for risk and infection mitigation should be tailored to match the individual allograft type.

References

1. Cendales L, et al. Implementation of vascularized composite allografts in the United States: recommendations from the ASTS VCA Ad Hoc Committee and the Executive Committee. Am J Transplant. 2011;11(1):13–7.
2. Lee WP, et al. Relative antigenicity of components of a vascularized limb allograft. Plast Reconstr Surg. 1991;87(3):401–11.
3. Murray JE. Organ transplantation (skin, kidney, heart) and the plastic surgeon. Plast Reconstr Surg. 1971;47(5):425–31.
4. Petruzzo P, et al. The international registry on hand and composite tissue transplantation. Transplantation. 2010;90(12):1590–4.
5. Petruzzo P, et al. First human double hand transplantation: efficacy of a conventional immunosuppressive protocol. Clin Transpl. 2003;17(5):455–60.
6. Petruzzo P, et al. Bilateral hand transplantation: six years after the first case. Am J Transplant. 2006;6(7):1718–24.
7. Whitaker IS, et al. Composite tissue allotransplantation: a review of relevant immunological issues for plastic surgeons. J Plast Reconstr Aesthet Surg. 2008;61(5):481–92.
8. Hautz T, et al. Immunologic aspects and rejection in solid organ versus reconstructive transplantation. Transplant Proc. 2010;42(9):3347–53.
9. Ravindra KV, Ildstad ST. Immunosuppressive protocols and immunological challenges related to hand transplantation. Hand Clin. 2011;27(4):467–79.. ix
10. Petruzzo P, Dubernard JM. The international registry on hand and composite tissue allotransplantation. Clin Transpl. 2011;22:247–53.
11. Schneeberger S, et al. Achievements and challenges in composite tissue allotransplantation. Transpl Int. 2011;24(8):760–9.
12. Guo S, et al. Human facial allotransplantation: a 2-year follow-up study. Lancet. 2008;372(9639):631–8.
13. Sicilia-Castro D, et al. Reconstruction of a severe facial defect by allotransplantation in neurofibromatosis type 1: a case report. Transplant Proc. 2011;43(7):2831–7.
14. Knoll BM, et al. Infections following facial composite tissue allotransplantation – single center experience and review of the literature. Am J Transplant. 2013;13(3):770–9.
15. Gordon CR, et al. The world's experience with facial transplantation: what have we learned thus far? Ann Plast Surg. 2009;63(5):572–8.
16. Tasigiorgos S, et al. Five-year follow-up after face transplantation. N Engl J Med. 2019;380(26):2579–81.
17. Brannstrom M, et al. Uterus transplantation: a rapidly expanding field. Transplantation. 2018;102(4):569–77.

18. Kanitakis J, et al. Clinicopathologic monitoring of the skin and oral mucosa of the first human face allograft: report on the first eight months. Transplantation. 2006;82(12):1610–5.
19. Cendales L, Levine M, Bartlett S, Cheeseman J, Drachenberg C, Hancock W, Joshi M, Kirk A, Leopardi F, Levin S, Uluer M, Selim A, Song M, Twaddell W, Wang L, Wang Z, Barth R. Skin as a harbinger of rejection of underlying structures in vascularized composite allografts: concordance or discordance? Am J Transplant. 2016;16(suppl 3):433.
20. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. 2011;335(1):2–13.
21. Devauchelle B, et al. First human face allograft: early report. Lancet. 2006;368(9531):203–9.
22. Dubernard JM, et al. Outcomes 18 months after the first human partial face transplantation. N Engl J Med. 2007;357(24):2451–60.
23. Siemionow M, et al. Near-total human face transplantation for a severely disfigured patient in the USA. Lancet. 2009;374(9685):203–9.
24. Lantieri L, et al. Feasibility, reproducibility, risks and benefits of face transplantation: a prospective study of outcomes. Am J Transplant. 2011;11(2):367–78.
25. Broyles JM, et al. Characterization, prophylaxis, and treatment of infectious complications in craniomaxillofacial and upper extremity allotransplantation: a multicenter perspective. Plast Reconstr Surg. 2014;133(4):543e–51e.
26. Avery RK. Update on infections in composite tissue allotransplantation. Curr Opin Organ Transplant. 2013;18(6):659–64.
27. Pomahac B, et al. Vascular considerations in composite midfacial allotransplantation. Plast Reconstr Surg. 2010;125(2):517–22.
28. Guibert EE, et al. Organ preservation: current concepts and new strategies for the next decade. Transfus Med Hemother. 2011;38(2):125–42.
29. Flyckt RL, et al. Deceased donor uterine transplantation: innovation and adaptation. Obstet Gynecol. 2016;128(4):837–42.
30. Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. J Physiol. 2017;595(2):451–63.
31. Hammond SP. Infections in composite tissue allograft recipients. Infect Dis Clin N Am. 2013;27(2):379–93.
32. Pomahac B, et al. Three patients with full facial transplantation. N Engl J Med. 2012;366(8):715–22.
33. Hemmersbach-Miller M, et al. Nocardia infections in the transplanted host. Transpl Infect Dis. 2018;20(4):e12902.
34. Steinbrink J, et al. Manifestations and outcomes of nocardia infections: comparison of immunocompromised and nonimmunocompromised adult patients. Medicine (Baltimore). 2018;97(40):e12436.
35. Bonatti H, et al. Local administration of cidofovir for human papilloma virus associated skin lesions in transplant recipients. Transpl Int. 2007;20(3):238–46.
36. Bonatti H, et al. Infectious complications in three double hand recipients: experience from a single center. Transplant Proc. 2009;41(2):517–20.
37. Muluneh B, et al. Successful clearance of cutaneous acyclovir-resistant, foscarnet-refractory herpes virus lesions with topical cidofovir in an allogeneic hematopoietic stem cell transplant patient. J Oncol Pharm Pract. 2013;19(2):181–5.
38. Epstein JB, et al. Complex management of resistant oral herpes simplex virus infection following hematopoietic stem cell transplantation: potential role of topical cidofovir. Support Care Cancer. 2016;24(8):3603–6.
39. Bonatti H, et al. Alternaria alternata soft tissue infection in a forearm transplant recipient. Surg Infect. 2007;8(5):539–44.
40. Wong EH, Revankar SG. Dematiaceous molds. Infect Dis Clin N Am. 2016;30(1):165–78.
41. Kanitakis J, Morelon E. Scabies in a bilateral hand allograft recipient: an additional mimicker of acute skin rejection in vascularized composite allotransplantation. Transpl Infect Dis. 2017;19(3):e12683–89.

42. Kanitakis J. The challenge of dermatopathological diagnosis of composite tissue allograft rejection: a review. J Cutan Pathol. 2008;35(8):738–44.
43. Kanitakis J. Transmission of rosacea from the graft in facial allotransplantation. Am J Transplant. 2011;11(6):1338–9.
44. Wohlfert EA, Blader IJ, Wilson EH. Brains and brawn: toxoplasma infections of the central nervous system and skeletal muscle. Trends Parasitol. 2017;33(7):519–31.
45. Montoya JG, et al. Toxoplasmic myocarditis and polymyositis in patients with acute acquired toxoplasmosis diagnosed during life. Clin Infect Dis. 1997;24(4):676–83.
46. Khurana S, Batra N. Toxoplasmosis in organ transplant recipients: evaluation, implication, and prevention. Trop Parasitol. 2016;6(2):123–8.
47. Gallino A, et al. Toxoplasmosis in heart transplant recipients. Eur J Clin Microbiol Infect Dis. 1996;15(5):389–93.
48. Schwartz BS, Mawhorter SD. Parasitic infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):280–303.
49. Burdette SD, et al. Staphylococcus aureus pyomyositis compared with non-Staphylococcus aureus pyomyositis. J Inf Secur. 2012;64(5):507–12.
50. Crum NF. Bacterial pyomyositis in the United States. Am J Med. 2004;117(6):420–8.
51. Eastlund T. Bacterial infection transmitted by human tissue allograft transplantation. Cell Tissue Bank. 2006;7(3):147–66.
52. Kainer MA, et al. Clostridium infections associated with musculoskeletal-tissue allografts. N Engl J Med. 2004;350(25):2564–71.
53. Tomford WW, et al. Frozen musculoskeletal allografts. A study of the clinical incidence and causes of infection associated with their use. J Bone Joint Surg Am. 1990;72(8):1137–43.
54. Hinsenkamp M, et al. Adverse reactions and events related to musculoskeletal allografts: reviewed by the World Health Organisation Project NOTIFY. Int Orthop. 2012;36(3):633–41.
55. Zamborsky R, et al. Infection in bone allograft transplants. Exp Clin Transplant. 2016;14(5):484–90.
56. James JI. Tuberculosis transmitted by banked bone. J Bone Joint Surg (Br). 1953;35-B(4):578.
57. Li CM, Ho YR, Liu YC. Transmission of human immunodeficiency virus through bone transplantation: a case report. J Formos Med Assoc. 2001;100(5):350–1.
58. Simonds RJ, et al. Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. N Engl J Med. 1992;326(11):726–32.
59. Karcher HL. HIV transmitted by bone graft. BMJ. 1997;314(7090):1300.
60. Schratt HE, et al. HIV infection caused by cold preserved bone transplants. Unfallchirurg. 1996;99(9):679–84.
61. Centers for Disease Control (CDC). Transmission of HIV through bone transplantation: case report and public health recommendations. MMWR Morb Mortal Wkly Rep. 1988;37(39):597–9.
62. Tugwell BD, et al. Transmission of hepatitis C virus to several organ and tissue recipients from an antibody-negative donor. Ann Intern Med. 2005;143(9):648–54.
63. Conrad EU, et al. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg Am. 1995;77(2):214–24.
64. Sanzen L, Carlsson A. Transmission of human T-cell lymphotrophic virus type 1 by a deep-frozen bone allograft. Acta Orthop Scand. 1997;68(1):72–4.
65. Shutkin NM. Homologous-serum hepatitis following the use of refrigerated bone-bank bone. J Bone Joint Surg Am. 1954;36-A(1):160–2.
66. Levitsky J, et al. The American Society of transplantation consensus conference on the use of hepatitis C viremic donors in solid organ transplantation. Am J Transplant. 2017;17(11):2790–802.
67. Woolley AE, et al. Heart and lung transplants from HCV-infected donors to uninfected recipients. N Engl J Med. 2019;380(17):1606–17.
68. Goldberg DS, et al. Trial of transplantation of HCV-infected kidneys into uninfected recipients. N Engl J Med. 2017;376(24):2394–5.

69. Cavadas PC, et al. The Spanish experience with hand, forearm, and arm transplantation. Hand Clin. 2011;27(4):443–53.. viii
70. Barker JH, et al. Composite tissue allotransplantation. Transplantation. 2002;73(5):832–5.
71. Wilck MB, Zuckerman RA. Herpes simplex virus in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):121–7.
72. Pergam SA, Limaye AP. Varicella zoster virus in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):138–46.
73. Dooling KL, et al. Recommendations of the advisory committee on immunization practices for use of herpes zoster vaccines. MMWR Morb Mortal Wkly Rep. 2018;67(3):103–8.
74. Srinivasan A, et al. Transmission of rabies virus from an organ donor to four transplant recipients. N Engl J Med. 2005;352(11):1103–11.
75. Koshy JC, Bell B. Hand Infections. J Hand Surg [Am]. 2019;44(1):46–54.
76. Birchall MA, et al. Laryngeal transplantation in 2005: a review. Am J Transplant. 2006;6(1):20–6.
77. Birchall M. Human laryngeal allograft: shift of emphasis in transplantation. Lancet. 1998;351(9102):539–40.
78. van der Merwe A, et al. Penile allotransplantation for penis amputation following ritual circumcision: a case report with 24 months of follow-up. Lancet. 2017;390(10099):1038–47.
79. Tuffaha SH, et al. Penile transplantation: an emerging option for genitourinary reconstruction. Transpl Int. 2017;30(5):441–50.
80. Szafran AA, Redett R, Burnett AL. Penile transplantation: the US experience and institutional program set-up. Transl Androl Urol. 2018;7(4):639–45.
81. Brannstrom M, et al. Global results of human uterus transplantation and strategies for pre-transplantation screening of donors. Fertil Steril. 2019;112(1):3–10.
82. Nguyen ML, Flowers L. Cervical cancer screening in immunocompromised women. Obstet Gynecol Clin N Am. 2013;40(2):339–57.
83. Brannstrom M, et al. First clinical uterus transplantation trial: a six-month report. Fertil Steril. 2014;101(5):1228–36.
84. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol. 2010;20(4):202–13.
85. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin Infect Dis. 2010;50(11):1439–47.
86. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13512.
87. Gordon CR, et al. Cytomegalovirus and other infectious issues related to face transplantation: specific considerations, lessons learned, and future recommendations. Plast Reconstr Surg. 2011;127(4):1515–23.
88. Gordon CR, Abouhassan W, Avery RK. What is the true significance of donor-related cytomegalovirus transmission in the setting of facial composite tissue allotransplantation? Transplant Proc. 2011;43(9):3516–20.
89. Schneeberger S, et al. Cytomegalovirus-related complications in human hand transplantation. Transplantation. 2005;80(4):441–7.
90. Jones JW, et al. Successful hand transplantation. One-year follow-up. Louisville Hand Transplant Team. N Engl J Med. 2000;343(7):468–73.
91. Lanzetta M, et al. Human hand transplantation: what have we learned? Transplant Proc. 2004;36(3):664–8.
92. Breidenbach WC, et al. Outcomes of the first 2 American hand transplants at 8 and 6 years posttransplant. J Hand Surg [Am]. 2008;33(7):1039–47.
93. Lantieri L, et al. Repair of the lower and middle parts of the face by composite tissue allotransplantation in a patient with massive plexiform neurofibroma: a 1-year follow-up study. Lancet. 2008;372(9639):639–45.

94. Ravindra KV, et al. Hand transplantation in the United States: experience with 3 patients. Surgery. 2008;144(4):638–43; discussion 643–4
95. BenMarzouk-Hidalgo OJ, et al. First face composite-tissue transplant recipient successfully treated for cytomegalovirus infection with preemptive valganciclovir treatment. Antimicrob Agents Chemother. 2011;55(12):5949–51.
96. Chelmonski A, Jablecki J, Szajerka T. Insidious course of cytomegalovirus infection in hand transplant recipient: case report, diagnostics, and treatment. Transplant Proc. 2011;43(7):2827–30.
97. Danziger-Isakov L, Kumar D. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13563.
98. CDC. Guidelines for vaccinating pregnant women. 2016. https://www.cdc.gov/vaccines/pregnancy/hcp-toolkit/guidelines.html
99. Avery RK, Michaels MG. Strategies for safe living following solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33:e13519.

Part VII

Considering Emerging Infections in Differential Diagnosis

Emerging Diagnostics for Transplant Infectious Diseases

62

Marwan M. Azar, David C. Gaston, and Maricar Malinis

Contents

Introduction 1536
Epidemiology and Impact of Antimicrobial Resistance in Transplant Populations 1536
Emerging Diagnostics for Bloodstream Infections 1537
Molecular Methods Performed on Positive Blood Culture Bottles 1537
Molecular Methods Performed on Blood Specimens 1548
Impact of Rapid Molecular Diagnostics on Clinical Outcomes in Transplant Populations 1549
Magnetic Resonance-Based Methods 1550
Metagenomic Sequencing 1552
Emerging Diagnostics for Respiratory Infections 1552
Aspergillus PCR 1553
Aspergillus PCR for Diagnosis of Resistance in *Aspergillus* Species 1558
Gas Chromatography-Mass Spectrometry of Exhaled Metabolites 1559
Multiplex PCR 1560
Metagenomic Sequencing 1560
Emerging Diagnostics for CNS Infections 1563
Multiplex PCR 1564
Metagenomic Sequencing 1564
Emerging Diagnostics for Gastrointestinal Infections 1565
Multiplex PCR 1565
Conclusion 1568
Key Points 1569
References 1570

M. M. Azar (✉) · D. C. Gaston
Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT, USA
e-mail: marwan.azar@yale.edu; david.gaston@yale.edu

M. Malinis
Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA
e-mail: maricar.malinis@yale.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_69

Introduction

Following solid organ and hematopoietic stem cell transplantation, innate and adaptive immune responses are significantly impaired, placing patients at high risk for infections with both community-acquired and opportunistic pathogens. In contrast to the general population, immunocompromised hosts often develop infections with multiple pathogens at once or with uncommon pathogens for which currently available conventional diagnostics are suboptimal. Owing to prolonged hospitalizations and greater exposure to antimicrobials, transplant recipients patients are also at higher risk for acquisition of multidrug-resistant (MDR) organisms. In light of the increased vulnerability to infection, diagnostics applicable to the practice of transplant infectious diseases are essential. Though culture and histopathology remain a bedrock of diagnosis, diagnostic microbiology has witnessed immense progress in the last decade, including a shift toward culture-independent methods such as molecular testing, mass spectrometry, and magnetic resonance-based technologies. Such methods have greatly expanded the array of organisms identified, enhanced the detection of resistance determinants, significantly shortened the time to diagnosis, and positively impacted the care of transplant patients with infections. On the horizon is another wave of diagnostic modalities with immense potential to enhance our diagnostic capabilities for transplant infectious diseases including highly multiplexed polymerase chain reaction (PCR) panels directly applicable to clinical specimens and with the potential for resistance detection, metagenomic next-generation sequencing (mNGS), and breath-based diagnosis of volatile metabolites among others. In this review, we examine emerging diagnostic methods for infectious diseases with a focus on the application to transplant patients.

Epidemiology and Impact of Antimicrobial Resistance in Transplant Populations

The emergence and spread of MDR organisms in the last decade have imposed an enormous clinical burden on patient populations worldwide. Partially a result of systematic misuse and overuse of available antibiotics, the crisis of antimicrobial resistance has been compounded by diminished antibiotic drug development, leading to a severely restricted antimicrobial armamentarium to treat MDR organisms. At the turn of the twenty-first century, infections with resistant Gram-positive organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) predominated as the foremost causes of nosocomial infections, with strains of vancomycin-intermediate *S. aureus* (VISA) now encountered worldwide [1]. In the last decade, however, MDR Gram-negative infections, driven by extended-spectrum beta-lactamase (ESBL) producing and carbapenem-resistant *Enterobacteriaceae* (CRE), have emerged as a serious threat to hospitalized patients. The negative impact of increasing multidrug resistance has been most pronounced among certain subsets of patients, including the critically ill and immunosuppressed, for whom ineffective empiric antibiotic therapy is strongly

correlated with increased mortality [2]. Recipients of solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) are particularly vulnerable to MDR infections due to underlying chronic disease, extended and recurrent hospitalizations, use of immunosuppressive modalities, ubiquity of intravascular catheters, risk of surgical site infections, repeated exposure to antimicrobials, and, in some cases, acquisition of donor-derived pathogens [3, 4]. Within transplant populations, allogeneic HSCT and heart and lung transplantation are associated with higher rates of resistance in Gram-negative bacteria, likely as a result of the aforementioned factors, including more intense immunosuppression [5, 6]. Resistance presents challenges to the selection of antibacterial prophylaxis and both empiric and targeted treatment modalities in any setting. Yet these are significantly amplified in the context of transplantation due to drug-drug interactions and poor baseline vital organ function which further limit antibiotic choices. Indeed, infections with drug-resistant organisms including MRSA, ESBL, CRE, *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, and *Acinetobacter* species have been associated with increased mortality for kidney, liver, and lung SOT and HSCT [5–9]. In parallel with the continued threat of bacterial resistance, drug-resistant fungi have become an important public health concern. *Candida* species are now the fifth most common nosocomial pathogen and a leading cause of mortality for transplant patients, especially in liver transplantation [10]. Recent years have seen the emergence of *C. auris*, an organism with the potential for resistance to all three major classes of antifungals (azoles, echinocandins, polyenes), and a proclivity for environmental persistence and nosocomial transmission including the possibility of donor-derived acquisition [11–13].

Since transplant patients with bacteremia or fungemia are at significantly higher risk for mortality than other populations, with a risk of death of up to 60% by some assessments, prompt identification of bloodstream pathogens is crucial in timely administration of effective antimicrobial therapy [24, 14].

Emerging Diagnostics for Bloodstream Infections

In the last decade, molecular-based diagnostics that rapidly identify bloodstream pathogens and their resistance determinants directly from positive blood cultures and on blood samples have been developed. These assays, along with non-PCR-based methods such as magnetic resonance and metagenomics, hold significant promise for improving diagnostic yield and ultimately outcomes of transplant patients.

Molecular Methods Performed on Positive Blood Culture Bottles

Rapid identification and antimicrobial susceptibility testing (AST) for bloodstream pathogens are critical in transitioning empiric to directed antimicrobial therapy, a key step in optimizing patient outcomes in the setting of sepsis. In traditional laboratory schemes, organism identification takes 48 hours or longer, and results of AST require

an additional 24 hours at least [15]. Such delays in the identification of bloodstream pathogens prompt treating clinicians to select empiric antimicrobial therapy for anticipated infecting pathogens based on the clinical syndrome and host, as well as local or facility-specific antimicrobial resistance patterns for selected pathogens. However, empiric therapy based on estimation of pretest probability is very often incorrect, with up to 50% of patients with bacteremia and up to 70% of patients with fungemia receiving inadequate empiric treatment prior to resulting of culture data [16, 24]. To circumvent the inherent delays associated with this testing paradigm, several molecular-based rapid diagnostic tests have been developed in an attempt to directly identify organisms from positive blood culture broth and provide resistance information without the need for culture. These techniques have the capacity to identify specific organisms (to the species or genus level) or groups of organisms. Importantly, many methods provide additional direct genotypic resistance information by identifying specific genetic determinants associated with phenotypic resistance, such as the *mecA* gene that confers methicillin resistance in *S. aureus* or direct phenotypic resistance data by providing extrapolated minimal inhibitory concentration (MIC) for specific antimicrobials. These molecular assays are considered moderate in terms of user complexity but benefit from minimal sample processing and hands-on time as well as automated test interpretation. Though little data exists in the context of transplant patients, these assays hold significant promise to accelerate diagnosis and resistance detection for this vulnerable population.

Several FDA-cleared rapid molecular tests for direct organism and resistant detection from blood culture are currently commercially available in the United States (Table 1). The *Quick*FISH and PNA FISH BC assays (OpGen, Maryland, USA, previously AdvanDx) for *Staphylococcus*, *Enterococcus,* Gram-negative, and *Candida* (*Candida Quick*FISH not FDA cleared at time of this writing) employ fluorescence in situ hybridization (FISH) using nucleic acid probes to identify organism-specific ribosomal RNA sequences, followed by automated examination and quantification under fluorescence microscopy, within a turnaround time of 2.5 hours and 20–30 minutes, respectively. The Gram-negative organisms identified are limited to *Escherichia coli, P. aeruginosa*, and *Klebsiella pneumoniae*, and *Candida* species are categorized into groups (*C. albicans/C. parapsilosis*, *C. glabrata/C. krusei*, and *C. tropicalis*). No AST information is provided using this assay; importantly the method does not differentiate between MRSA and methicillin-sensitive *S. aureus* (MSSA). Other limitations include the high limit of detection of 10^5 CFU/mL and the possibility of false negatives with prolonged times between specimen collection and processing. With the PNA FISH assay, *Micrococcus* may be misidentified as *Staphylococcus*, and *S. anginosus* may be misidentified as *Enterococcus.*

The Xpert MRSA/SA blood culture assay by Cepheid (Carlsbad, USA) identifies *S. aureus* from positive blood cultures within a 50-minute time frame. Using PCR, the assay detects DNA sequences for staphylococcal protein A (SpA) as well as staphylococcal chromosomal cassette (SCC*mec*) and *mecA*, genes specific to MRSA. This assay therefore provides some resistance information to guide directed treatment of *S. aureus* bacteremia. Test sensitivity in FDA trial data was reported at

Table 1 Direct organism identification and detection of resistance from positive blood culture

Manufacturer, assay	Methodology	Organisms identified	Direct genotypic resistance detection	Direct phenotypic resistance detection	Turnaround time	FDA Performance Data (in comparison with culture)	Caveats
AdvanDx, *Quick*FISH BC assays for *Staphylococcus*, *Enterococcus*, Gram-negative, and *Candida*	FISH using PNA probes to organism-specific ribosomal RNA sequences followed by examination by fluorescence microscopy	*S. aureus*, Coagulase-negative staphylococci, *Enterococcus faecalis*, Other Enterococci, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Candida albicans*, *C. glabrata*, *C. krusei* (not FDA cleared)	None	None	20 min	Se (*S. aureus*): 99.3% Se (CoNS): 98.3% Sp (Staphylococci): 95.6% Se (*E. faecalis*): 100% Se (other Enterococci): 97.5% Sp: 100% Se (*E. coli*): 96.8% Se (*P. aeruginosa*): 98.1% Se (*K. pneumoniae*): 100% Sp: 99%	Does not differentiate MSSA from MRSA Candida *QuickFISH* not FDA cleared at time of publication False negatives can occur with mixed cultures and with prolonged times between specimen collection and processing *P. fulva* may be misidentified as *P. aeruginosa*
AdvanDx, PNA FISH BC assays for *S. aureus/ CNS, E. faecalis/ OE*, Gram-negative, and *Candida*	FISH using PNA probes to organism-specific ribosomal RNA sequences followed by examination by fluorescence microscopy	*S. aureus*, Coagulase-negative staphylococci, *E. faecalis*, Other Enterococci, *E. coli*, *P. aeruginosa*, *C. albicans* and/or *C. parapsilosis*, *C. glabrata* and/or *C. krusei* and *C. tropicalis*	None	None	30 min	Se (*S. aureus*): 100% Se (CoNS): 100% Sp (Staphylococci): 81.8% Se (*E. faecalis*): 100% Se (other enterococci): 100% Sp: 100% Se (*E.coli*): 100% Se (*P.aeruginosa*): 96.9% Se (*K.pneumoniae*): 98.7%	Does not differentiate MSSA from MRSA False negatives can occur with mixed cultures *Micrococcus* may be misidentified as *Staphylococcus* *S. anginosus* may be misidentified as *Enterococcus*

(continued)

Table 1 (continued)

Manufacturer, assay	Methodology	Organisms identified	Direct genotypic resistance detection	Direct phenotypic resistance detection	Turnaround time	FDA Performance Data (in comparison with culture)	Caveats
						Sp: 97.5% Se (*C.albicans/ C.parapsilosis*): 100% Se (*C.glabrata/ C.krusei*): 97.9% Se: (*C.tropicalis*) Sp: 100%	
Cepheid, Xpert MRSA/SA blood culture assay	PCR for staphylococcal protein A (spa), the gene for methicillin/ oxacillin resistance (mecA) and staphylococcal chromosomal cassette (SCCmec)	*S. aureus*	Methicillin (mecA; SCCmec)	None	50 min	Se: 100% Sp: 100%	False negative for MRSA can occur if MSSA and MRSA present in same sample
BioFire, FilmArray blood culture identification panel	Multiplexed PCR identification of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance	*S. aureus*, *Streptococcus* spp. (*Streptococcus agalactiae*, *S. pneumoniae*, and *S. pyogenes*), *Enterococcus*, *Listeria monocytogenes*, *Acinetobacter baumannii*, *Enterobacter*	Methicillin (mecA), vancomycin (vanA/vanB), and carbapenems (KPC)	None	1 h	Se (*S. aureus*): 98.4% Se (Staphylococci): 96.5% *Se* (Streptococci): 97.5% Se (*Enterococcus*): 97.7% Se (*Listeria monocytogenes*): 100% Se (*A. baumannii*): 100% Se (*E. cloacae*	False negatives can occur with mixed cultures Does not provide *Enterococcus* species *Enterococcus* may be misidentified as *Staphylococcus* *E. aerogenes*, *S. marcescens* and *Raoultella*

		cloacae complex, *E. coli*, *K. oxytoca*, *K. pneumoniae*, *Proteus*, *Serratia marcescens*, *Haemophilus influenzae*, *Neisseria meningitidis* (encapsulated), *P. aeruginosa*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*				complex): 97.4% Se (*E. coli*): 98% Se (*K. oxytoca*): 92.2% Se (*K. pneumoniae*): 97.1% Se (*Proteus*): 100% Se (S. *marcescens*): 98.7% Se (*H. influenzae*): 100% Se (*N. meningitides*): 100% Se (*P. aeruginosa*) 98.1% Se (*C. albicans*): 100% Se (*C. glabrata*): 100% Se (*C. krusei*): 100% Se (*C. parapsilosis*):100% Se (*C. tropicalis*): 100% Se (mecA): 98.4% Se (vanA/vanB): 100% Se (KPC): 100% Sp: 99.1–100%	*ornithinolytica* may be misidentified as *K. pneumoniae*
Luminex, Verigene GP blood culture assay	Rapid microarray-based detection of specific nucleic acid targets	*S. aureus*, *S. lugdunensis*, coagulase-negative Staphylococci, *Streptococcus* spp., *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus*	Methicillin (mecA), vancomycin (vanA and vanB)	None	2.5 h	Se (*S. aureus*): 99.1% Se (mecA): 94.2% Se (*S. lugdunensis*): 95.0% Se (*Staphylococcus* spp.): 98% Se: (*Streptococcus* spp.): 95% Se (*Listeria* spp.): 100%	False negatives can occur with mixed cultures *L. grayi* may not be identified *E. avium* may be misidentified as *E. faecium*

(continued)

Table 1 (continued)

Manufacturer, assay	Methodology	Organisms identified	Direct genotypic resistance detection	Direct phenotypic resistance detection	Turnaround time	FDA Performance Data (in comparison with culture)	Caveats
		agalactiae, *Streptococcus anginosus* group, *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria* spp.				Se (*E. faecalis*): 96.9% Se (*E. faecium*): 97.1% Se (vanA): 94.2% Se (vanB):97.3% Sp: 99.5–100%	
Luminex, Verigene GN blood culture assay	Rapid microarray-based detection of specific nucleic acid targets	*E. coli*, *K. oxytoca*, *K. pneumoniae*, *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Acinetobacter* spp., *P. aeruginosa*	ESBL (CTX-M), carbapenem (KPC, IMP, NDM, OXA, VIM)	None	2 h	Se (*E. coli*): 99.8% Se (*K. oxytoca*): 92.2% Se (*K. pneumoniae*): 93.1% Se (*Proteus* spp.): 100% Se (*Enterobacter* spp.): 97.6% Se (*Citrobacter* spp.): 100% Se (*Acinetobacter* spp.): 98.2% Se (*Pseudomonas aeruginosa*): 97.6% Se (CTX-M): 98.7% Se (OXA): 95.3% Se (KPC): 100% Se (NDM): 100% Se (IMP): 100% Se (VIM): 100% Sp: 99.4–100%	False negatives can occur with mixed cultures *E. coli* is not distinguished from *Shigella* spp.

GeneMark Diagnostics, ePlex BCID-GP	Multiplexed PCR identification of multiple bacterial nucleic acids and select genetic determinants of antimicrobial resistance via competitive nucleic acid hybridization using a sandwich assay format	*Bacillus cereus* group, *B. subtilis* group, *Corynebacterium*, *Cutibacterium acnes*, *Enterococcus* spp., *E. faecalis*, *E. faecium*, *Lactobacillus*, *Listeria* spp., *L. monocytogenes*, *Micrococcus* spp., *Staphylococcus* spp., *S. aureus*, *S. lugdunensis*, Coagulase-negative staphylococci, *Streptococcus* spp., *S. agalactiae*, *S. anginosus*, *S. pneumoniae*, *S. pyogenes*, Pan Gram-negative, Pan *Candida*	Methicillin (mecA, mecC), vancomycin (vanA, vanB)	None	90 min	Se (*Bacillus cereus* group): 98.3% Se (*B. subtilis* group): 100% Se (*Corynebacterium*): 84.5% Se (*Cutibacterium acnes*): 93.5% Se (*Enterococcus* spp.): 97.6% Se (*E. faecalis*): 95.8% Se (*E. faecium*): 98.4% Se (*Lactobacillus*): 97.8% Se (*Listeria* spp.): 98.7% Se (*L. monocytogenes*): 100% Se (*Micrococcus* spp.): 93.0% Se (*Staphylococcus* spp.): 98% Se (*S. aureus*): 97.4% Se (*S. lugdunensis*): 100% Se (*Streptococcus* spp.): 97.4% Se (*S. agalactiae*): 96.4% Se (*S. anginosus*): 95.6% Se (*S. pneumoniae*): 95.7% Se (*S. pyogenes*): 98.1%	Relatively low sensitivity for *Corynebacterium* spp. Lower sensitivity for vanA detection in *E. faecalis* (88%)

(continued)

Table 1 (continued)

Manufacturer, assay	Methodology	Organisms identified	Direct genotypic resistance detection	Direct phenotypic resistance detection	Turnaround time	FDA Performance Data (in comparison with culture)	Caveats
						Se (Pan Candida): 92.3% Se (Pan Gram-negative): 95.6% Se (mecA): 97.2% Se (mecC): 100% Se (vanA): 96.8% Se (vanB): 100% Sp: 91.7–100%	
GeneMark Diagnostics, ePlex BCID-GN	Multiplexed PCR identification of multiple bacterial nucleic acids and select genetic determinants of antimicrobial resistance via competitive nucleic acid hybridization using a sandwich assay format	*Acinetobacter baumannii*, *Bacteroides fragilis*, *Citrobacter*, *Cronobacter sakazakii*, *Enterobacter* (non-cloacae complex), *E. cloacae*, *E. coli*, *Fusobacterium necrophorum*, *H. influenzae*, *K. oxytoca*, *K. pneumoniae*, *Morganella morganii*, *Neisseria meningitides*,	ESBL (CTX-M), carbapenem (KPC, IMP, NDM, OXA, VIM)	None	90 min	Panel not FDA cleared at time of publication	Panel not FDA cleared at time of publication

		Proteus spp., *P. mirabilis*, *P. aeruginosa*, *Salmonella*, *Serratia* spp., *S. marcescens*, *Stenotrophomonas maltophilia*, Pan Gram-positive, Pan *Candida*					
GeneMark Diagnostics, ePlex BCID-FP	Multiplexed PCR identification of multiple bacterial nucleic acids and select genetic determinants of antimicrobial resistance via competitive nucleic acid hybridization using a sandwich assay format.	*C. albicans*, *C. auris*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus neoformans*, *C. gatti*, *Fusarium*, *Rhodotorula*	None	None	90 min	Se (*C. albicans*): 97.1% Se (*C. auris*): 100% Se (*C. dubliniensis*): 100% Se (*C. famata*): 100% Se (*C. glabrata*): 98.3% Se (*C. guilliermondii*): 98.0% Se (*C. kefyr*): 100% Se (*C. krusei*): 100% Se (*C. lusitaniae*): 98.0% Se (*C. parapsilosis*): 98.3% Se (*C. tropicalis*): 100% Se (*Cryptococcus neoformans*): 100% Se (*C. gatti*): 100% Se (*Fusarium*): 98.6% Se (*Rhodotorula*):96.2% Sp: 99.8–100%	

(continued)

Table 1 (continued)

Manufacturer, assay	Methodology	Organisms identified	Direct genotypic resistance detection	Direct phenotypic resistance detection	Turnaround time	FDA Performance Data (in comparison with culture)	Caveats
Accelerate Diagnostics, Accelerate PhenoTest BC kit	FISH using PNA probes to organism-specific ribosomal RNA followed by antimicrobial susceptibility testing by time-lapse record of bacterial growth	*S. aureus*, *S. lugdunensis*, coagulase-negative staphylococci *E. faecalis*, *E. faecium*, *Streptococcus* spp., *P. aeruginosa*, *A. baumannii*, *Klebsiella* spp., *E. coli*, *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *S. marcescens*, *C. albicans* and *C. glabrata*	None	Yes – MICs provided	90 min (identification) 6.5 h (AST)	Se (*S. aureus*): 97.9% Se (*S. lugdunensis*): 97.5% Se (coagulase-negative staphylococci): 95.3% Se (*E. faecalis*): 97% Se (*E. faecium*): 98.3% Se (*Streptococcus* spp.): 97.2% Se (*P. aeruginosa*): *100%* Se (*A. baumannii*): 98.6% Se (*Klebsiella* spp.): 96.1% Se (*E. coli*): 97.3% Se (*Enterobacter* spp.): 97.3% Se (*Proteus* spp.): 97.7% Se (*Citrobacter* spp.): 96.8% Se (*S. marcescens*): 100% Se (*C. albicans*): 100% Se (*C. glabrata*): 100% Sp: 96–100%	False negatives can occur with mixed cultures *S. pneumoniae* not differentiated from other *Streptococcus* spp. No AST for *Candida* or *Streptococcus* spp. Overcalls resistance to ceftazidime and cefepime Too little data to determine sensitivity for daptomycin resistance

AST antimicrobial susceptibility testing; *ESBL* extended-spectrum beta-lactamase; *FDA* Food and Drug Administration; *FISH* fluorescence in situ hybridization; *MRSA* methicillin-resistant *S. aureus*; *MSSA* methicillin-sensitive *S. aureus*; *PCR* polymerase chain reaction; *PNA* protein nucleic acid; *RNA* ribonucleic acid; *SE* sensitivity; *SP* specificity

100%, but real-world incidence of false negatives (~0.5–1%) can be significant in a high-volume center and has the potential to lead to inappropriate narrowing of empiric antibiotics, so internal verification of the assay and ongoing surveillance for false negatives should be performed [17]. False negatives may occur with novel or rare MRSA strains whose resistance is based on variant resistance genes (such as *mecC*) and in cases of coinfection with MSSA and MRSA.

The FilmArray (bioMerieux, France, Biofire Diagnostics, USA) blood culture identification panel utilizes multiplex PCR to detect bacterial and yeast nucleic acids specific to *S. aureus*, Streptococci, *Enterococcus*, *Listeria*, Enterobacteriales, *Acinetobacter*, *Pseudomonas*, *Hemophilus influenzae*, and *Candida* species in a turnaround time of 1 hour . In previous iterations, only one sample could be processed at a time, but a new version of the instrument (FilmArray Torch) allows for much greater throughput. Select genetic determinants of antimicrobial resistance are provided including methicillin (*mecA*), vancomycin (*vanA/B*), and carbapenems (KPC). The assay delivers species identification for the five most clinically important *Candida* species. Species level identification for *Enterococcus* is not provided, and this genus may be misidentified as *Staphylococcus*. False-positive detection of the *mecA* gene in Staphylococci can occur.

The Verigene GP and GN assays (Luminex Corporation, USA) employ a microarray-based detection of specific nucleic acid targets whereby specific target sequences within the clinical sample bind to capture probes in defined spots on a microarray plate. Probe-target hybridization is detected and quantified by detection of gold-labeled targets to determine relative abundance of nucleic acid sequences in the target. The GP and GN assays are associated with turnaround times of 2.5 and 2 hours, respectively. Gram-positive organisms identified include *S. aureus* and coagulase-negative Staphylococci (including *S. lugdunensis*), Streptococci including *S. pneumonia*, *E. faecalis*, and *E. faecium*, and *Listeria* species. The GP assay also detects genetic markers of resistance including *mecA, vanA,* and *vanB*. Gram-negative organisms include several species of Enterobacteriacae, *P. aeruginosa*, and *Acinetobacter* species. The GN assay further detects genes associated with ESBL (CTX-M) and carbapenemase production (KPC, IMP, NDM, OXA, VIM). Notably, the assay does not distinguish *E.* coli from *Shigella* species.

The recently FDA-approved ePlex (GenMarkDx., Carlsbad, USA) blood culture identification (BCID) panels by GenMark Diagnostics provides a more expansive coverage of infecting pathogens than other commercial tests, with a similar turnaround time (90 min) and a considerable throughout capacity of 244 patient samples in a 24-hour period. The assay is based on nucleic acid amplification followed by competitive DNA hybridization and electrochemical detection. In addition to common Gram-positive pathogens, the BCID-GP panel includes *Bacillus*, *Lactobacillus*, *Cutibacterium acnes* (formerly *Propionibacterium acnes*), and *Corynebacterium* species. The Gram-negative panel (BCID-GN), which is undergoing FDA review at the time of this writing, includes an expanded array of organisms including anaerobes (*Fusobacterium*, *Bacteroides*), *Cronobacter*, and *Stenotrophomonas maltophilia*. The fungal pathogen panel (BCID-FP) includes an array of clinically important *Candida* species. This includes *C. auris*, as well as other clinically

important yeast (*Cryptococcus*, *Rhodotorula*) and molds (*Fusarium*) that are significant causes of disease in transplant populations. In addition to common resistance determinants, ePlex BCID-GP detects *mecC*, a novel *mecA* homologue encoding for a variant penicillin-binding protein (PBP2a). Real-world experience using the ePlex assays will be important in defining its utility and identifying potential caveats.

The Accelerate PhenoTest BC kit (Accelerate Diagnostics, Arizona, USA) represents the first FDA-approved rapid molecular test on blood that provides phenotypic resistance information in addition to organism identification directly from blood culture. The assay employs FISH-based technology for identification, which is obtained within 90 minutes. While FISH identification is underway, remaining clinical sample is combined with growth media in individual flow cells containing single concentrations of each test antibiotic. The cell morphology and light intensity of growing bacteria in each flow cell are imaged every 10 minutes for up to 4.5 hours, creating a time-lapse record of bacterial growth. Software algorithms then derive MIC values and apply appropriate interpretive criteria, delivering AST information approximately 5 hours after identifying the infecting pathogens. The array of organisms identified is comparable to some of the other genotypic methods, including the Verigene. The assay does not differentiate *S. pneumoniae* from other *Streptococcus* species. No AST is provided for *Streptococcus* or *Candida* species, and resistance to ceftazidime and cefepime may be overestimated. The assay achieved essential and categorical agreements of >95% in comparison with Vitek 2 (bioMerieux), a routine identification and AST method used in clinical laboratories in the United States [18].

For all the aforementioned assays, false negatives may occur in the setting of polymicrobial bacteremia, though the FilmArray and ePlex assays perform better in this setting, a Gram stain of blood culture broth must be used for confirmation.

Molecular Methods Performed on Blood Specimens

Molecular methods performed directly on whole blood specimens rather than on positive blood culture broth have several potential advantages. By eliminating the need for a growth phase, they allow for more rapid identification of bloodstream pathogens and are less susceptible to poor organism growth as with fastidious or unculturable organisms or growth inhibition from antimicrobials. Such methods also require lower volumes of blood compared to assays based on blood culture collection [19]. There are currently no FDA-approved molecular methods for direct BSI identification in the United States, but several are available in Europe and Asia. Among these, the LightCycler SeptiFast (Roche Molecular System, Basel, Switzerland) identifies 19 bacterial and 6 fungal pathogens directly from whole blood (1.5 mL sample) with a turnaround time of 6 hours. The assay is based on detection of ITS regions within ribosomal DNA via fluorescence resonance energy transfer probes. This method demonstrated higher positivity rates than blood culture in immunocompromised patients including organ transplant recipients but lower rates in normal hosts with endocarditis [20]. The SepsiTest (Molzym, Bremen, Germany)

identifies a vast array of pathogens including bacteria, fungi (including molds and yeast), and parasites (*Plasmodium* spp.) within 8–12 hours via broad range PCR of ribosomal DNA followed by sequencing. The assay achieved a sensitivity and specificity of 87.0 and 85.8%, respectively, in comparison to blood cultures in a multicenter study [21]. The Magicplex™ Sepsis Real-time Test (Seegene, Seoul, Korea) similarly targets a wide panel of pathogens as well as three resistance determinants (*vanA, vanB*, and *mecA*) and is applied directly to 1 mL of whole blood. The assay has not been well evaluated in transplant population but was poorly sensitive in comparison to blood culture in one study [22].

Methods that are directly applied to whole blood have several inherent disadvantages. First, diagnostic sensitivity is often suboptimal due to low bacterial concentration in whole blood and the low specimen volumes (~1 mL) processed by these assays as compared to blood culture (~10 mL). Second, high concentrations of human DNA and other blood constituents can act as interfering factors for PCR-based amplification, yielding false-negative results. Third, in the absence of growth amplification of live organisms, contaminating DNA or residual DNA from killed organisms may be preferentially amplified leading to false-positive results [19].

Impact of Rapid Molecular Diagnostics on Clinical Outcomes in Transplant Populations

For patients with bloodstream infections, the implementation of rapid molecular diagnostics on positive blood culture into standard microbiology practices reduces the time to targeted therapy as compared to routine microbiology techniques [23]. When combined with direct communication of results to treating clinicians and pharmacists, these assays are also associated with a significant reduction in mortality [24, 25]. Implementing rapid testing in conjunction with antimicrobial stewardship programs further reduces time to antimicrobial de-escalation, decreasing exposure to unnecessarily broad antibiotics [26]. This results in decreased selective pressure, a key driver of antimicrobial resistance, and reduces cost associated with antimicrobial treatment, lengths of hospitalization, and overall healthcare expenses [27]. Evidence for improved outcomes among transplant patients is limited as most studies either include this subpopulation as part of broader analyses or exclude them from stewardship interventions [23, 28]. However, since transplant patients are at increased risk for developing infections with MDR organisms as compared to other patient populations, they are at higher risk of receiving empiric therapy that does not have activity against highly resistant organisms. Transplant patients are also at higher risk for morbidity and mortality resulting from inadequate empiric therapy for bloodstream infections. Therefore, this population is conceivably more likely to benefit from the use of rapid molecular diagnostics than other groups [29]. Immunosuppressed patients are also more likely to develop infections with opportunistic pathogens encompassed within testing panels but for which traditional microbiologic identification is slow or suboptimal. This is particularly true for

bloodstream infections due to fungi, especially *Candida* species, which are more common in SOT and HSCT populations. Since conventional blood culture methods for candidemia are only 50% sensitive and *Candida* species often require an additional 24–48 hours to grow, the diagnosis is very often missed or delayed, with expected negative effects on clinical outcomes [30]. Several assays encompass targets for the most common clinically important *Candida* species, including *C. albicans* and *C. glabrata*, while newer assays now include *C. auris*, an emerging pathogen associated with high mortality in immunocompromised patients and for which microbial identification with traditional diagnostic methods is challenging [12, 31]. Rapid molecular tests on blood or positive blood culture bottles carry the potential to revolutionize the management of candidemia in transplant populations by improving the sensitivity of detection, reducing time to diagnosis and ultimately leveraging results to guide initiation and selection of appropriate antifungal therapy. Since rapid molecular tests were validated against blood cultures in FDA trial data, additional studies will be needed to determine if they can surpass the sensitivity of this "gold standard" and uncover the "missing 50% of invasive candidiasis" [30].

Magnetic Resonance-Based Methods

The T2MR platform (T2 Biosystems, Lexington, MA) detects genetic material directly from blood samples using PCR amplification of fungal and bacterial nucleic acid followed by measurement of T2 magnetic resonance emanating from DNA probe complexes [32]. The platform is a non-culture-based stand-alone device, validated for whole blood specimens, and provides results within a 4-hour time frame. At present, two panels (T2Candida and T2Bacteria) are commercially available for clinical use, while a T2Candida auris panel is currently designated as research use only (RUO). T2 assays represent significant progress in the ability to directly detect pathogens from blood without the need for culture and carry the potential to reduce the time to diagnosis of bloodstream infections, an important cause of morbidity and mortality in the transplant population.

The T2Candida panel detects five clinically significant *Candida* species, including *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* with the amplification step targeting internal transcribed spacer 2 (ITS2) rDNA. The assay does not provide resistance information other than susceptibility patterns inferred from species level identification. In a multicenter trial comparing detection of candidemia both from patients (including transplant recipients) with suspected candidemia as well as spiked samples, T2Candida achieved overall sensitivity and specificity of 91.1% and 99.4%, as well as a significantly reduced turnaround time for the final assay result compared to conventional blood cultures [33]. Discordant results included two samples identified by blood culture that were missed by the T2Candida panel as well as 29 samples labeled positive by the T2Candida assay which were not identified by conventional methods. Some of these cases were later shown to represent *Candida* infections through other methods of detection.

A subsequent study that included transplant recipients detected more episodes of candidemia as compared to conventional culture methods in patients on antifungals [34]. This system may have utility for early diagnosis, management, and antifungal stewardship in transplant populations at risk for *Candida* bloodstream infections, but prospective trials are needed to determine if rapid and enhanced diagnosis translates to reduced morbidity, mortality, or cost savings. The primary utility and cost savings of the T2MR platform may be related to antimicrobial stewardship.

Despite the significant theoretical advantages of the T2Candida assay, particularly in a transplant population with higher risk for candidemia, translating the reported diagnostic performance into real-world utility may be problematic for several reasons. First, though the initial validation study reported a sensitivity exceeding 90%, the true sensitivity is likely to be lower as a majority of samples included in the study (44%) were nonclinical, contrived specimens which contained a yeast concentration significantly higher than the mean concentration in initial blood cultures of candidemic patients (11–100 Candida CFU/mL vs. 1 CFU/mL) [33]. Even a small decrease in sensitivity and therefore in negative predictive value reduces the utility of the assay in guiding initiation or continuation of antifungals in transplant patients with a high-pretest probability for candidemia, rendering such an assay much less useful diagnostically. Second, in its current iteration, T2Candida does not detect several clinically important *Candida* species (such as *C. kefyr*, *C. lusitaniae*, *C. guilliermondii*, and *C. auris*), which comprise up to 10% of *Candida* infections in SOT patients [35]. Finally, in both clinical trials and real-world settings, the T2Candida has been susceptible to a high frequency of technical errors as well as indeterminate or invalid readings that require repeat testing and are disruptive to workflow.

T2Candida auris is currently a research-use-only assay that detects the emerging and potentially MDR organism, *Candida auris*. A validation study investigating the ability to detect *C. auris* from cutaneous sites demonstrated a sensitivity of 89% and a specificity of 98% in comparison to CDC methods [36]. Given the propensity of *C. auris* to persist on environmental surfaces, to lead to hospital-based outbreaks, and potentially to donor-derived transplant infections, this assay has significant implications for hospital epidemiology of transplant patients [11, 13].

The more recently FDA-approved assay for the T2MR system, T2Bacteria, can detect *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli.* No resistance information is provided. This assay was validated in a single-center study in comparison with conventional methods of bacterial identification from blood, achieving an overall sensitivity of 83.3% and specificity of 97.6%. Notably, the sensitivity and specificity varied by the pathogen detected, ranging from 50% with *K. pneumoniae* to 100% with *E. faecalis*, *P. aeruginosa*, and *S. aureus* [37]. As with molecular methods applied to whole blood and blood culture, the T2Bacteria panel carries the potential to shorten time to diagnosis of BSI and may prove highly useful in transplant populations, but data will be needed to determine if theoretical advantages translate into real-world benefit.

Metagenomic Sequencing

Metagenomic next-generation sequencing is an unbiased, hypothesis-independent approach to pathogen detection that is directly applicable to clinical samples. In contrast with PCR-based and 16S ribosomal RNA gene-based sequencing which target specific primers, mNGS is capable of sequencing all the nucleic acids in a clinical specimen and entire pathogen genomes. The majority of mNGS-based assays sequence only DNA, excluding organisms with RNA genomes. To address this, complementary DNA can be synthesized from total RNA. In addition to pathogen identification, mNGS has the potential to identify genetic determinants associated with phenotypic resistance [38–40]. Due to its capacity to identifying a multitude of pathogens in clinical samples, careful interpretation of data and clinical correlation is needed. Current mNGS platforms may vary with regard to sequencing technologies and software packages required for data analyses [41]. Metagenomic methods have been applied to blood samples in transplant patients. A prospective study of allogeneic HSCT recipients evaluated the value of a commercially available mNGS assay on plasma (Karius**,** Karius Inc., Menlo Park, California) obtained prior to conditioning, at time of graft infusion, weekly intervals after transplant, and at time of acute illness [42]. Plasma mNGS added diagnostic value in 3 of 20 cases, including the identification of uncommon presentation of *Chlamydia trachomatis* and of MRSA infection prior to diagnosis by blood cultures. In addition to bacterial pathogens, plasma mNGS detected CMV and EBV in concordance with standard PCRs. In a multicenter blinded prospective study of 101 immunocompromised patients including 22 SOT and 18 HSCT, blood samples tested within 30 days of enrollment by an mNGS assay identified more clinically relevant viruses and bacteria than standard testing (36/101 vs. 11/101) [43]. In this study, mNGS was associated with a high negative predictive value compared with conventional diagnostics (64/65, 95% CI 0.95-1). These findings suggest that negative mNGS results may have a role in antimicrobial and diagnostic stewardship.

Emerging Diagnostics for Respiratory Infections

In the evaluation of community-acquired pneumonia, 40–60% of cases remain undiagnosed despite extensive testing with traditional microbiologic tests [44]. Sputum cultures and blood cultures provide diagnostic information in only 15% of cases of community-acquired pneumonia, while bronchoscopy provides additive diagnostic value in 50% of cases. The diagnostic yield of microscopy with staining of induced sputum for *Pneumocystis jirovecii* pneumonia is around 50%, while that of serum galactomannan for *Aspergillus* infection can be as low as 30%, depending on the population. *Aspergillus* PCR assays, gas chromatography-mass spectrometry of exhaled metabolites, multiplex PCR, and mNGS have the potential to significantly improve diagnostic sensitivity for respiratory pathogens.

Aspergillus PCR

Aspergillus PCR for Diagnosis of Invasive Pulmonary Aspergillosis

Invasive pulmonary aspergillosis (IPA) is the most common fungal infection among HSCT recipients and one of the most common among SOT patients, particularly lung transplant recipients. Conventional methods for diagnosis of IPA include histopathology demonstrating septated hyphae and growth on culture followed by species identification. However, the diagnosis of aspergillosis can sometimes be challenging using these methods alone. Indeed, barriers to biopsy, such as severe thrombocytopenia, are common in this population. Additionally, culture yield is variable, especially with antecedent antifungals, and histopathological findings cannot reliably distinguish *Aspergillus* species from other hyaline molds. Galactomannan antigen testing on serum and bronchoalveolar lavage (BAL) fluid serves as an additional diagnostic tool and an important adjunct for diagnosis, but the sensitivity is suboptimal and significantly impacted by treatment with triazoles.

Molecular testing with PCR on blood or respiratory specimens is an emerging method to diagnose IPA. Multiple genetic targets have been investigated, including 18 s rRNA, 18 s rDNA, 5.8S rDNA, 28 s rRNA, mitochondrial DNA, and internal transcribed spacer (ITS) regions [45]. Use of PCR for routine diagnosis of IPA has not been uniformly accepted by society guidelines. Whereas guidelines proposed by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group in collaboration with the National Institute for Allergy and Infectious Diseases Mycosis Study Group (EORTC/MSG) advocate against PCR-based diagnosis given the lack of standardized testing procedures, more recent ESCMID-ECMM-ERS guidelines indicate that PCR should be considered in conjunction with other diagnostic testing [46, 47]. The 2016 Infectious Disease Society of America (IDSA) guidelines caution against the routine use of PCR without utilizing other standardized methods of diagnosis, but committee members differed in opinions regarding the utility of this platform with one group favoring use of PCR in conjunction with other standard methods and the other group indicating that lack of standardization among heterogeneous PCR methods needed to be addressed prior to being implemented for routine use [48].

Indeed, the primary challenge facing acceptance of PCR for the identification of *Aspergillus* species genetic material is assay standardization, which allows for uniform test performance and interpretation across institutions and in clinical trials. A Cochrane review of PCR methods for diagnosing *Aspergillus* species infection from blood demonstrated a mean sensitivity and specificity of 80.5% and 78.5%, respectively, when compared to EORTC/MSG recommendations for diagnosis as a reference standard [49]. Following publication of the initial EORTC/MSG guidelines, the European Aspergillus PCR Initiative (EAPCRI) was formed in an effort to progress toward increased standardization. Comparison of protocols from involved centers yielded specific recommendations for processing serum and whole blood samples [50, 51]. Notably, the DNA extraction protocol was identified as the most significant component of standardization. Utilizing standardized protocols, the

sensitivity and specificity of diagnosing invasive aspergillosis by PCR were found to be similar to that of *Aspergillus* galactomannan and beta-D-glucan assays at the time these assays were included in guideline recommendations [52]. Accordingly, future guidelines are likely to incorporate *Aspergillus* PCR as a routine tool for the diagnosis of invasive disease.

Though several reference laboratories in the United States offer *Aspergillus* PCR testing in the form of laboratory-developed tests (LDT), there are currently no FDA-approved assays. In recent years, several commercially available PCR assays for identification of *Aspergillus* species in blood or bronchoalveolar lavage have become available in Europe, including the MycAssay Aspergillus assay (Microgen Bioproducts Ltd., Cambridge, United Kingdom), the AsperGenius assay (PathoNostics, Maastricht, Netherlands), the MycoGENIE assay (Ademtech, Pessac, France), the SeptiFast assay (Roche Molecular System, Basel, Switzerland), and the RenDx Fungiplex assay (Renishaw Diagnostics Ltd., Glasgow, United Kingdom) (Table 2). These PCR assays incorporate internal standardization protocols validated by the manufacturer, allowing a degree of reproducibility across institutions. Many kits are validated on multiple real-time PCR-capable instruments, permitting a given laboratory to utilize the assay on existing devices.

The AsperGenius assay, a real-time PCR targeting the 18S rDNA, detects *A. fumigatus* complex, *A. terreus*, and other *Aspergillus* species (*A. flavus* and *A. niger*). The assay was initially evaluated on BAL samples in comparison with positive galactomannan samples in patients with suspected invasive aspergillosis. The sensitivity and specificity ranged from 80% to 88.9% and 89.3% to 93.3%, respectively, depending on the patient population studied, with a higher sensitivity in leukemic and HSCT patients [53]. A larger retrospective study involving five European hospitals evaluated the performance of the AsperGenius assay on BAL in comparison with galactomannan and culture data in patients primarily with hematologic malignancies and HSCT and found a sensitivity and specificity of 80% and 84%, respectively [54]. This assay has also been applied to serum, suggesting broader potential application [55, 56].

The MycoGENIE assay (Ademtech, France) identifies presence of *Aspergillus fumigatus* by targeting 28 s rRNA in clinical samples. This assay was validated using culture-positive bronchoalveolar lavage samples with a sensitivity of 92.9% and a specificity of 90.1% [57]. However, the sensitivity on BAL was only 40% for proven or probable IPA in a retrospective study from a majority of patients status-post HSCT. An increase in sensitivity and specificity was observed when the assay was combined with galactomannan antigen testing [58].

The MycAssay (Myconostica, Lab21, Manchester, UK), an early commercial kit detecting 15 *Aspergillus* species, targets 18 s rRNA. Validation studies used samples from patients primarily with hematologic malignancies and compared the assay to galactomannan and institution-derived PCR testing. Initial evaluation using samples spiked with *Aspergillus fumigatus* and samples from patients with possible/probable IPA demonstrated similar performance to an institutionally developed PCR panel [59]. However, subsequent investigations found a lower sensitivity and specificity for diagnosis of invasive aspergillosis as compared to galactomannan antigen testing

Table 2 Commercially available PCR assays and platforms for *Aspergillus* species identification

Assay and manufacturer	Methodology	Target	Species identified/ readout	Resistance mutations identified	Tested specimens and performance
AsperGenius (PathoNostics)	Real-time multiplex PCR kit	28 s rDNA; CYP51A	*A. fumigatus*, *A. terreus*, *Aspergillus* spp.	*CYP51A* mutations: 1) L98H 2) Tandem repeat 34 3) T289A 4) Y121F	BAL 80.0–88.9% sensitive, 84.0–93.3% specific per patient population; compared against BAL galactomannan and/or culture [53, 54] Serum 78.6% sensitive, 100% specific for serum samples of patients with proven/probable/ possible disease [55] Plasma 80% sensitive, 77.8% specific for plasma samples of patients with proven/probable/ possible disease [56]
A. fumigatus Bio-Evolution (Bio-Evolution)	Real time PCR kit	ITS1 rDNA	*A. fumigatus*	None	BAL 100% sensitive, 81% specific for BAL samples of patients with probable/possible disease [62]
MycoGENIE (Ademtech)	Real-time multiplex PCR kit	28 s rRNA; CYP51A	*A. fumigatus*, *Aspergillus* sp.	CYP51A mutations 1) Tandem repeat 34 2) L98H	BAL 100% sensitive, 71% specific for BAL samples of patients with probable/possible disease [62] 40% sensitive, 69% specific for BAL samples of patients with proven/probable disease [58]

(continued)

Table 2 (continued)

Assay and manufacturer	Methodology	Target	Species identified/ readout	Resistance mutations identified	Tested specimens and performance
					Serum 100% sensitive, 84.6% specific for serum samples of patients with proven/probable disease [57]
MycAssay Aspergillus (Myconostica/ Lab21)	Real-time PCR kit	18 s rRNA	*Aspergillus* spp.	None	BAL 86.7–93% sensitive, 82.4–87.6% specific (range dependent on number of samples tested) for BAL samples of patients with probable disease [99] 60–70% sensitive, 90.5–100% specific for BAL samples of patients with proven/probable disease [59] 94.1% sensitive, 98.6% specific for BAL samples of patients with proven/probable disease [61] Serum 43.8% sensitive, 63.2% specific for serum samples of patients with proven/probable/ possible disease [60] 65.4% sensitive, 57.8% specific for serum of patients with proven/probable disease [100] Biopsy

(continued)

Table 2 (continued)

Assay and manufacturer	Methodology	Target	Species identified/ readout	Resistance mutations identified	Tested specimens and performance
					82–90% sensitive, 64–79% specific for biopsy specimens as compared to microscopy and culture based diagnosis [101]
PLEX-ID Broad Fungal (Abbott)	PCR with electrospray ionization mass spectrometry; PLEX-ID platform	mtSSU rRNA, proprietary primers	*A. fumigatus, A. flavus, A. nidulans, A. niger, A. terreus* Other non-*Aspergillus* species detected as well	None	Respiratory samples Correctly identified of 11 of 12 standard samples and 6 of 8 samples from respiratory samples [102] No studies conducted to evaluating sensitivity and specificity
RenDx Fungiplex (Renishaw)	PCR with surface enhanced resonance Raman scattering spectroscopy	18 s rRNA, 28 s rRNA	*A. fumigatus A. terreus, A. niger, A. flavus* Other non-*Aspergillus* species detected as well	None	Serum/plasma 81.3% sensitive, 87.5% specific for serum/plasma specimens of patients with proven/probable/ possible disease [66]
SeptiFast (Roche)	Real-time multiplex PCR; Roche LightCycler platform	ITS rDNA	*A. fumigatus* Other non-*Aspergillus* species detected as well	None	No studies conducted to evaluating sensitivity and specificity for detection of *A. fumigatus*. Variability is reported between detection *A. fumigatus* with SeptiFast and comparative assays

Abbreviations: *BAL* bronchoalveolar lavage; *mtSSU rRNA* mitochondrial short subunit ribosomal ribonucleic acid; *ITS* internal transcribed spacer; *PCR* polymerase chain reaction; *rDNA* ribosomal deoxyribonucleic acid; *rRNA* ribosomal ribonucleic acid

[60, 61]. The MycoGENIE assay demonstrated similar performance to the *A. fumigatus* Bio-Evolution assay (Bio-Evolution, France) in a retrospective analysis using BAL samples [62].

The Roche SeptiFast assay is based on real-time multiplex PCR using the Roche LightCycler platform and is capable of detecting multiple bacterial and fungal pathogens, including *A. fumigatus.* This assay has been investigated against blood culture, individual laboratory-developed PCR assays, and galactomannan testing in patients with hematologic malignancy and neutropenic fever, some of whom were recipients of or being conditioned for HSCT [63, 64]. Positive results for *A. fumigatus* were reported in these studies, but positive results were rarer than for bacterial pathogens or *Candida* species, reflecting either lower prevalence or lower sensitivity for aspergillosis. Notably, this platform was also found to support the diagnosis of invasive aspergillosis in two patients with hepatic transplantation [65]. A primary limitation is the ability to only detect *A. fumigatus*, whereas patients with SOT or HSCT are at an increased risk for other *Aspergillus* species as well.

The RenDx Fungiplex assay (Renishaw, United Kingdom) utilizes PCR and surface-enhanced resonance Raman scattering spectroscopy to detect amplified DNA. The assay was developed to detect ten fungal organisms, including *A. fumigatus* and *A. terreus*, and demonstrated a sensitivity of 85.7% when detecting *Aspergillus* species from blood in a group of patients including recipients of HSCT [66].

In a review of published literature, the sensitivities and specificities of aforementioned *Aspergillus* PCR assays were overall comparable but consistently higher in BAL than in serum and likely lower sensitivity in non-hematologic patients as well as in those on antifungal treatment or prophylaxis [67]. Active investigation is ongoing to determine the feasibility of incorporating these and other developing platforms into the difficult process of diagnosing invasive aspergillosis. At present they do not represent stand-alone diagnostic methods, and results must be evaluated in the context of the clinical presentation and all other available data.

Aspergillus PCR for Diagnosis of Resistance in *Aspergillus* Species

The need for improved diagnostics is further highlighted by the increasing prevalence of azole-resistant *Aspergillus* species worldwide [68]. The first azole-resistant *Aspergillus fumigatus* clinical isolates were reported in the United States and the Netherlands in 1997 and 1998 [69, 70]. Since then, the prevalence of resistance has risen to 10–15% in some areas of Europe. At this time, resistance appears to be uncommon in the United States, with surveys of clinical isolates reporting a resistance prevalence of less than 1.8% [58, 71]. Mutations in the CYP51A (ERG11) gene encoding sterol demethylase are primarily responsible for resistance seen in clinical *A. fumigatus* isolates. Resistance develops in the setting of selective pressure from long-term and widespread azole use such as with environmental application of triazoles as fungicides in mass agriculture and less commonly with conditions favoring active reproduction in vivo such as with long-term treatment of patients

with aspergilloma. Some CYP51A mutations are associated with minimal inhibitory concentrations (MIC) that exceed the epidemiologic cutoff value (ECV) of ≤ 1 μg/mL for azoles established by the Clinical & Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) by multiple dilutions. The presence of such mutations has been linked to treatment failure and increased mortality in patients with IPA [15].

Among the aforementioned *Aspergillus* PCR assays, the AsperGenius and the MycoGENIE assays have the added capability of detecting azole resistance based on the presence of *CYP51A* mutations. The MycoGENIE assay detects two resistance-associated mutations in *CYP51A*, whereas the AsperGenius assay identifies four *CYP51A* point mutations representing prevalent mutation types. In a retrospective study, samples with positive results for resistance-associated mutations using this assay were associated with significantly poorer patient outcomes [54].

Given the low prevalence of azole-resistant *Aspergillus* isolates in the United States, current IDSA guidelines do not recommend routine susceptibility testing. Susceptibility testing was suggested in cases of suspected azole resistance (without further specifications on when to suspect), lack of response to normally effective antifungals, or for epidemiologic purposes. With ongoing widespread clinical and industry use of triazoles in the United States, the prevalence of azole resistance is likely to increase, leading to a greater need for resistance detection.

Gas Chromatography-Mass Spectrometry of Exhaled Metabolites

Molds produce organic compounds during routine metabolism, many of which are released as volatile metabolites during exhalation. Monoterpenes and sesquiterpenes, the primary metabolites produced by *Aspergillus* species, can be detected using breath-based diagnostics to establish a diagnosis of invasive aspergillosis. The first report of this method involved recipients of SOT and HSCT with proven or suspected IPA [72]. The study compared volatile organic compounds produced in vitro with those detected from patient breath samples using gas chromatography-mass spectrometry. Sesquiterpene compounds were detected from varying in vitro culture conditions as well as in breath samples. Breath-based detection of volatile organic compounds achieved a sensitivity of 94% and specificity of 93% for identifying patients with IPA as compared to clinical diagnosis by EORTC/MSG criteria. Some sesquiterpene metabolites were only identified in the in vivo breath samples corresponding to activation of alternate metabolic pathways in the context of an immune response and lung microbiome [73]. Notably, sesquiterpenes are produced in a species-specific manner by *Aspergillus* species, potentially allowing diagnostic discrimination beyond the genus level. Development of this method for clinical diagnosis has many challenges involving consistent breath collection, which can be affected by multiple factors including age, race, respiratory rate, and tidal volumes among others, and standardization of analysis methods used to identify the volatile organic compounds [73]. However, this platform has the potential to significantly improve the diagnostic yield for IPA and could be expanded to other invasive

fungal infections, particularly those due to Mucorales species for which few targeted diagnostics are available.

Multiplex PCR

Respiratory multiplex PCR can be performed on nasopharyngeal swab samples, nasal washes/aspirates, and BAL specimens. There are currently five FDA-approved respiratory multiplex assays, including NxTAG Respiratory Pathogen Panel (RPP) (Luminex, TX, USA), Verigene Respiratory (RP) Flex (Nanosphere, IL, USA), FilmArray Respiratory Panel (RP) (BioFire Diagnostics, UT, USA), eSensor Respiratory Viral Panel (RVP) (Genmark, CA, USA), and, the recently approved, Unyvero Lower Respiratory (Curetis, CA, USA) (Table 3). The former four panels include predominantly viral targets, while Unyvero also detects bacterial pathogens and genes associated with antimicrobial resistance (Table 4) [74]. Such assays have the advantage of providing earlier diagnoses than culture- or immunofluorescence-based methods, as well as the potential to detect multiple coinfecting pathogens with early institution of appropriate therapy and enforcement of isolation practices, measures that are especially critical for the care of immunocompromised hosts. However, studies of these assays among immunocompromised patients are limited. The FilmArray RP was evaluated in 87 immunocompromised patients (48% HSCT, 37% SOT and 18% with hematologic malignancies) using either BAL or nasopharyngeal samples [75]. In this cohort, only viral pathogens were detected. The positive and negative predictive values were 86% and 100%, respectively. Unyvero was evaluated in BAL samples of 522 immunocompromised patients with suspected lower respiratory tract infections [76]. Compared to conventional methods, the assay result was more expedient (available within 5 hours), but sensitivity was poor at 68.4% (specificity was 86%), notably failing to identify cases of *Streptococcus pneumoniae* infection.

Metagenomic Sequencing

In patients without a microbiologic diagnosis using conventional testing, mNGS may have a role in providing an early and definitive diagnosis, which is especially important in lung transplant recipients for whom respiratory infection is associated with allograft dysfunction and mortality. Using a laboratory-developed mNGS assay in a cohort of lung transplant recipients with suspected respiratory infection, Lewdowonska et al. [77] identified respiratory viral pathogens in only 5 of 29 cases (Table 5). Langelier et al. [78] enrolled 22 HSCT recipients with respiratory illness whose BAL was tested by standard microbiologic testing and mNGS. Standard microbiologic testing identified clinically relevant pathogens (as determined by the treating physician) in six cases, all of which were concordant with mNGS. Conversely, six other cases identified by mNGS were missed by conventional testing. Studies of mNGS for the diagnosis of fungal infections in transplant recipients are limited. Li et al. evaluated 20 patients by lung biopsy, 5 of which were

Table 3 Respiratory multiplex PCR assays

	FilmArray RP	NxTAG RPP	Verigene RP Flex	eSensor RVP	Unyvero lower respiratory
Viruses					
Adenovirus (ADV)	X	X	X	X (ADV B/E/C)	
Coronavirus HKU1	X	X			
Coronavirus 229E	X	X			
Coronavirus OC43	X	X			
Coronavirus NL63	X	X			
Human metapneumovirus	X	X	X	X	
Human rhinovirus/ enterovirus	X	X	X	X	
Influenza A	X	X	X	X	
Influenza A/H1	X	X	X	X	
Influenza A/H3	X	X	X	X	
Influenza A/H1–2009	X			X	
Influenza B	X	X	X	X	
Parainfluenza viruses 1,2,3,4	X	X	X	X	
Respiratory syncytial virus	X	X (RSV A/B)	X (RSV A/ B)	X (RSV A/B)	
Bocavirus		X			
Gram-positive bacteria					
Staphylococcus aureus					X
Streptococcus pneumoniae					X
Enterobacteriaceae					
Citrobacter freundii					X
Escherichia coli					X
Enterobacter cloacae complex					X
Enterobacter aerogenes					X
Proteus spp.					X
Klebsiella oxytoca					X
Klebsiella variicola					X
Serratia marcescens					X
Morganella morganii					X
Non-fermenting bacteria					
Moraxella catarrhalis					X

(continued)

Table 3 (continued)

	FilmArray RP	NxTAG RPP	Verigene RP Flex	eSensor RVP	Unyvero lower respiratory
Pseudomonas aeruginosa					X
Acinetobacter baumannii complex					X
Stenotrophomonas maltophilia					X
Legionella pneumophila					X
Others/fungi					
Chlamydophila pneumoniae	X				X
Mycoplasma pneumoniae	X	X			X
Haemophilus influenzae					X
Pneumocystis jirovecii					X
Bordetella parapertussis	X		X		
Bordetella pertussis	X		X		
Bordetella holmesii			X		

Table 4 Unyvero resistance gene panel

Resistance	Gene
Carbapenem	KPC
	NDM
	OXA-23
	OXA-24
	OXA-48
	OXA-58
	VIM
Third-generation cephalosporins	CTX-M
Oxacillin/cefoxitin	mecA
Penicillin	TEM

allogeneic HSCT recipients [79]. Histopathology demonstrated fungal elements in 5/5, while fungal culture was only positive in 2/5. mNGS identified fungal species as follows: *Rhizopus microsporus* (2), *Mucor racemosus* (2), and *Rhizopus oryzae* (1). Although available studies are small and require validation in larger cohort, they emphasize the diagnostic potential of mNGS in comparison with standard diagnostics. In addition to pathogen identification, one study stressed the potential role of mNGS in the identification of genetic determinants related to host immune response of immunocompromised patients with confirmed lower respiratory infection [78].

Table 5 Utility of metagenomic next-generation sequencing (mNGS) on respiratory specimens for diagnosis of pneumonia in SOT and HSCT recipients

Source	No of cases	Transplant	Clinical presentation	Clinical specimen	Pathogen(s)
Lewandowska et al. [77]	29	Lung transplant	Respiratory illness	Throat swab (27) BAL (2)	*Respiratory pathogens* Rhinovirus A and B (4/29) Human coronavirus (1/29) *Non-respiratory pathogens* Human herpes virus-7 (18/29) Torque teno virus (9/29)
Langelier et al. [78]	22	HSCT	Acute respiratory illness	BAL	6/22 cases identified by conventional and mNGS 6/22 cases were novel pathogens identified by mNGS 10/22 cases identified by mNGS of uncertain or unlikely pathogenicity
Li et al. [79]	5	Allogeneic HSCT	Lower respiratory infection	Lung tissue	Fungal infection diagnosed in 5/5 with mNGS

BAL bronchoalveolar lavage; *HSCT* hematopoietic stem cell transplantation; *SOT* solid organ transplantation

Explify™ Respiratory (IDbyDNA, San Francisco, USA) is a commercially available mNGS assay validated for BAL and detects hundreds of bacterial, fungal, and viral respiratory pathogens in a single run with result turnaround time of 2–3 days. In a study among immunocompromised children with suspected pneumonia (42% of which had underlying hematologic malignancies), mNGS testing demonstrated high agreement with conventional microbiologic tests (90.2% for bacterial, 94.1% for virus, 66.7% for fungal detection). The assay also identified putative and possible infectious causes in 44% of children with suspected respiratory infections whose conventional testing was negative [80].

Emerging Diagnostics for CNS Infections

Identification of the causative agent of encephalitis and differentiation between infectious and noninfectious processes are critical in delineating the treatment approach for transplant recipients and potential deceased donors with encephalitis/meningitis syndromes. The conventional diagnostic approach to encephalitis consists of testing

tailored to a patient's clinical presentation and epidemiologic exposures including targeted antigen-based, PCR, and serologic assays. Despite extensive concurrent and sequential testing, the underlying pathogen remains unclear in up to 60% of encephalitis cases [81]. Novel diagnostics for CNS infections, on the other hand, have the capacity to test for dozens or even hundreds of pathogens simultaneously. These include targeted multiplexed PCR assays and mNGS.

Multiplex PCR

Multiplex PCR assays that include several viral, bacterial, and fungal targets are now available. The FilmArray meningitis/encephalitis (ME) panel (Biofire Diagnostics) tests for six bacterial (*E. coli K1*, *H. influenzae*, *L. monocytogenes*, *N. meningitides*, *S. pneumoniae*, *S. agalactiae*), seven viral (HSV1-2, VZV, CMV, HHV6, *Enterovirus*, human parechovirus), and two fungal pathogens (*C. neoformans* and *C. gattii*) on cerebrospinal fluid (CSF) with a turnaround time of 1 h. In a multicenter study of 1560 prospectively collected CSF samples utilizing FilmArray ME panel versus conventional techniques (i.e., culture for bacteria and monoplex PCR for other agents [82], the FilmArray ME panel had a sensitivity of 100% for 9/14 pathogens (*E. coli* K1, *H. influenzae*, *S. pneumoniae*, CMV, HSV-1, HSV-2, human parechovirus, VZV, *Cryptococcus neoformans/gattii*). Enterovirus and HHV-6 had an agreement of 95.7% and 85.7%, respectively. The specificity was $\geq$99.2% for all 14 pathogens. This study did not include immunocompromised hosts, but other studies include limited data for this population. Chang et al. evaluated the ME panel in hospitalized patients, which included eight immunocompromised patients (HIV/AIDS, steroid use, active chemotherapy, transplant). The ME panel detected HHV-6 in an HSCT recipient with symptoms of encephalitis, while the concurrent HHV-6 PCR was negative [83]. There are emerging data that the sensitivity of ME panel for *Cryptococcus* is relatively poor compared to conventional testing. The ME panel failed to detect *Cryptococcus* in CSF twice, resulting in delayed diagnosis in a kidney transplant recipient [84]. The diagnosis was later confirmed by the conventional cryptococcal antigen lateral flow assay. Another study retrospectively evaluated patients who underwent CSF or serum testing for *Cryptococcus* with ME panel CSF culture and antigen testing [85]. Twelve of the 14 cases who met inclusion criteria were HIV-positive. Of the 14 cases with positive CSF cryptococcal antigens, 5 had positive ME and culture. Among the nine cases with negative ME, two had positive culture, and seven had negative culture. The sensitivity was calculated at 71.4% while specificity was 100%. Based on these two reports, a negative ME result for *Cryptococcus* does not rule out cryptococcal meningitis, especially in at-risk patients.

Metagenomic Sequencing

mNGS carries the potential for hypothesis-independent testing of hundreds of pathogens simultaneously and may be particularly useful when applied to normally

sterile specimens such as CSF. The value of mNGS in the diagnosis of encephalitis in SOT and HSCT recipients has already been demonstrated (Table 6). In a review of the current literature, 8 of 17 cases diagnosed with mNGS involved novel pathogens including arenavirus [3/17], astrovirus [4/17], and human betacoronavirus [1/17]. Unexpected etiologies such as *Candida* and Hepatitis E encephalitis were found in two cases. Donor-derived transmission of tick-borne virus encephalitis [86] and possibly due to arenavirus [87] was also reported. These cases illustrate the potential use of mNGS in diagnosing meningoencephalitis of unclear etiology in potential organ donors, a high-risk category that normally precludes utilization of organs due to concern for transmission of life-threatening infectious pathogens not detected by conventional testing.

Emerging Diagnostics for Gastrointestinal Infections

Diarrheal disease is associated with significant morbidity and mortality for immunocompromised hosts. In SOT recipients, diarrhea is associated with poorer graft survival and increased risk for death [88]. Establishing a diagnosis early and accurately allows providers to distinguish infectious from noninfectious causes including immunosuppressive drug-related (e.g., mycophenolate) or graft-versus-host disease-related diarrhea, which require special intervention. In comparison with conventional testing, which includes stool culture, microscopy, and antigen testing, molecular assays can identify multiple pathogens simultaneously with shorter turnaround times.

Multiplex PCR

There are currently three FDA-approved multiplex tests for gastrointestinal pathogens: FilmArray Gastrointestinal Panel (Biofire Diagnostics, Utah, USA), xTag Gastrointestinal Pathogens Panel (GPP) (Luminex, Texas, USA), and Verigene Enteric Pathogens (Nanosphere, Illinois, USA) (Table 7). Studies in immunocompromised patients with gastrointestinal illness are limited. Mengelle et al. evaluated xTag GPP in 102 adult organ transplant recipients with diarrhea [89]. Twenty-five of 147 samples (17%) collected were positive for at least one pathogen. Toxigenic *C. difficile*, norovirus, and *Salmonella* spp. were detected in 7.5%, 4.1%, and 2.7%, respectively. Coste et al. evaluated xTAG GPP in 49 adult kidney transplant recipients with 54 severe diarrhea events [90]. Among 39 positive samples, multiplex PCR identified multiple infections in 15 (38%), providing additional diagnoses compared to traditional tests. The most common pathogens identified by multiple PCR were enteropathogenic *E. coli* (15/39, 38%), *Campylobacter* spp. (15/39, 38%), and norovirus (14/39, 36%). Despite the potential for additional diagnoses, there are drawbacks to increased sensitivity. Since nucleic acids of viable and nonviable pathogens including free DNA/RNA is not distinguished [91], disease, asymptomatic carriage, or shedding (particularly with certain viruses) can be confounded. This

Table 6 Utility of metagenomic next-generation sequencing (mNGS) on CSF for diagnosis of encephalitis in SOT and HSCT recipients

Source	Case	Age (years)	Transplant type	Clinical presentation	Clinical specimen	Pathogen	Clinical outcome
Palacios et al. [87]	1	63	Kidney transplant	Fever, encephalopathy, sepsis	Brain biopsy, CSF, serum, kidney, liver	Arenavirus	Died
Palacios et al. [87]	2	64	Liver transplant	Fever, encephalopathy, myoclonus	Brain biopsy, CSF, serum, kidney and liver	Arenavirus	Died
Palacios et al. [87]	3	44	Kidney transplant	Fever, encephalopathy	Brain biopsy, CSF, kidney and liver	Arenavirus	Died
Wilson et al. [103]	4	14	Allogeneic HSCT (twice), SCID	Fever, headache, myalgia, status epilepticus	CSF and serum	*Leptospira santarosai*	Survived
Brown et al. [104]	5	1.5	PBSCT	Irritability, dystonia, encephalopathy	Brain biopsy	Astrovirus	Died
Naccache et al. [105]	6	42	Allogeneic HSCT	Sensorineural deafness, tinnitus, encephalopathy	Brain biopsy, CSF	Astrovirus	Died
Christopeit et al. [106]	7	65	Allogeneic HSCT	Encephalopathy, sepsis	CSF	*Candida tropicalis*	Died
Lum et al. [107]	8	4 months	Allogeneic HSCT	Encephalopathy, dystonia, respiratory failure	Post-mortem brain biopsy	Astrovirus	Died
Salzberg et al. [108]	9	67	SOT (did not specify organ)	Facial paralysis	Brain	EBV	Unknown
Sato et al. [109]	10	4	Cord HSCT	Fever, seizure, recurrent diarrhea	CSF, serum, throat swab, stool, urine	Astrovirus	Survived

Morfopoulou et al. [110]	11	11 months	Cord HSCT	Encephalopathy	Brain biopsy	Human betacoronavirus	Died
Wilson et al. [111]	12	14	Kidney transplant	Fever, myalgia, rash, encephalopathy, language impairment, urinary retention	CSF	West Nile Virus	Survived
Murkey et al. [112]	13	58	Lung transplant	Fever, headache, photophobia, neck stiffness	CSF	Hepatitis E (possible donor-derived transmission)	Survived
Morfopoulou et al. [113]	14	1.6	Allogeneic HSCT	Fever, rash, encephalopathy, seizure	Brain	Vaccine- related mumps virus	Died
Lipowski et al. [86]	15	27	Kidney transplant	Fever, headache, vertigo, nystagmus, dysarthria, aphasia, cranial nerve III and IV palsy	CSF, postmortem brain biopsy, serum	TBEV – donor-derived transmission	Died
Lipowski et al. [86]	16	54	Liver transplant	Fever, headache, dysarthria, dysphagia, tetraplegia, progressed to coma	CSF, postmortem brain biopsy, serum	TBEV – donor-derived transmission	Died
Lipowski et al. [86]	17	48	Kidney transplant	Fever, headache, lower extremity weakness, nystagmus, progressed to coma	CSF, serum	TBEV – donor-derived transmission	Died
Lipowski et al. [86]	18	44/donor	N/A	N/A	Postmortem brain biopsy	TBEV	Died due to MVA

Abbreviations: *CSF* cerebrospinal fluid; *PBSCT* peripheral blood stem cell transplant; *SCID* severe combined immunodeficiency; *HSCT* hematopoietic stem cell transplant; *MVA* motor vehicle accident; *TBEV* tick-borne encephalitis virus

Table 7 Gastrointestinal multiplex PCR assays

	FilmArray GI	*xTAG GPP*	*Verigene EP*
Virus			
Adenovirus 40/41	X	X	X
Norovirus genogroups I and II	X	X	X
Rotavirus A	X	X	X
Sapovirus	X		
Astrovirus	X		
Bacteria			
Campylobacter	X	X	
Clostridiodes difficile (toxin A/B)	X	X	
Plesiomonas shigelloides	X		
Salmonella	X	X	X
Yersinia enterocolitica	X		X
Vibrio spp.	X	X	X
Enteroaggregative *E. coli*	X		
Enteropathogenic *E. coli*	X		
Enterotoxigenic *E. coli*	X	X	
Shiga-like toxin producing *E. coli*	X	X	X
E. coli 0157	X	X	
Enteroinvasive *E. coli/Shigella*		X	X
Parasite			
Cryptosporidium	X	X	
Cyclospora cayetanensis	X		
Entamoeba histolytica	X	X	
Giardia lamblia	X	X	

includes detection of *C. difficile* [92–94] which may represent colonization rather than infection. Detection of more than one pathogen [95], though sometimes useful, may render result interpretation difficult. As opposed to culture techniques, multiplex PCR assays do not provide antimicrobial susceptibility, information that is essential for antimicrobial stewardship and epidemiologic monitoring. Notably, GI panels that include adenovirus such as GPP and FilmArray assays only target adenovirus types F40/41. However, other adenovirus types especially within subgroup C cause most infections among transplant patients, so these assays do not rule out adenovirus gastroenteritis [96].

Conclusion

In the past decade, several novel diagnostic modalities have emerged with the potential to revolutionize the diagnosis of infectious diseases in transplant patients in a way comparable to the effect of MALDI-TOF MS on general microbiological diagnostics. Though significant strides have been made, most modalities continue

to have certain drawbacks that limit their applicability. Molecular methods applicable directly to blood samples for the diagnosis of BSI benefit from very short processing times but suffer from lower sensitivity due to circumvention of a growth phase, while assays performed on positive blood cultures are generally more sensitive except for fastidious or unculturable organisms. T2 magnetic resonance-based testing is rapid and highly sensitive, but translating the reported diagnostic performance into real-world utility has been problematic. mNGS has significant potential for rapid and broad detection of pathogens in multiple specimen types, but current methods remain less sensitive than targeted PCR tests that are based on amplification [97], and cost of commercial mNGS assays is prohibitive. Breath-based diagnosis has been shown to improve the diagnosis of invasive aspergillosis, yet there remain important challenges to its application. Despite their shortcomings, emerging technologies have already demonstrated a diagnostic advantage as well as a positive impact on patient outcomes [98]. Though data on test performance characteristics and clinical impact among transplant patients remains limited, it is likely that these modalities will translate into even greater utility given the increased risk for infection and propensity for multiple and uncommon pathogens in this population.

Key Points

- Transplant patients are at increased risk for community-acquired and opportunistic pathogens, multiple pathogens simultaneously, and multidrug-resistant organisms.
- Diagnostic microbiology has witnessed a shift toward culture-independent methods in the past decade.
- Molecular methods applicable directly to blood samples for the diagnosis of BSI benefit from very short processing times but suffer from lower sensitivity due to circumvention of a growth phase.
- Molecular assays performed on positive blood cultures are more sensitive except for fastidious or unculturable organisms.
- T2 magnetic resonance-based testing is rapid and highly sensitive, but translating the reported diagnostic performance into real-world utility has been problematic.
- Metagenomic next-generation sequencing has massive potential for rapid and broad detection of pathogens in multiple specimen types, but current methods remain less sensitive than targeted PCR tests.
- Breath-based diagnosis has been shown to improve the diagnosis of invasive aspergillosis, yet there remain important challenges to its application.
- Despite shortcomings, emerging technologies have already demonstrated a diagnostic advantage as a well as a positive impact on patient outcomes.
- Though data on test performance characteristics and clinical impact among transplant patients remains scarce, it is likely that these modalities will translate into even greater utility in this population.

References

1. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem. 2014;6:25–64.
2. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34(6):1589–96.
3. Viehman JA, Clancy CJ, Clarke L, Shields RK, Silveira FP, Kwak EJ, et al. Surgical site infections after liver transplantation: emergence of multidrug-resistant Bacteria and implications for prophylaxis and treatment strategies. Transplantation. 2016;100(10):2107–14.
4. Camargo JF. Donor-derived infections in solid organ transplant recipients: challenging the 30-day paradigm. Transpl Infect Dis. 2017;19(2):e12836.
5. Lanini S, Costa AN, Puro V, Procaccio F, Grossi PA, Vespasiano F, et al. Incidence of carbapenem-resistant gram negatives in Italian transplant recipients: a nationwide surveillance study. PLoS One. 2015;10(4):e0123706.
6. Averbuch D, Tridello G, Hoek J, Mikulska M, Akan H, Yanez San Segundo L, et al. Antimicrobial resistance in gram-negative rods causing bacteremia in hematopoietic stem cell transplant recipients: intercontinental prospective study of the infectious diseases working Party of the European Bone Marrow Transplantation Group. Clin Infect Dis. 2017;65(11): 1819–28.
7. van Duin D, van Delden C, Practice ASTIDCo. Multidrug-resistant gram-negative bacteria infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):31–41.
8. Liu T, Zhang Y, Wan Q. Methicillin-resistant Staphylococcus aureus bacteremia among liver transplant recipients: epidemiology and associated risk factors for morbidity and mortality. Infect Drug Resist. 2018;11:647–58.
9. Alexander BD, Petzold EW, Reller LB, Palmer SM, Davis RD, Woods CW, et al. Survival after lung transplantation of cystic fibrosis patients infected with *Burkholderia cepacia* complex. Am J Transplant. 2008;8(5):1025–30.
10. Bassetti M, Peghin M, Carnelutti A, Righi E, Merelli M, Ansaldi F, et al. Invasive Candida infections in liver transplant recipients: clinical features and risk factors for mortality. Transplant Direct. 2017;3(5):e156.
11. Azar MM, Turbett SE, Fishman JA, Pierce VM. Donor-derived transmission of Candida auris during lung transplantation. Clin Infect Dis. 2017;65(6):1040–2.
12. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis. 2017;64(2):134–40.
13. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, et al. First hospital outbreak of the globally emerging Candida auris in a European hospital. Antimicrob Resist Infect Control. 2016;5:35.
14. Weinstein MP, Murphy JR, Reller LB, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. II. Clinical observations, with special reference to factors influencing prognosis. Rev Infect Dis. 1983;5(1):54–70.
15. Savinelli T, Parenteau S, Mermel LA. What happens when automated blood culture instrument detect growth but there are no technologists in the microbiology laboratory? Diagn Microbiol Infect Dis. 2004;48(3):173–4.
16. Cunney RJ, McNamara EB, Alansari N, Loo B, Smyth EG. The impact of blood culture reporting and clinical liaison on the empiric treatment of bacteraemia. J Clin Pathol. 1997;50(12):1010–2.
17. Buchan BW, Allen S, Burnham CA, McElvania TeKippe E, Davis T, Levi M, et al. Comparison of the next-generation Xpert MRSA/SA BC assay and the GeneOhm StaphSR assay to routine culture for identification of Staphylococcus aureus and methicillin-resistant S. aureus in positive-blood-culture broths. J Clin Microbiol. 2015;53(3):804–9.

18. Charnot-Katsikas A, Tesic V, Love N, Hill B, Bethel C, Boonlayangoor S, et al. Use of the accelerate Pheno system for identification and antimicrobial susceptibility testing of pathogens in positive blood cultures and impact on time to results and workflow. J Clin Microbiol. 2018;56(1):e01166-17.
19. Peker N, Couto N, Sinha B, Rossen JW. Diagnosis of bloodstream infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches. Clin Microbiol Infect. 2018;24(9):944–55.
20. Korber F, Zeller I, Grunstaudl M, Willinger B, Apfalter P, Hirschl AM, et al. SeptiFast versus blood culture in clinical routine – a report on 3 years experience. Wien Klin Wochenschr. 2017;129(11–12):427–34.
21. Wellinghausen N, Kochem AJ, Disque C, Muhl H, Gebert S, Winter J, et al. Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol. 2009;47(9):2759–65.
22. Zboromyrska Y, Cillóniz C, Cobos-Trigueros N, Almela M, Hurtado JC, Vergara A, et al. Evaluation of the Magicplex™ Sepsis real-time test for the rapid diagnosis of bloodstream infections in adults. Front Cell Infect Microbiol. 2019;9:56.
23. Banerjee R, Teng CB, Cunningham SA, Ihde SM, Steckelberg JM, Moriarty JP, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. Clin Infect Dis. 2015;61(7):1071–80.
24. Buehler SS, Madison B, Snyder SR, Derzon JH, Cornish NE, Saubolle MA, et al. Effectiveness of practices to increase timeliness of providing targeted therapy for inpatients with bloodstream infections: a laboratory medicine best practices systematic review and meta-analysis. Clin Microbiol Rev. 2016;29(1):59–103.
25. Walker T, Dumadag S, Lee CJ, Lee SH, Bender JM, Cupo Abbott J, et al. Clinical impact of laboratory implementation of Verigene BC-GN microarray-based assay for detection of gram-negative Bacteria in positive blood cultures. J Clin Microbiol. 2016;54(7):1789–96.
26. Messacar K, Parker SK, Todd JK, Dominguez SR. Implementation of rapid molecular infectious disease diagnostics: the role of diagnostic and antimicrobial stewardship. J Clin Microbiol. 2017;55(3):715–23.
27. Felsenstein S, Bender JM, Sposto R, Gentry M, Takemoto C, Bard JD. Impact of a rapid blood culture assay for gram-positive identification and detection of resistance markers in a pediatric hospital. Arch Pathol Lab Med. 2016;140(3):267–75.
28. Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA. An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant Staphylococcus aureus/S. aureus blood culture test in patients with S. aureus bacteremia. Clin Infect Dis. 2010;51(9):1074–80.
29. Doern CD. The slow march toward rapid phenotypic antimicrobial susceptibility testing: are we there yet? J Clin Microbiol. 2018;56(4):e01329-17.
30. Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013;56(9):1284–92.
31. Mizusawa M, Miller H, Green R, Lee R, Durante M, Perkins R, et al. Can multidrug-resistant Candida auris be reliably identified in clinical microbiology laboratories? J Clin Microbiol. 2017;55(2):638–40.
32. Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D, et al. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med. 2013;5(182):182ra54.
33. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis. 2015;60(6):892–9.
34. Clancy CJ, Pappas PG, Vazquez J, Judson MA, Kontoyiannis DP, Thompson GR 3rd, et al. Detecting infections rapidly and easily for Candidemia trial, Part 2 (DIRECT2): a prospective, multicenter study of the T2Candida panel. Clin Infect Dis. 2018;66(11):1678–86.

35. Neofytos D, Fishman JA, Horn D, Anaissie E, Chang CH, Olyaei A, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transpl Infect Dis. 2010;12(3):220–9.
36. Sexton DJ, Bentz ML, Welsh RM, Litvintseva AP. Evaluation of a new T2 magnetic resonance assay for rapid detection of emergent fungal pathogen Candida auris on clinical skin swab samples. Mycoses. 2018;61(10):786–90.
37. De Angelis G, Posteraro B, De Carolis E, Menchinelli G, Franceschi F, Tumbarello M, et al. T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood. J Antimicrob Chemother. 2018;73(suppl_4):iv20–iv6.
38. Falgenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Kasbohrer A, Roesler U, et al. Colistin resistance gene mcr-1 in extended-spectrum beta-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis. 2016;16(3):282–3.
39. Harrison EM, Paterson GK, Holden MT, Larsen J, Stegger M, Larsen AR, et al. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel mecA homologue mecC. EMBO Mol Med. 2013;5(4):509–15.
40. Laabei M, Recker M, Rudkin JK, Aldeljawi M, Gulay Z, Sloan TJ, et al. Predicting the virulence of MRSA from its genome sequence. Genome Res. 2014;24(5):839–49.
41. Deurenberg RH, Bathoorn E, Chlebowicz MA, Couto N, Ferdous M, Garcia-Cobos S, et al. Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol. 2017;243:16–24.
42. Fung M, Zompi S, Seng H, Hollemon D, Parham A, Hong DK, et al. Plasma cell-free DNA next-generation sequencing to diagnose and monitor infections in allogeneic hematopoietic stem cell transplant patients. Open Forum Infect Dis. 2018;5(12):ofy301.
43. Parize P, Muth E, Richaud C, Gratigny M, Pilmis B, Lamamy A, et al. Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. Clin Microbiol Infect. 2017;23(8):574.e1–6.
44. Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, et al. Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. Clin Infect Dis. 2016;62(7):817–23.
45. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol. 2014;52(10):3731–42.
46. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis. 2002;34(1):7–14.
47. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect. 2018;24(Suppl 1):e1–e38.
48. Patterson TF, Thompson GR 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Executive summary: practice guidelines for the diagnosis and Management of Aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):433–42.
49. Cruciani M, Mengoli C, Loeffler J, Donnelly P, Barnes R, Jones BL, et al. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. Cochrane Database Syst Rev. 2015;2015(10):CD009551.
50. White PL, Bretagne S, Klingspor L, Melchers WJ, McCulloch E, Schulz B, et al. Aspergillus PCR: one step closer to standardization. J Clin Microbiol. 2010;48(4):1231–40.
51. White PL, Mengoli C, Bretagne S, Cuenca-Estrella M, Finnstrom N, Klingspor L, et al. Evaluation of Aspergillus PCR protocols for testing serum specimens. J Clin Microbiol. 2011;49(11):3842–8.
52. White PL, Wingard JR, Bretagne S, Loffler J, Patterson TF, Slavin MA, et al. Aspergillus polymerase chain reaction: systematic review of evidence for clinical use in comparison with antigen testing. Clin Infect Dis. 2015;61(8):1293–303.

53. Chong GL, van de Sande WW, Dingemans GJ, Gaajetaan GR, Vonk AG, Hayette MP, et al. Validation of a new Aspergillus real-time PCR assay for direct detection of Aspergillus and azole resistance of Aspergillus fumigatus on bronchoalveolar lavage fluid. J Clin Microbiol. 2015;53(3):868–74.
54. Chong GM, van der Beek MT, von dem Borne PA, Boelens J, Steel E, Kampinga GA, et al. PCR-based detection of Aspergillus fumigatus Cyp51A mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay(R) in 201 patients with haematological disease suspected for invasive aspergillosis. J Antimicrob Chemother. 2016;71(12):3528–35.
55. White PL, Posso RB, Barnes RA. Analytical and clinical evaluation of the PathoNostics AsperGenius assay for detection of invasive aspergillosis and resistance to azole antifungal drugs during testing of serum samples. J Clin Microbiol. 2015;53(7):2115–21.
56. White PL, Posso RB, Barnes RA. Analytical and clinical evaluation of the PathoNostics AsperGenius assay for detection of invasive aspergillosis and resistance to azole antifungal drugs directly from plasma samples. J Clin Microbiol. 2017;55(8):2356–66.
57. Dannaoui E, Gabriel F, Gaboyard M, Lagardere G, Audebert L, Quesne G, et al. Molecular diagnosis of invasive aspergillosis and detection of azole resistance by a newly commercialized PCR kit. J Clin Microbiol. 2017;55(11):3210–8.
58. Mikulska M, Furfaro E, De Carolis E, Drago E, Pulzato I, Borghesi ML, et al. Use of Aspergillus fumigatus real-time PCR in bronchoalveolar lavage samples (BAL) for diagnosis of invasive aspergillosis, including azole-resistant cases, in high risk haematology patients: the need for a combined use with galactomannan. Med Mycol. 2019;57:987.
59. White PL, Perry MD, Moody A, Follett SA, Morgan G, Barnes RA. Evaluation of analytical and preliminary clinical performance of Myconostica MycAssay Aspergillus when testing serum specimens for diagnosis of invasive aspergillosis. J Clin Microbiol. 2011;49(6):2169–74.
60. Danylo A, Courtemanche C, Pelletier R, Boudreault AA. Performance of MycAssay Aspergillus DNA real-time PCR assay compared with the galactomannan detection assay for the diagnosis of invasive aspergillosis from serum samples. Med Mycol. 2014;52(6):577–83.
61. Torelli R, Sanguinetti M, Moody A, Pagano L, Caira M, De Carolis E, et al. Diagnosis of invasive aspergillosis by a commercial real-time PCR assay for Aspergillus DNA in bronchoalveolar lavage fluid samples from high-risk patients compared to a galactomannan enzyme immunoassay. J Clin Microbiol. 2011;49(12):4273–8.
62. Denis J, Forouzanfar F, Herbrecht R, Toussaint E, Kessler R, Sabou M, et al. Evaluation of two commercial real-time PCR kits for Aspergillus DNA detection in Bronchoalveolar lavage fluid in patients with invasive pulmonary aspergillosis. J Mol Diagn. 2018;20(3):298–306.
63. Elges S, Arnold R, Liesenfeld O, Kofla G, Mikolajewska A, Schwartz S, et al. Prospective evaluation of the SeptiFAST multiplex real-time PCR assay for surveillance and diagnosis of infections in haematological patients after allogeneic stem cell transplantation compared to routine microbiological assays and an in-house real-time PCR method. Mycoses. 2017;60(12):781–8.
64. Lamoth F, Jaton K, Prod'hom G, Senn L, Bille J, Calandra T, et al. Multiplex blood PCR in combination with blood cultures for improvement of microbiological documentation of infection in febrile neutropenia. J Clin Microbiol. 2010;48(10):3510–6.
65. Steinmann J, Buer J, Rath PM, Paul A, Saner F. Invasive aspergillosis in two liver transplant recipients: diagnosis by SeptiFast. Transpl Infect Dis. 2009;11(2):175–8.
66. White PL, Hibbitts SJ, Perry MD, Green J, Stirling E, Woodford L, et al. Evaluation of a commercially developed semiautomated PCR-surface-enhanced raman scattering assay for diagnosis of invasive fungal disease. J Clin Microbiol. 2014;52(10):3536–43.
67. Rath PM, Steinmann J. Overview of commercially available PCR assays for the detection of Aspergillus spp. DNA in patient samples. Front Microbiol. 2018;9:740.
68. Verweij PE, Chowdhary A, Melchers WJ, Meis JF. Azole resistance in Aspergillus fumigatus: can we retain the clinical use of Mold-active antifungal azoles? Clin Infect Dis. 2016;62(3):362–8.

69. Denning DW, Venkateswarlu K, Oakley KL, Anderson MJ, Manning NJ, Stevens DA, et al. Itraconazole resistance in Aspergillus fumigatus. Antimicrob Agents Chemother. 1997;41(6):1364–8.
70. Verweij PE, van de Sande-Bruisma N, Kema GH, Melchers WJ. [Azole resistance in Aspergillus fumigatus in the Netherlands – increase due to environmental fungicides?]. Ned Tijdschr Geneeskd 2012;156(25):A4458.
71. Wiederhold NP, Gil VG, Gutierrez F, Lindner JR, Albataineh MT, McCarthy DI, et al. First detection of TR34 L98H and TR46 Y121F T289A Cyp51 mutations in Aspergillus fumigatus isolates in the United States. J Clin Microbiol. 2016;54(1):168–71.
72. Koo S, Thomas HR, Daniels SD, Lynch RC, Fortier SM, Shea MM, et al. A breath fungal secondary metabolite signature to diagnose invasive aspergillosis. Clin Infect Dis. 2014;59(12):1733–40.
73. Acharige MJT, Koshy S, Ismail N, Aloum O, Jazaerly M, Astudillo CL, et al. Breath-based diagnosis of fungal infections. J Breath Res. 2018;12(2):027108.
74. Curetis. 2019. p. https://www.curetisusa.com/unyvero-system/
75. Hammond SP, Gagne LS, Stock SR, Marty FM, Gelman RS, Marasco WA, et al. Respiratory virus detection in immunocompromised patients with FilmArray respiratory panel compared to conventional methods. J Clin Microbiol. 2012;50(10):3216–21.
76. Affolter K, Schumann DM, Tamm M, Jahn K, Siebeneichler A, Junker L, et al. Multiplex PCR on the Bronchoalveolar lavage fluid of immunocompromised patients. Chest. 2018;154(3):722–5.
77. Lewandowska DW, Schreiber PW, Schuurmans MM, Ruehe B, Zagordi O, Bayard C, et al. Metagenomic sequencing complements routine diagnostics in identifying viral pathogens in lung transplant recipients with unknown etiology of respiratory infection. PLoS One. 2017;12(5):e0177340.
78. Langelier C, Zinter MS, Kalantar K, Yanik GA, Christenson S, O'Donovan B, et al. Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients. Am J Respir Crit Care Med. 2018;197(4):524–8.
79. Li H, Gao H, Meng H, Wang Q, Li S, Chen H, et al. Detection of pulmonary infectious pathogens from lung biopsy tissues by metagenomic next-generation sequencing. Front Cell Infect Microbiol. 2018;8:205.
80. Xie H, Flygare S, Mei Y, Matsuzaki H, Yandell M, Graf EH. Detection of Previously Missed Pathogens in Immunocompromised Children with Pneumonia by a Fully-Validated Next-Generation Sequencing Test. A27 BRONCHIECTASIS AND LUNG INFECTION. p. A7561-A.
81. Glaser CA, Honarmand S, Anderson LJ, Schnurr DP, Forghani B, Cossen CK, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. Clin Infect Dis. 2006;43(12):1565–77.
82. Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, et al. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of Bacteria, viruses, and yeast in cerebrospinal fluid specimens. J Clin Microbiol. 2016;54(9):2251–61.
83. Chang D, Okulicz JF, Nielsen LE, White BK. A tertiary care Center's experience with novel molecular meningitis/encephalitis diagnostics and implementation with antimicrobial stewardship. Mil Med. 2018;183(1–2):e24–e7.
84. O'Halloran JA, Franklin A, Lainhart W, Burnham CA, Powderly W, Dubberke E. Pitfalls associated with the use of molecular diagnostic panels in the diagnosis of Cryptococcal meningitis. Open Forum Infect Dis. 2017;4(4):ofx242.
85. Walker M, Sheets J, Hamer D, O'Neal C. Performance of the Biofire Filmarray meningitis/encephalitis panel in Cryptococcal meningitis diagnosis. Open Forum Infect Dis. 2018;5(Supplement 1):S599.
86. Lipowski D, Popiel M, Perlejewski K, Nakamura S, Bukowska-Osko I, Rzadkiewicz E, et al. A cluster of fatal tick-borne encephalitis virus infection in organ transplant setting. J Infect Dis. 2017;215(6):896–901.

87. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. N Engl J Med. 2008;358(10):991–8.
88. Bunnapradist S, Neri L, Wong W, Lentine KL, Burroughs TE, Pinsky BW, et al. Incidence and risk factors for diarrhea following kidney transplantation and association with graft loss and mortality. Am J Kidney Dis. 2008;51(3):478–86.
89. Mengelle C, Mansuy JM, Prere MF, Grouteau E, Claudet I, Kamar N, et al. Simultaneous detection of gastrointestinal pathogens with a multiplex Luminex-based molecular assay in stool samples from diarrhoeic patients. Clin Microbiol Infect. 2013;19(10):E458–65.
90. Coste JF, Vuiblet V, Moustapha B, Bouin A, Lavaud S, Toupance O, et al. Microbiological diagnosis of severe diarrhea in kidney transplant recipients by use of multiplex PCR assays. J Clin Microbiol. 2013;51(6):1841–9.
91. Liesman RM, Binnicker MJ. The role of multiplex molecular panels for the diagnosis of gastrointestinal infections in immunocompromised patients. Curr Opin Infect Dis. 2016;29(4):359–65.
92. Atlas C, Aad G, Abajyan T, Abbott B, Abdallah J, Abdel Khalek S, et al. Jet energy measurement and its systematic uncertainty in proton-proton collisions at [Formula: see text] TeV with the ATLAS detector. Eur Phys J C Part Fields. 2015;75:17.
93. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol. 2015;53(3):915–25.
94. Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, et al. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-acquired gastroenteritis. Clin Microbiol Infect. 2015;21(8):719–28.
95. Wessels E, Rusman LG, van Bussel MJ, Claas EC. Added value of multiplex Luminex Gastrointestinal Pathogen Panel (xTAG(R) GPP) testing in the diagnosis of infectious gastroenteritis. Clin Microbiol Infect. 2014;20(3):O182–7.
96. McMillen T, Lee YJ, Kamboj M, Babady NE. Limited diagnostic value of a multiplexed gastrointestinal pathogen panel for the detection of adenovirus infection in an oncology patient population. J Clin Virol. 2017;94:37–41.
97. Greninger AL. The challenge of diagnostic metagenomics. Expert Rev Mol Diagn. 2018;18(7):605–15.
98. Simner PJ, Miller S, Carroll KC. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis. 2018;66(5):778–88.
99. Guinea J, Padilla C, Escribano P, Munoz P, Padilla B, Gijon P, et al. Evaluation of MycAssay Aspergillus for diagnosis of invasive pulmonary aspergillosis in patients without hematological cancer. PLoS One. 2013;8(4):e61545.
100. Aslan M, Oz Y, Aksit F, Akay OM. Potential of polymerase chain reaction and galactomannan for the diagnosis of invasive aspergillosis in patients with febrile neutropenia. Mycoses. 2015;58(6):343–9.
101. Lass-Florl C, Follett SA, Moody A, Denning DW. Detection of Aspergillus in lung and other tissue samples using the MycAssay Aspergillus real-time PCR kit. Can J Microbiol. 2011;57(9):765–8.
102. Simner PJ, Uhl JR, Hall L, Weber MM, Walchak RC, Buckwalter S, et al. Broad-range direct detection and identification of fungi by use of the PLEX-ID PCR-electrospray ionization mass spectrometry (ESI-MS) system. J Clin Microbiol. 2013;51(6):1699–706.
103. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med. 2014; 370(25):2408–17.
104. Brown JR, Morfopoulou S, Hubb J, Emmett WA, Ip W, Shah D, et al. Astrovirus VA1/HMO-C: an increasingly recognized neurotropic pathogen in immunocompromised patients. Clin Infect Dis. 2015;60(6):881–8.

105. Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, Grant P, et al. Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. Clin Infect Dis. 2015;60(6):919–23.
106. Christopeit M, Grundhoff A, Rohde H, Belmar-Campos C, Grzyska U, Fiehler J, et al. Suspected encephalitis with Candida tropicalis and Fusarium detected by unbiased RNA sequencing. Ann Hematol. 2016;95(11):1919–21.
107. Lum SH, Turner A, Guiver M, Bonney D, Martland T, Davies E, et al. An emerging opportunistic infection: fatal astrovirus (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient. Transpl Infect Dis. 2016;18(6):960–4.
108. Salzberg SL, Breitwieser FP, Kumar A, Hao H, Burger P, Rodriguez FJ, et al. Next-generation sequencing in neuropathologic diagnosis of infections of the nervous system. Neurol Neuroimmunol Neuroinflamm. 2016;3(4):e251.
109. Sato M, Kuroda M, Kasai M, Matsui H, Fukuyama T, Katano H, et al. Acute encephalopathy in an immunocompromised boy with astrovirus-MLB1 infection detected by next generation sequencing. J Clin Virol. 2016;78:66–70.
110. Morfopoulou S, Brown JR, Davies EG, Anderson G, Virasami A, Qasim W, et al. Human coronavirus OC43 associated with fatal encephalitis. N Engl J Med. 2016;375(5):497–8.
111. Wilson MR, Zimmermann LL, Crawford ED, Sample HA, Soni PR, Baker AN, et al. Acute West Nile virus meningoencephalitis diagnosed via metagenomic deep sequencing of cerebrospinal fluid in a renal transplant patient. Am J Transplant. 2017;17(3):803–8.
112. Murkey JA, Chew KW, Carlson M, Shannon CL, Sirohi D, Sample HA, et al. Hepatitis E virus-associated meningoencephalitis in a lung transplant recipient diagnosed by clinical metagenomic sequencing. Open Forum Infect Dis. 2017;4(3):ofx121.
113. Morfopoulou S, Mee ET, Connaughton SM, Brown JR, Gilmour K, Chong WK, et al. Deep sequencing reveals persistence of cell-associated mumps vaccine virus in chronic encephalitis. Acta Neuropathol. 2017;133(1):139–47.

Rashes to Recognize in the Immunocompromised Transplant Patient: Focus on the Solid Organ Transplant Recipient

63

Melodi Javid Whitley, Adela R. Cardones, and Sarah A. Myers

Contents

Introduction 1578
Superficial Fungal Infections 1578
Disease Epidemiology 1578
Clinical Impact in Transplant 1578
Disease Diagnostics and Clinical Evaluation 1578
Management 1579
Viral Infections 1580
Disease Epidemiology 1580
Clinical Impact in Transplant 1581
Disease Diagnostics and Clinical Evaluation 1581
Management 1582
Bacterial Infections 1583
Disease Epidemiology 1583
Clinical Impact in Transplant 1584
Disease Diagnostics and Clinical Evaluation 1584
Management 1585
Special Considerations for the Blood and Marrow Transplant Patient 1586
Cutaneous Graft-Versus-Host Disease 1587
Cytomegalovirus Reactivation Among HSCT Patients 1588
Conclusion 1588
Key Points 1589
References 1590

M. J. Whitley · A. R. Cardones · S. A. Myers (✉)
Department of Dermatology, Duke University Medical Center, Durham, NC, USA
e-mail: melodi.whitley@duke.edu; adela.cardones@duke.edu; sarah.myers@duke.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_62

Introduction

Due to immunosuppression, solid organ transplant (SOT) and hematopoietic stem cell transplant recipients are highly susceptible to a host of cutaneous infections. Often, exaggerated and atypical cutaneous manifestations are due to impaired immunological surveillance of common and ubiquitous pathogens. In the immediate post-transplant period, patients are most likely to present with cutaneous infections due to nosocomial pathogens, while opportunistic pathogens become more prevalent once patients have had longer exposure to immunosuppression. Here, we will explore the incidence, diagnosis, and management of cutaneous infections that are commonly observed in SOT recipients, and compare them against findings seen in the stem cell transplant population.

Superficial Fungal Infections

Disease Epidemiology

Superficial fungal infections are quite common in SOT recipients. In one series of 223 renal transplant recipients (RTRs), 60% of transplanted patients developed a superficial fungal infection with the majority presenting within the first year after transplantation [1, 2]. The majority of these cases are related to organisms normally found on the human skin or mucosa, such as oral candidiasis [3]; however *Malassezia* and dermatophytes, such as *Trichophyton rubrum*, are also frequently seen. Penetrative injuries to the skin, however, can introduce a much wider variety of soil and environmental fungi that should also be considered.

Clinical Impact in Transplant

Disseminated disease is unlikely to result from superficial fungal infections and therefore reduction in immunosuppression is usually not warranted outside of severe or atypical cases. However, cutaneous infections can carry high symptomatic morbidity and therefore attention to and treatment of these conditions is important. Rarely, sporotrichoid spread of cutaneously inoculated molds can be associated with secondary dissemination and may warrant immunosuppression reduction.

As mentioned, deep and disseminated fungal infections are not discussed in this section. Please refer to ▶ Chaps. 45, "*Aspergillus* in Solid Organ and Stem Cell Transplant Patients: Emerging Options for Diagnosis and Management," and ▶ 47, "Non-*Aspergillus* Fungal Infections in Transplant Patients" for a more extensive discussion of these infections and their cutaneous extension.

Disease Diagnostics and Clinical Evaluation

Superficial fungal infections can have diverse cutaneous presentations. *Malassezia*, for example, classically presents as tinea versicolor with hyper- and hypo-pigmented

patches and plaques with fine scales that are commonly on the trunk. SOT recipients may present with tinea versicolor in unusual locations, like the genitalia [4]. *Malassezia* may also often manifest as a folliculitis-type rash, with erythematous papules and pustules on the face and upper trunk [2, 5]. This presentation can easily be confused with steroid acne, a not infrequent skin issue in the transplant population, owing to the use of prednisone as a component of the immunosuppressive regimen. *Malassezia* is also implicated in seborrheic dermatitis which was shown to affect about 10% of patients in one series of renal transplant recipients [6]. Seborrheic dermatitis presents with erythema and greasy scale, primarily on the scalp and face with trunk and back involvement reported as a feature of *Malassezia* infections as well [6]. Onychomycosis can also appear in SOT recipients, [7] although this condition, characterized by thickening and yellow discoloration of the nails, is most often caused by dermatophyte species like *Trichophyton rubrum* [3]. Other common presentations of dermatophyte infection include tinea cruris, tinea corporis, and tinea pedis [3].

Atypical dermatophyte infections are also seen in SOT recipients as in the of case of *T. rubrum* causing molluscum-like umbilicated papules on the face [8]. Superficial dermatophyte infections can lead to severe dermatophytosis in such a t-cell immunosuppressed host, presenting with lower extremity nodules that may ulcerate or become necrotic [9]. Tinea corporis can also present as diffuse erythema and scaling without classic annular lesions and can prove difficult to eradicate due to host immunosuppression.

Mucocutaneous candidiasis presents with erosions and a creamy exudate on the oral mucosa [10] and on the intertriginous regions with erythema, vesicles, pustules, and typical "satellite" lesions. There should be high suspicion for candidiasis early in the post-transplant course [11], especially after more intense induction immunosuppression, such as thymoglobulin or alemtuzumab.

The diagnosis of superficial fungal infections is largely clinical, but in unusual or refractory cases it can be aided at the bedside by potassium hydroxide (KOH) preparation and microscopy of skin scrapings. On KOH preparation, *Malassezia* species will reveal yeast-like bodies and hyphae [9], as opposed to dermatophytes which will show septate hyphae [12], and candida where hyphae and pseudohyphae are present. If the clinical diagnosis is not clear, tissue culture and histopathology can help provide certainty.

Management

Topical treatments are preferred for control of limited superficial fungal infections. These include the use of antifungal shampoos like ketoconazole and selenium sulfide as well as azole or allylamine antifungal preparations [2, 3]. For extensive or refractory disease, systemic therapy with oral fluconazole or itraconazole for *Malassezia* and oral terbinafine for dermatophyte infections is indicated [3]. Discussion with the transplant team regarding drug interactions is important if systemic therapy is considered, especially when considering the azole family. From a

dermatologic perspective, it is important for the physician to recognize the risks associated with voriconazole, specifically. Namely, voriconazole use is associated with significant photosensitivity and an overall 1.86-fold increase in squamous cell carcinoma incidence among lung and hematopoietic cell transplant patients, a population that is already at a significantly increased risk of cutaneous squamous cell carcinoma [13]. Importantly, these risks are positively correlated with the dose and duration of treatment [13]. Therefore, patients who do receive antifungal therapy with voriconazole should undergo regular full body skin examinations.

For oral candidiasis lesions, nystatin swish and swallow is the treatment of choice [10], and systemic therapy is limited to patients where drug-resistant candida is suspected or extension into the retropharynx or esophagus is documented.

Viral Infections

Disease Epidemiology

SOT recipients are susceptible to primary cutaneous infection and reactivation of common human viruses. Herpes simplex virus (HSV) and varicella zoster virus (VZV) are the most common offenders within the first year post-transplant, in the absence of prophylaxis, while oncogenic viruses like HPV and HHV-8 tend to be more relevant after years of immunosuppression [14]. Different studies of VZV incidence range from 5% to 40% in the first 1–2 years after transplant. CMV infection most often presents with systemic symptoms in the early post-transplant period but can have associated skin findings.

For a more extensive discussion on the systemic impacts of HSV, VZV, CMV, or HHV8 please refer to ► Chap. 26, "Herpes Simplex and Varicella Zoster in Transplant Candidates and Recipients."

Most SOT recipients are latently infected with alpha-herpes viruses HSV-1, HSV-2, and VZV prior to transplant [15] and reactivation is seen during acute and high levels of immunosuppression. CMV is the most common viral infection among SOT recipients, affecting up to 60% of patients, and cutaneous manifestations may be seen, although very rarely [16]. The now common use of viral prophylaxis for CMV, either valganciclovir or more recently letermovir, has reduced the rates of several viral infections in SOT recipients, including reduction in the rate of cutaneous HSV or VZV infections from nearly half of patients to less than 10% in the year after transplant [17–19].

Renal transplant recipients are 3.4 times more likely than the nontransplant population to have HPV-related genital warts and this increased risk persists for at least ten years after transplant [20]. The prevalence of HPV on cervical smears is about 30% with high-risk subtypes detected in the majority of cases [21, 22]. Whether the declines in HPV rates seen in some countries with high penetrance of HPV vaccination in early teenage years translates into lower incidence in the transplanted population is not yet known.

Clinical Impact in Transplant

Diffuse, morbilliform and nonspecific eruptions should alert the provider to possible systemic viral infection, especially in the acute post-transplant course. The rare occurrence of solid organ transplantation–associated graft-versus-host disease (GVHD) can similarly present with morbilliform, confluently erythematous, and desquamative eruptions. It is important to include GVHD in the differential of a suspected viral exanthem as it is critical to recognize this entity early [23]. Miliaria (crystallina and rubra) may also present similarly. Therefor, biopsy or viral smears and/or staining may be needed to differentiate all of these competing diagnoses.

Skin-limited viral infections, like HSV ulcers, can occasionally serve as a site for secondary bacterial infection in the susceptible host, therefore a thorough history and physical exam is important to identify any lesions and begin prompt treatment. HPV-associated warts are a risk factor for progression to squamous cell carcinoma, and these lesions are reported to be more common among non-White SOT recipients [24, 25]. Importantly, the majority of patients deny the presence of genital lesions although about half will have a lesion on physical exam [24]. Therefore, routine genital examination is important for the detection of HPV-associated genital lesions.

In one study of SOT recipients with HSV infection, immunosuppressive regimens were reduced in about 50% of patients in response to infection [19]. Interestingly, specifically for renal transplant recipients with refractory warts, replacement of cyclosporine with sirolimus has shown to result in resolution of cutaneous lesions [26]. Such studies are not available for other organ groups, although feasibly the same outcome may be expected. Indications for possible reduction of immunosuppression include large and multiple lesions, patient discomfort/impairment, as well as anal and vulvar lesions associated with AIN or VIN. Although rare, cutaneous manifestations of CMV may be a harbinger of graft rejection and therefore, careful reconsideration of the immunosuppressive regimen is advised [14].

Disease Diagnostics and Clinical Evaluation

The typical lesions of HSV-1 and HSV-2 are grouped vesicles or ulcers in the oral or genital regions, respectively [15]. Primary VZV infection, or varicella, is characterized by a diffuse vesicular eruption preceded by a viral prodrome, whereas VZV reactivation, or herpes zoster, presents with a unilateral and dermatomal distribution of painful vesicles preceded by dysesthesia in the same distribution [15]. Disseminated VZV, which does not respect the typical dermatomal distribution, is more common in solid organ recipients than in the immunocompetent population [15]. The diagnosis of HSV or VZV can often be made clinically, but should be confirmed with laboratory testing if there is any doubt or if the patient is acutely ill. Confirmation may also be important in determining infection control implications and isolation restrictions for an infected patient. It may also impact decision-making for the patient with other systemic symptoms in terms of confirming or refuting a diagnosis.

The diagnostic test of choice for all herpes viruses is PCR. Viral culture is now limited to cases where acyclovir-resistance is suspected [18, 27]. Tissue pathology can be confirmatory of the clinical and laboratory findings.

HPV-associated warts in SOT recipients are similar to those seen in immunocompetent individuals with distribution to the face, hands, and feet [14]. However, warts may be greater in number and larger in size with possible progression to acquired epidermodysplasia verruciformis, or generalized verrucosis, a condition characterized by the presence of greater than 20 verrucous lesions and associated with increased risk of cutaneous squamous cell carcinoma [28–30]. The diagnosis is clinical, but histopathologic evaluation can be confirmatory and serve to rule out carcinoma in particularly atypical appearing lesions, or in lesions that have grown rapidly or exhibit bleeding.

Molluscum contagiosum is characterized by isolated or scattered flesh colored umbilicated papules caused by Poxvirus and is diagnosed clinically. Up to ~7% of SOTRs with molluscum contagiosum may have an atypical presentation with more extensive disease, larger nodules, or a folliculitis-like appearance [31, 32].

Cutaneous manifestations of CMV infection are rare in SOT recipients but have been reported. Ulcerated lesions are most commonly reported with the majority of the lesions located in the genital area [33–35]. Ulcers located on the face and extremities have been reported as well [36, 37]. Punch biopsy for histopathologic examination as well as viral culture should be performed if cutaneous CMV is suspected as the differential is broad for such ulcerated lesions. CMV viremia should also be confirmed with hematologic testing.

Management

- Prophylaxis: Recent guidelines for the management of HSV in SOT recipients recommend acyclovir for prophylaxis in HSV-1- and/or HSV-2-positive patients who are not already receiving prophylaxis for CMV [15, 18]. Duration varies according to the organ type, and the degree to which they are immunosuppressed, but typically may continue for 3–6 months. Vaccination for primary VZV, or varicella, should be considered in the pre-transplant setting for the 2–4% of patients that are seronegative [27]. This is a live attenuated vaccine and is contraindicated after transplant but is effective in inducing a memory T-cell response in transplant candidates [38]. Vaccination for herpes zoster using the live attenuated Zostavax vaccine is now rare, and contraindicated in patients who are already transplanted due to rare circumstances where reactivation of attenuated virus occurs. [39]. The new herpes zoster subunit vaccine, Shingrix, is being investigated for use in the post-transplant setting with early promising results [39]. Certainly, it should be standard of care for patients who are over the age of 50 and contemplating transplant.

  Regarding HPV, current US guidelines recommend a three-dose vaccine regimen for patients between the ages of 9 and 26 years; this includes SOT recipients [40]. Between 27 and 45 years of age, shared decision-making is recommended for

HPV vaccination. One study showed that the quadrivalent vaccine was well tolerated in transplanted patients up to the age of 35, but with a sustained response in only about half of patients [41]. Interestingly, successful use of the four-valent and nine-valent vaccines as treatment for patients with active lesions has been reported [28, 42]. Furthermore, the nine-valent vaccine has been injected intratumorally simultaneously with systemic vaccination, resulting in tumor resolution in an immunocompetent patient [43]. These studies support the likely benefit of HPV vaccination for both the reduction of HPV-associated benign and malignant lesions in at-risk transplant recipients. Larger prospective trials in this population will be critical to better define who benefits and when the dosing should occur.

The utility of CMV prophylaxis is well established and should be prescribed per guidelines.

For more details on the management of CMV, please refer to ▶ Chap. 25, "Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment" on CMV management.

- Treatment: For HSV and VZV, oral therapy is indicated for limited mucocutaneous disease although IV Foscarnet may be appropriate for acyclovir-resistant or recalcitrant cases [15, 18]. Options for oral therapy of HSV and VZV include acyclovir, valacyclovir, and famciclovir [15]. First-line therapy for HPV-associated warts is localized destruction of tissue that is most often accomplished with cryotherapy. Intralesional Candida injections (available as a candida antigen solution in the USA) have also been used safely in transplant recipients with HPV-associated warts [44]. They are hypothesized to work by stimulating Tcell immunity that cross-targets infected cells. For treatment of recalcitrant warts, there are several reported therapies with limited success which include topical and intralesional cidofivir [45, 46], oral isotretinoin [47], and oral cimetidine [48]. Treatment options for molluscum contagiosum include topical cantharidin, cryotherapy, curettage, and pulsed dye laser [49]. First-line treatment for CMV is ganciclovir and duration is dependent on the clearance of viremia. For ganciclovir-resistant disease, CMV-specific immunoglobulin is an option and there are several promising novel antiviral agents currently undergoing clinical studies [50].

Bacterial Infections

Disease Epidemiology

All transplant recipients are susceptible to nosocomial bacterial pathogens like *Staphylococcus aureus*, especially in the very early post-transplant period when surgical site infections represent the most common site involved [14]. In one study of renal graft recipients, skin was the most common site of *Staphylococcus* aureus infection and the median time to infection after transplant was 29 days [51]. Interestingly, *Staphylococcus aureus* colonization tends to be higher in SOT compared to immunocompetent patients [52], with an increased risk of infection as a direct result [53]. *Nocardia* is another gram-positive opportunistic pathogen that has

been recognized in SOTRs with skin being the second most common site of infection [54]. Increasingly, transplant recipients are also recognized as being at increased risk for cutaneous mycobacterial infections, particularly in the first two years post-transplant when immunosuppression is at its greatest, and transplant surgical wounds are healing [14, 55]. Such infections include both *Mycobacterium tuberculosis* outside of north America, but also nontuberculous mycobacteria (NTM) such as *Mycobacterium abscessus* and less commonly *Mycobacterium chelonae* [56–59].

Clinical Impact in Transplant

Early recognition and treatment of cutaneous bacterial infection in an immunosuppressed host is important, with data suggesting that anti-rejection therapy plays a role in the loss of control of skin and soft tissue microbes. The skin frequently serves as the first recognizable portal of entry for subsequent severe systemic infection. Indeed, solid organ transplant recipients with skin and soft tissue infection, such as necrotizing fasciitis, are more than twice as likely to develop septic shock than immunocompetent controls [60]. Importantly, infection with *Staphylococcus aureus* has been associated with increased risk of graft loss [51, 53], through multiple direct and indirect pathways, highlighting the need for prevention and early recognition of bacterial infections which present on the skin.

Disease Diagnostics and Clinical Evaluation

The same bacterial pathogen can cause several different types of cutaneous infections with varying presentations. *Staphylococcus aureus,* for example, can be the pathogen in folliculitis, impetigo, erysipelas, cellulitis, and toxic shock syndrome or necrotizing fasciitis/panniculitis [14]. Folliculitis is characterized by erythematous follicular–based papules and pustules on the trunk and extremities. Impetigo is a superficial bacterial infection caused by common skin flora, including *Staphylococcus* and *Streptococcus* species, and characterized by honey-colored crusting of eroded plaques. Erysipelas describes bacterial infection limited to the upper dermis and superficial lymphatic system that presents with sharply defined, bright red plaques. On the other hand, cellulitis is infection of the deeper dermis and subcutaneous tissue that presents with ill-defined, pink, and tender plaques. Less common presentations of cutaneous infections due to *Staphylococcus* and *Streptococcus* include ecthyma, which is characterized by painful ulcers on the lower extremities, and necrotizing fasciitis, which is a polymicrobial infection that invades into the deep soft tissues.

Ecthyma gangrenosum is a rare presentation most classically associated with *Pseudomonas aeruginosa* infection, although it has been documented with other gram-negative infections especially. It is characterized by a rapidly developing hemorrhagic blister that may develop into a necrotic ulcer. This should be considered a sign of Pseudomonal bacteremia and has been reported in the transplant population [52, 61].

Cutaneous manifestations of *Nocardia* can be primary or secondary to disseminated infection. Primary cutaneous nocardiosis may present with cellulitis, abscesses, exophytic growths, blisters, or ulcers and may spread along the lymphatics in a "sporotrichoid" distribution [62–65]. Mycetomas, or draining nodules and plaques, may develop on the lower extremities.

Cutaneous manifestations of *Mycobacterium tuberculosis* are rare but can present with abscesses, ulcers, or verrucous nodules with possible sporotrichoid spread [55, 66, 67]. Miliary tuberculosis may present with diffuse erythematous, papular or vesicular rash [68]. Finally, tuberculids are cutaneous lesions caused by a hypersensitivty reaction in patients infected with M.tuberculosis. The pathogenesis of these reactive manifestations remains unknown. Typically these lesions respond to anti-tuberculosis treatment.

Scrofuloderma describes the development of ulcerating, draining nodules overlying affected lymph nodes. NTM infections in transplant recipients have been reported to present with extremity nodules and ulcers as well surgical site infections [56–59].

For a more detailed review of tuberculous and nontuberculous mycobacterial disease in transplants, please refer to ▶ Chaps. 18, "*Mycobacterium tuberculosis* in Transplantation," and ▶ 20, "Slow-Growing Nontuberculous Mycobacteria in Transplant."

When bacterial infection is suspected, cultures obtained via surface swabs are of limited value given the risk of contamination. Therefore, punch biopsy of the presenting lesion for tissue culture, speciation, and sensitivities as well as histopathologic analysis is recommended for diagnosis. Blood cultures should also be obtained prior to starting treatment if there is any concern for systemic involvement, e.g., fevers, hypotension, tachycardia, and rigors.

Management

- Prophylaxis: There is precedence and data to support the role of *Staphylococcus* decolonization as a cost-saving measure, but some studies have shown lack of efficacy [69, 70]. For example, a local decolonization protocol involves combination of 4% chlorhexidine for daily bathing, chlorhexidine mouth rinse, and 2% intra-nasal mupirocin twice daily, for 5 days. Some experts combine this with an anti-staphylococcal antibiotic, such as doxycycline, for those with larger or multiple pustules at the outset, although this is not uniformly done. Patients who are receiving prophylaxis with TMP-SMX for *Pneumocystis jiroveci* pneumonia may have a decreased rate of *Nocardia* infection. There is data to suggest some benefit to daily TMP-SMX for secondary prophylaxis in patients with a prior episode of nocardiosis [71].
- Treatment: For superficial infections like folliculitis and impetigo, topical antibiotics like mupirocin and clindamycin are often sufficient [52]. Oral therapy should be considered when the involved surface area is greater than that which can be covered easily with topical treatments or in those with refractory disease.

For purulent infections, incision and drainage is primarily indicated. Empiric oral or intravenous antibiotics may be helpful for complicated or more severe infections, for example, those >2 cm or with extensive cellulitis, or where multiple abscesses are present. Whether solid organ transplant alone puts the patient at sufficiently high enough risk to undertake broad spectrum empiric antibiotics is unclear, but in general patients with systemic immunosuppression should be managed with more caution [72]. In the case of cellulitis, erysipelas, or necrotizing infection, empiric oral or intravenous antibiotic treatment with an anti-staphylococcal penicillin, first-generation cephalosporin, or clindamycin should be initiated as first line [72]. Trimethoprim/sulfamethoxezole may also be used. For severe infections, broad spectrum empiric coverage should be initiated with prompt narrowing of coverage upon availability of culture and sensitivity data. Intravenous antibiotics are indicated with any sign of bacteremia or sepsis. Surgical debridement may also be necessary for infections [54] that appear to be necrotizing, especially those where the tissue exhibits crepitus, or the patient has signs of sepsis, therefore multidisciplinary care of these patients is necessary.

Special Considerations for the Blood and Marrow Transplant Patient

Hematopoietic stem cell transplantation (HSCT) is a commonly used treatment for hematologic malignancies, although it is also used for nonmalignant hematologic and autoimmune conditions [73–75]. Just like patients who undergo solid organ transplantation, the immunosuppressed state of HSCT patients puts them at risk for a variety of cutaneous and systemic infections that have skin manifestations. There are major immunologic differences among SOTRs and HSCT patients, however, that affect the type and timing of their cutaneous diseases. SOTRs expect to be on chronic immunosuppressive therapy for years. However, the goal of HSCT is to restore the patient's immune compartment, without requirement for prolonged immunosuppressive therapy. The exception is for patients who develop chronic graft-versus-host disease (GVHD), a common complication of allogeneic HSCT. Patient who develops chronic GVHD may require years of immunosuppressive therapy depending on the severity of their disease [76]. In contrast to SOTRs, patients who undergo HSCT go through an acute phase of profound immunosuppression. Patients who undergo HSCT are often treated with myeloablative chemotherapy, with or without total body irradiation, prior to infusion of either autologous or allogeneic hematopoietic stem cells. The post-HSCT neutropenic phase typically lasts for 1 month, but T and B cell recovery may not occur until after several months to even years after HSCT [77]. During the neutrophil recovery period, patients may develop an inflammatory reaction associated with immunologic engraftment, characterized by a morbilliform eruption or diffuse macular erythema and edema of the skin, fever, and manifestations of capillary leak syndrome [78].

Cutaneous Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) is a common complication of allogeneic HSCT and is a leading cause of nonrelapse mortality among these patients. This is a multisystem disease, but the skin is the most commonly involved organ, affecting up to 76% of patients with acute GVHD [79]. Acute GVHD has traditionally been defined as occurring within the first 100 days after allogeneic HSCT. The most commonly described appearance of acute cutaneous GVHD is a morbilliform reaction (see Fig. 1). This makes it difficult to differentiate from a cutaneous drug reaction. Drug hypersensitivity syndrome or drug reaction with systemic symptoms (DRESS) is a particularly challenging differential diagnosis, and severe cutaneous acute GVHD can be clinically and histologically similar to Stevens Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [80]. A phototoxic drug reaction, especially to voriconazole, should be recognized, and can occur in both acute and chronic settings [81]. While acute phototoxicity presents with photodistributed erythema and bullae, chronic phototoxicity is characterized by dyspigmentation, poikilodermatous change, and even cutaneous malignancies. Certain clinical features, such as facial involvement, the presence of diarrhea, and hyperbilirubinemia can help distinguish acute GVHD from a morbilliform drug reaction [82]. The cutaneous eruption in acute GVHD can also be characterized by follicular scaly papules and can simulate a bacterial or fungal folliculitis [83]. In our experience, however, the follicular form of acute GVHD tends to be more monomorphic, and less pustular when compared to a bacterial folliculitis. Other atypical manifestations of acute GVHD may be more papulosquamous in appearance, and may mimic pityriasis rubra pilaris, acquired ichthyosis, and psoriasis (see Fig. 2) [84].

Chronic cutaneous GVHD is a long-term immunologic complication of allogeneic HSCT [85]. In contrast to acute GVHD, the skin manifestations of chronic GVHD mimic a variety of autoimmune cutaneous disorders [84, 85]. The diagnosis

Fig. 1 Acute GVHD

Fig. 2 Eczematous acute GVHD

of chronic cutaneous GVHD relies on clinical features, although a skin biopsy may sometimes be helpful. The sclerotic form of chronic cutaneous GVHD is the most severe phenotype, and is associated with increased morbidity [86]. Other diagnostic manifestations include poikiloderma, lichen planus-, morphea-, and lichen sclerosus-like changes (for sclerotic GVHD, refer to Fig. 3).

Cytomegalovirus Reactivation Among HSCT Patients

CMV reactivation remains a common challenge in the post-HSCT period. As many as 40% of allogeneic HSCT patients have CMV reactivation within 1 year of their transplant when both donor and recipient have positive serologies for CMV [87]. The median time to CMV reactivation is 41 days. The classic cutaneous manifestation of CMV consists of oral ulcerations and round, crusted perianal papules [88, 89]. However, these papules can appear in other parts of the body, and disseminated forms of the disease may occur, especially in the setting of total body irradiation [90]. Vasculitis and vasculopathic cutaneous lesions from CMV have been described, and are not surprising given the tendency of CMV to localize and proliferate in endothelial cells [89, 91, 92]. Coinfection with bacteria and other herpes viruses are not uncommon, and is a potential pitfall in diagnosis [89, 90].

Conclusion

Transplant recipients are at an increased risk for common community-acquired and nosocomial cutaneous infections. The presentation of such diseases may be typical or atypical and importantly may follow a more aggressive clinical course. Patients may not report the presence of lesions, especially those in sensitive body areas, therefore routine and thorough skin examinations are indicated in all SOT and HSCT recipients. Vaccination and antimicrobial prophylaxis are two important strategies for preventing

Fig. 3 Sclerotic chronic GVHD

cutaneous infections, and the use of HPV vaccines in this population warrants further study. Treatment of cutaneous infections in transplant requires a multidisciplinary and multimodal approach with careful consideration of any changes in the immunosuppressive regimen, depending on the severity and morbidity of the infection.

Key Points

- Solid organ transplant recipients are at an increased risk of common community-acquired and nosocomial cutaneous fungal, viral, and bacterial infections.
- Drug reactions, viral infection, a cutaneous eruption from immune reconstitution, and GVHD are important differential diagnoses to consider in a HSCT patient who presents with a rash.
- Cutaneous manifestations may have typical or atypical presentations, but often follow a more aggressive course in this population.
- Diagnosis is often clinical but skin biopsy, tissue culture, and PCR are important useful diagnostic tools.
- Voriconazole use for severe fungal infections is associated with a significantly higher risk of squamous cell carcinoma in SOTRs.

- The use of CMV prophylaxis has decreased the rates of HSV and VZV infection in SOTRs.
- HPV vaccination may be a useful tool in preventing and treating HPV-associated benign and malignant lesions in SOTRs but further study is needed to understand the safety and efficacy.
- SOTRs may not report cutaneous lesions, especially in sensitive body areas, therefore routine and thorough skin examination is indicated in all patients.
- Decisions regarding treatment modalities and reduction in immunosuppression requires a multidisciplinary approach.

References

1. Imko-Walczuk BB, Predota A, Okuniewska A, Jaskiewicz J, Zegarska B, Placek W, et al. Superficial fungal infections in renal transplant recipients. Transplant Proc. 2014;46(8):2738–42.
2. Rhie S, Turcios R, Buckley H, Suh B. Clinical features and treatment of *Malassezia folliculitis* with fluconazole in orthotopic heart transplant recipients. J Heart Lung Transplant. 2000;19(2): 215–9.
3. Ilyas M, Sharma A. Cutaneous fungal infections in solid organ transplant recipients. Transplant Rev (Orlando). 2017;31(3):158–65.
4. Ryu HW, Cho JW, Lee KS. Pityriasis versicolor on penile shaft in a renal transplant recipient. Ann Dermatol. 2012;24(3):345–7.
5. Mota R, Reifenberger J, Homey B, Bruch-Gerharz D. Malassezia folliculitis in an immunocompromised patient. Hautarzt. 2011;62(10):725–7.
6. Lally A, Casabonne D, Newton R, Wojnarowska F. Seborrheic dermatitis among Oxford renal transplant recipients. J Eur Acad Dermatol Venereol. 2010;24(5):561–4.
7. Ertam I, Aytimur D, Alper S. Malassezia furfur onychomycosis in an immunosuppressed liver transplant recipient. Indian J Dermatol Venereol Leprol. 2007;73(6):425–6.
8. Ergen EN, Donaldson SL, Stratton MS, Pavlidakey PG, Elewski BE. Image gallery: Molluscum contagiosum-like facial lesions in a patient with a renal transplant: deep dermatophytosis due to *Trichophyton rubrum*. Br J Dermatol. 2018;178(5):e328.
9. Rouzaud C, Chosidow O, Brocard A, Fraitag S, Scemla A, Anglicheau D, et al. Severe dermatophytosis in solid organ transplant recipients: a French retrospective series and literature review. Transpl Infect Dis. 2018;20(1):e12799.
10. Ponticelli C, Bencini PL. Nonneoplastic mucocutaneous lesions in organ transplant recipients. Transpl Int. 2011;24(11):1041–50.
11. Zwald F, Brown MD Advances in transplant dermatology: clinical and practical implications. New York/Berlin/Heidelberg: Springer; 2014. pages cm p.
12. Lipner SR, Scher RK. Onychomycosis: clinical overview and diagnosis. J Am Acad Dermatol. 2019;80(4):835–51.
13. Tang H, Shi W, Song Y, Han J. Voriconazole exposure and risk of cutaneous squamous cell carcinoma among lung or hematopoietic cell transplant patients: a systematic review and meta-analysis. J Am Acad Dermatol. 2019;80(2):500–7. e10
14. Naldi L, Venturuzzo A, Invernizzi P. Dermatological complications after solid organ transplantation. Clin Rev Allergy Immunol. 2018;54(1):185–212.
15. Abad CL, Razonable RR. Alpha herpes virus infections among renal transplant recipients. Semin Nephrol. 2016;36(5):344–50.
16. Ilyas M, Maganty N, Sharma A. Cutaneous infections from viral sources in solid organ transplant recipients. J Clin Virol. 2017;97:33–7.
17. Martin-Gandul C, Stampf S, Hequet D, Mueller NJ, Cusini A, van Delden C, et al. Preventive strategies against cytomegalovirus and incidence of alpha-herpesvirus infections

in solid organ transplant recipients: a nationwide cohort study. Am J Transplant. 2017;17(7): 1813–22.
18. Lee DH, Zuckerman RA, Practice ASTIDCo. Herpes simplex virus infections in solid organ transplantation – guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13526.
19. Netchiporouk E, Tchervenkov J, Paraskevas S, Sasseville D, Billick R. Evaluation of herpes simplex virus infection morbidity and mortality in pancreas and kidney-pancreas transplant recipients. Transplant Proc. 2013;45(9):3343–7.
20. Larsen HK, Thomsen LT, Haedersdal M, Dehlendorff C, Schwartz Sorensen S, Kjaer SK. Risk of genital warts in renal transplant recipients-A registry-based, prospective cohort study. Am J Transplant. 2019;19(1):156–65.
21. Meeuwis KA, Hilbrands LB, IntHout J, Slangen BF, Hendriks IM, Hinten F, et al. Cervicovaginal HPV infection in female renal transplant recipients: an observational, self-sampling based, cohort study. Am J Transplant. 2015;15(3):723–33.
22. Aggarwal R, Suri V, Awasthi S, Naru J, Nijhawan R, Minz M, et al. Prevalence and genotypes of HPV in female renal transplant recipients in North India. Int J Gynecol Pathol. 2014;33(5): 537–42.
23. Kim GY, Schmelkin LA, Davis MD, El-Azhary RA, Wieland CN, Leise MD, et al. Clinical and histopathologic manifestations of solid organ transplantation-associated graft-versus-host disease involving the skin: a single-center retrospective study. J Cutan Pathol. 2018;45(11): 817–23.
24. Nadhan KS, Larijani M, Abbott J, Doyle AM, Linfante AW, Chung CL. Prevalence and types of genital lesions in organ transplant recipients. JAMA Dermatol. 2018;154(3):323–9.
25. Pritchett EN, Doyle A, Shaver CM, Miller B, Abdelmalek M, Cusack CA, et al. Nonmelanoma skin cancer in nonwhite organ transplant recipients. JAMA Dermatol. 2016;152(12):1348–53.
26. Shahidi S, Moeinzadeh F, Mohammadi M, Gholamrezaei A. Sirolimus-based immunosuppression for treatment of cutaneous warts in kidney transplant recipients. Iran J Kidney Dis. 2011; 5(5):351–3.
27. Zuckerman RA, Limaye AP. Varicella zoster virus (VZV) and herpes simplex virus (HSV) in solid organ transplant patients. Am J Transplant. 2013;13 Suppl 3:55–66; quiz
28. Maor D, Brennand S, Goh MS, Fahey V, Tabrizi SN, Chong AH. A case of acquired epidermodysplasia verruciformis in a renal transplant recipient clearing with multimodal treatment including HPV (Gardasil) vaccination. Aust J Dermatol. 2018;59(2):147–8.
29. Mendes AD, Bittencourt Mde J, Moure ER, D'Macedo CM, Yamaki IN, Araujo DM. Acquired epidermodysplasia verruciformis in a renal transplant recipient – case report. An Bras Dermatol. 2014;89(1):144–6.
30. Sri JC, Dubina MI, Kao GF, Rady PL, Tyring SK, Gaspari AA. Generalized verrucosis: a review of the associated diseases, evaluation, and treatments. J Am Acad Dermatol. 2012;66(2):292–311.
31. Feldmeyer L, Kamarashev J, Boehler A, Irani S, Speich R, French LE, et al. Molluscum contagiosum folliculitis mimicking tinea barbae in a lung transplant recipient. J Am Acad Dermatol. 2010;63(1):169–71.
32. Piaserico S, Sandini E, Peserico A, Alaibac M. Cutaneous viral infections in organ transplant patients. G Ital Dermatol Venereol. 2014;149(4):409–15.
33. Prasad N, Jain M, Gupta A, Sharma RK, Agarwal V. An unusual case of CMV cutaneous ulcers in a renal transplant recipient and review of literature. NDT Plus. 2010;3(4):379–82.
34. Moscarelli L, Zanazzi M, Rosso G, Farsetti S, Caroti L, Annunziata F, et al. Can skin be the first site of CMV involvement preceding a systematic infection in a renal transplant recipient? NDT Plus. 2011;4(1):53–5.
35. Pinca R, Crawford RI, Au S. Cytomegalovirus scrotal ulcer in a renal transplant patient. J Cutan Med Surg. 2016;20(6):567–9.
36. Mena Lora A, Khine J, Khosrodad N, Yeldandi V. Unusual manifestations of acute cytomegalovirus infection in solid organ transplant hosts: a report of two cases. Case Rep Transplant. 2017;2017:4916973.

37. Ezzatzadegan Jahromi S, Lotfi Z, Torabi-Nezhad S, Aslani FS, Pourabbas B. A potential role for cytomegalovirus in a facial ulcer in a renal transplant recipient. Transpl Infect Dis. 2016;18(3): 457–60.
38. Kho MM, Zuijderwijk JM, van der Eijk AA, de Kuiper R, Boer-Verschragen MJ, Weimar W, et al. Humoral and cellular response after varicella vaccination in VZV IgG seronegative kidney transplant candidates. Vaccine. 2017;35(1):71–6.
39. Wang L, Verschuuren EAM, van Leer-Buter CC, Bakker SJL, de Joode AAE, Westra J, et al. Herpes zoster and immunogenicity and safety of zoster vaccines in transplant patients: a narrative review of the literature. Front Immunol. 2018;9:1632.
40. Meites E, Kempe A, Markowitz LE. Use of a 2-dose schedule for human papillomavirus vaccination – updated recommendations of the advisory committee on immunization practices. MMWR Morb Mortal Wkly Rep. 2016;65(49):1405–8.
41. Kumar D, Unger ER, Panicker G, Medvedev P, Wilson L, Humar A. Immunogenicity of quadrivalent human papillomavirus vaccine in organ transplant recipients. Am J Transplant. 2013;13(9):2411–7.
42. Ferguson SB, Gallo ES. Nonavalent human papillomavirus vaccination as a treatment for warts in an immunosuppressed adult. JAAD Case Rep. 2017;3(4):367–9.
43. Nichols AJ, Gonzalez A, Clark ES, Khan WN, Rosen AC, Guzman W, et al. Combined systemic and intratumoral administration of human papillomavirus vaccine to treat multiple cutaneous basaloid squamous cell carcinomas. JAMA Dermatol. 2018;154(8):927–30.
44. Alikhan A, Griffin JR, Newman CC. Use of Candida antigen injections for the treatment of verruca vulgaris: a two-year mayo clinic experience. J Dermatol Treat. 2016;27(4):355–8.
45. Blouin MM, Cloutier R, Noel R. Intralesional cidofovir in the treatment of cutaneous warts in a renal transplant patient. J Cutan Med Surg. 2012;16(6):462–4.
46. Cleary A, Watson R, McMahon CJ. Successful treatment of refractory cutaneous warts using topical 3% cidofovir in a child after heart transplant. J Heart Lung Transplant. 2014;33(9):971–2.
47. Herold M, Nielson C, Longo MI. Isotretinoin and candida immunotherapy for recalcitrant warts in solid organ transplant recipients. Dermatol Ther. 2019;32(2):e12803.
48. Das BB, Anton K, Soares N, Riojas S, McDermott J, Knox L, et al. Cimetidine: a safe treatment option for cutaneous warts in Pediatric Heart Transplant Recipients. Med Sci (Basel). 2018;6(2):30.
49. Fisher C, McLawhorn JM, Adotama P, Stasko T, Collins L, Levin J. Pulsed dye laser repurposed: treatment of refractory molluscum contagiosum in renal transplant patient. Transpl Infect Dis. 2019;21(2):e13036.
50. Haidar G, Singh N. Viral infections in solid organ transplant recipients: novel updates and a review of the classics. Curr Opin Infect Dis. 2017;30(6):579–88.
51. Florescu DF, Qiu F, West SB, Richards S, Florescu MC, Stevens B, et al. *Staphylococcus aureus* infections in kidney transplantation: a matched case controlled study. Scand J Infect Dis. 2012;44(6):427–32.
52. Ilyas M, Maganty N, Ginsberg Z, Sharma A. Skin Infections due to bacteria in solid organ transplant recipients: a review. Dermatology. 2017;233(5):358–65.
53. Shields RK, Clancy CJ, Minces LR, Kwak EJ, Silveira FP, Abdel Massih RC, et al. *Staphylococcus aureus* infections in the early period after lung transplantation: epidemiology, risk factors, and outcomes. J Heart Lung Transplant. 2012;31(11):1199–206.
54. Coussement J, Lebeaux D, van Delden C, Guillot H, Freund R, Marbus S, et al. Nocardia infection in solid organ transplant recipients: a multicenter European Case-control study. Clin Infect Dis. 2016;63(3):338–45.
55. Pettit CJ, Mazurek K, Kaffenberger B. Cutaneous manifestations of infections in solid organ transplant recipients. Curr Infect Dis Rep. 2018;20(7):16.
56. Kim JE, Sung H, Kim MN, Won CH, Chang SE, Lee MW, et al. Synchronous infection with *Mycobacterium chelonae* and Paecilomyces in a heart transplant patient. Transpl Infect Dis. 2011;13(1):80–3.

57. Garrison AP, Morris MI, Doblecki Lewis S, Smith L, Cleary TJ, Procop GW, et al. *Mycobacterium abscessus* infection in solid organ transplant recipients: report of three cases and review of the literature. Transpl Infect Dis. 2009;11(6):541–8.
58. Morales P, Gil A, Santos M. *Mycobacterium abscessus* infection in transplant recipients. Transplant Proc. 2010;42(8):3058–60.
59. Chatzikokkinou P, Luzzati R, Sotiropoulos K, Katsambas A, Trevisan G. Disseminated cutaneous infection with *Mycobacterium chelonae* in a renal transplant recipient. Cutis. 2015;96(5):E6–9.
60. Sarani B, Strong M, Pascual J, Schwab CW. Necrotizing fasciitis: current concepts and review of the literature. J Am Coll Surg. 2009;208(2):279–88.
61. Hizli S, Isikay S, Arikan C. Ecthyma gangrenosum in an infant after liver transplantation. Pediatr Int. 2016;58(9):950–2.
62. Ayoade F, Mada P, Joel Chandranesan AS, Alam M. Sporotrichoid skin infection caused by Nocardia brasiliensis in a Kidney Transplant Patient. Diseases. 2018;6(3):68.
63. Chikowski RE, Sprigle AM, Krishna SM, Nunley JR. Primary cutaneous Nocardia vinacea in a renal transplant recipient: report of a rare case and review of the literature. Clin Exp Dermatol. 2014;39(4):534–6.
64. Lyngdoh WV, Mawlong MG, Sailo SL, Khyriem AB. Sub-cutaneous abscess caused by Nocardia asteroides in renal transplant recipient. Indian J Med Sci. 2011;65(4):172–4.
65. Hartford OA, Pothiawala GA, Goldman GD. Ulcerated facial nodules in a renal transplant recipient – quiz case. Diagnosis: primary cutaneous nocardiosis. Arch Dermatol. 2010;146(1):81–6.
66. Ram R, Uppin S, Swarnalatha G, Desai M, Harke M, Prasad N, et al. Isolated skin ulcers due to *Mycobacterium tuberculosis* in a renal allograft recipient. Nat Clin Pract Nephrol. 2007;3(12):688–93.
67. Voigtlander T, Cornberg M, Gottlieb J, Welte T, Suerbaum S, Bange FC. When a respiratory pathogen turns to the skin: cutaneous tuberculosis in a lung transplant patient. Ther Adv Respir Dis. 2015;9(5):260–2.
68. Yodmalai S, Chiewchanvit S, Mahanupab P. Cutaneous miliary tuberculosis in a renal transplant patient: a case report and literature review. Southeast Asian J Trop Med Public Health. 2011;42(3):674–8.
69. Clancy CJ, Bartsch SM, Nguyen MH, Stuckey DR, Shields RK, Lee BY. A computer simulation model of the cost-effectiveness of routine *Staphylococcus aureus* screening and decolonization among lung and heart-lung transplant recipients. Eur J Clin Microbiol Infect Dis. 2014;33(6):1053–61.
70. Paterson DL, Rihs JD, Squier C, Gayowski T, Sagnimeni A, Singh N. Lack of efficacy of mupirocin in the prevention of infections with *Staphylococcus aureus* in liver transplant recipients and candidates. Transplantation. 2003;75(2):194–8.
71. Restrepo A, Clark NM, Infectious Diseases Community of Practice of the American Society of Transplantation. Nocardia infections in solid organ transplantation: guidelines from the Infectious Diseases Community of Practice of the American Society of Transplantation. Clin Transplant. 2019;33:e13509.
72. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis. 2014;59(2):e10–52.
73. Sullivan KM, Goldmuntz EA, Keyes-Elstein L, McSweeney PA, Pinckney A, Welch B, et al. Myeloablative autologous stem-cell transplantation for severe scleroderma. N Engl J Med. 2018;378(1):35–47.
74. Leonard A, Tisdale JF. Stem cell transplantation in sickle cell disease: therapeutic potential and challenges faced. Expert Rev Hematol. 2018;11(7):547–65.
75. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.

76. Uhm J, Hamad N, Shin EM, Michelis FV, Shanavas M, Gupta V, et al. Incidence, risk factors, and long-term outcomes of sclerotic graft-versus-host disease after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2014;20(11):1751–7.
77. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation. Curr Opin Hematol. 2012;19(4):324–35.
78. Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. Bone Marrow Transplant. 2001;27(9):893–8.
79. MacMillan ML, Robin M, Harris AC, DeFor TE, Martin PJ, Alousi A, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. Biol Blood Marrow Transplant. 2015;21(4):761–7.
80. Blanchard SK, Sullivan KM, Hooten JN, Holcomb ZE, Rowe Nichols K, Selim MA, et al. High-dose intravenous immunoglobulin as adjuvant treatment for grade IV acute cutaneous graft-versus-host disease. Br J Dermatol. 2019;181(4):869–71.
81. Cowen EW, Nguyen JC, Miller DD, McShane D, Arron ST, Prose NS, et al. Chronic phototoxicity and aggressive squamous cell carcinoma of the skin in children and adults during treatment with voriconazole. J Am Acad Dermatol. 2010;62(1):31–7.
82. Byun HJ, Yang JI, Kim BK, Cho KH. Clinical differentiation of acute cutaneous graft-versus-host disease from drug hypersensitivity reactions. J Am Acad Dermatol. 2011;65(4):726–32.
83. Friedman KJ, LeBoit PE, Farmer ER. Acute follicular graft-vs-host reaction. A distinct clinicopathologic presentation. Arch Dermatol. 1988;124(5):688–91.
84. Strong Rodrigues K, Oliveira-Ribeiro C, de Abreu Fiuza Gomes S, Knobler R. Cutaneous graft-versus-host disease: diagnosis and treatment. Am J Clin Dermatol. 2018;19(1):33–50.
85. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transplant. 2015;21(3):389–401. e1
86. Inamoto Y, Storer BE, Petersdorf EW, Nelson JL, Lee SJ, Carpenter PA, et al. Incidence, risk factors, and outcomes of sclerosis in patients with chronic graft-versus-host disease. Blood. 2013;121(25):5098–103.
87. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, et al. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. Blood. 2016;127(20):2427–38.
88. Drozd B, Andriescu E, Suarez A, De la Garza Bravo MM. Cutaneous cytomegalovirus manifestations, diagnosis, and treatment: a review. Dermatol Online J. 2019;25(1):2.
89. Choi YL, Kim JA, Jang KT, Kim DS, Kim WS, Lee JH, et al. Characteristics of cutaneous cytomegalovirus infection in non-acquired immune deficiency syndrome, immunocompromised patients. Br J Dermatol. 2006;155(5):977–82.
90. Kim JK, Ahmad A, Selim MA, Olsen EA, Cardones AR. Disseminated cutaneous cytomegalovirus infection following total body electron beam irradiation for mycosis fungoides. JAMA Dermatol. 2015;151(12):1380–1.
91. Jarvis MA, Nelson JA. Human cytomegalovirus tropism for endothelial cells: not all endothelial cells are created equal. J Virol. 2007;81(5):2095–101.
92. Weigand DA, Burgdorf WH, Tarpay MM. Vasculitis in cytomegalovirus infection. Arch Dermatol. 1980;116(10):1174–6.

Ophthalmic Infections in Transplant

64

Jacob E. Lemieux, Lucia Sobrin, and Miriam B. Barshak

Contents

Introduction 1595
Epidemiology of Ocular Infections After Organ Transplantation 1596
Ocular Anatomy 1596
Approach to the Patient with Suspected Ocular Infection 1598
Herpesvirus Infections 1598
Fungal Infections 1602
Bacterial Infections 1606
Parasitic Infections 1608
Summary and Key Points 1608
References 1609

Introduction

Ocular infections are rare but serious complications of solid organ and hematopoietic stem cell transplantation. Infection must be considered as a part of the differential diagnosis of ocular pathology after transplant, and any ocular symptoms in this patient population should prompt evaluation by an ophthalmologist. Here we summarize the epidemiology, clinical manifestations, and treatment of the most common causes of ocular infection among transplant recipients. While different frameworks may be useful for conceptualizing this class of infections, such as dividing infections by anatomic site, causative organism, or chronicity of symptoms, we subdivide this review chapter by causative organism for two reasons. First, the causative organism dictates diagnosis and

J. E. Lemieux · M. B. Barshak (✉)
Division of Infectious Disease, Massachusetts General Hospital, Boston, MA, USA
e-mail: JELEMIEUX@PARTNERS.ORG; mbarshak@partners.org

L. Sobrin
Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA, USA
e-mail: Lucia_sobrin@meei.harvard.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_63

treatment. Second, infections in immunocompromised hosts are frequently not restricted to a single anatomic subdivision within the eye. We provide easily referenced tables and, where possible, offer guiding principles for diagnosis and management.

Epidemiology of Ocular Infections After Organ Transplantation

The transplant recipient is at risk for a variety of community-acquired and opportunistic pathogens including viruses, bacteria, fungi, parasites, and mycobacteria. Several large surveys provide insight into the type, frequency, and morbidity of these infectious ocular complications after transplant. In a retrospective series from Australia, Ng et al. evaluated 860 heart, lung, and liver transplants, identifying ocular complications in 2%; among those, 65% were opportunistic infections comprised of viral retinitis (77%) and fungal chorioretinitis (22%) [1]. Papanicolaou studied 684 patients who underwent orthotopic liver transplantation in the United States and identified ocular infections in 1.3%, with cytomegalovirus (CMV) retinitis the most commonly identified infection followed by fungal endophthalmitis [2]. Fungal infections tended to occur early (mean 42 days), whereas viral infections occurred later (mean 128 days) [2]. Tarabishy et al. conducted a retrospective analysis of 545 lung transplant recipients in the United States [3]. Among 46 who underwent ophthalmologic evaluation, 6 patients with infectious ocular complications were identified, 3 had herpesvirus infections (1 CMV retinitis, 1 varicella zoster virus (VZV) keratouveitis, and 1 zoster ophthalmicus due to VZV), and 3 had fungal infections including 2 cases of disseminated *Pseudallescheria boydii* infection and 1 rhinocerebral mucormycosis. Tabbara et al. conducted a retrospective analysis of 620 patients who underwent allogeneic hematopoietic stem cell transplantation in Saudi Arabia. The authors found a similar pattern in which CMV retinitis was the most common infection (0.7%) followed by fungal endophthalmitis (0.2%) [4]. Chung et al. surveyed 1198 cases of solid organ and bone marrow transplantation, finding 21 (1.8%) infections including 11 cases of CMV retinitis, 5 cases of fungal chorioretinitis, 3 cases of acute retinal necrosis (ARN), 1 case of progressive outer retinal necrosis, and 1 case of toxoplasma retinochoroiditis [5]. In addition to these large series, case reports and smaller series describe a long list of other organisms, such as parasites, spirochetes, and atypical bacteria that have been found to cause ocular infection but appear to do so less commonly [6–11]. In summary, the available data identify ocular infectious complications in 1–2% of transplant recipients, frequently as an organ-specific manifestation of a systemic infection, and highlight the role of herpesviruses and opportunistic fungi but also a variety of other pathogens.

Ocular Anatomy

A clear understanding of ocular anatomy is essential for accurate diagnosis and treatment of infectious complications because ocular infections tend to be classified and identified by the affected anatomic site(s). The eye is divided into anterior and

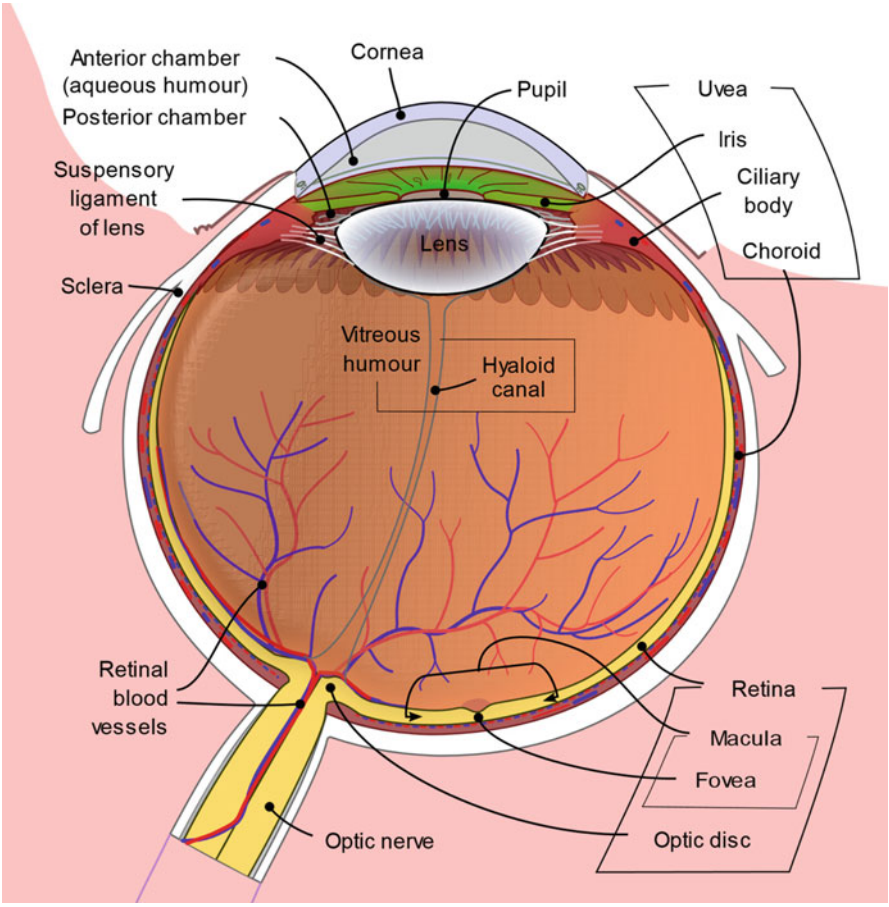


Fig. 1 Anatomy of the eye "Schematic diagram of the human eye with English annotations" by Rhcastilhos and Jmarchn is licensed under CC BY-SA 3.0. https://commons.wikimedia.org/w/index.php?curid=1597930. No changes were made. Retinitis refers to inflammation of the retina and retinal vessels. In the transplant population, retinitis is most commonly caused by herpesviruses and *Toxoplasma*. Endophthalmitis refers to inflammation of the aqueous or vitreous humors and is commonly caused by fungi such as *Candida* spp. in the transplant population. Anterior uveitis refers to inflammation of the iris and ciliary body; posterior uveitis refers to inflammation of the choroid, retina, or vitreous. Panuveitis denotes inflammation affecting both the anterior and posterior uvea. Among transplant recipients, a variety of pathogens can cause uveitis, including viruses, fastidious bacteria, mycobacteria, fungi, and parasites

posterior segments. The anterior segment contains the cornea, anterior chamber (containing the aqueous humor), iris, lens, and ciliary body. The posterior segment contains the vitreous, retina, and choroid (Fig. 1). The uvea is the term for the continuous pigmented layer of the eye that is comprised of the iris and ciliary body (anterior uvea) and choroid (posterior uvea). Anterior uveitis therefore denotes inflammation of the iris and/or ciliary body. Posterior uveitis refers to inflammation

of the choroid, retina, and/or vitreous. Panuveitis denotes inflammation involving both the anterior and posterior uvea. Endophthalmitis refers to bacterial or fungal infection of the aqueous or vitreous humors and is subdivided into endogenous endophthalmitis (from bloodstream seeding) and exogenous endophthalmitis (infection inoculated from the external world). Dacryocystitis refers to inflammation, often in the presence of obstruction, of the lacrimal sac. Since ocular infections often have characteristic exam findings that guide the differential diagnosis, it is imperative that the medical specialist clearly understands the findings of the clinical exam. Direct communication with the ophthalmologist who examined the patient is recommended, as it is, in our experience, almost always the most important source of information for diagnosing ocular infections and monitoring the response to treatment.

Approach to the Patient with Suspected Ocular Infection

Any visual symptom(s) in a transplant recipient should prompt timely evaluation by an ophthalmologist, given the wide spectrum and sight- or life-threatening nature of potential conditions. The initial ophthalmologic exam should describe the findings in detail and will often lead to a clinical diagnosis. The anatomic site(s) involved, timing after transplant, and associated systemic signs and symptoms all provide vital clues to the underlying pathophysiologic process and etiologic agent. Since the eye is frequently a site of metastatic infection from a systemic, disseminated process, a concurrent medical evaluation is essential. The results of this initial evaluation should be used to narrow and prioritize the differential diagnosis to establish a working clinical diagnosis, empiric treatment regimen, and confirmatory testing plan. If infection is being considered, an aggressive effort to obtain a diagnosis based on focused microbiologic testing should be made before initiating empiric treatment. This may include specific PCRs and serologies and/or cultures for viral causes and broad stains and cultures for bacterial, fungal, and mycobacterial causes. In cases where there is a greater degree of diagnostic uncertainty and/or the risk of missed or incorrect diagnosis is high, invasive procedures to obtain appropriate diagnostic specimens may be warranted.

Herpesvirus Infections

Herpesviruses are the most commonly reported infection in this patient group [1, 4, 5]. This is likely due to their ubiquity, propensity to reactivate, and potential to acquire resistance to antiviral drugs. Individual herpesviruses are preferentially associated with particular clinical syndromes – CMV with retinitis, VZV and HSV with ARN, VZV with zoster ophthalmicus, and HSV and VZV with keratitis – but there is also overlap between clinical syndromes caused by each pathogen, particularly in immunocompromised hosts [12–15]. A confirmed microbiological diagnosis is usually desirable and is imperative when the response to empiric treatment is inadequate. In comparison to

immunocompetent patients, the spectrum of infections is broader, and the infections may be more severe, harder to treat, and more likely to develop resistance to first-line treatments [13].

Retinitis: CMV is the most commonly identified agent in most series. CMV typically causes a characteristic retinitis that presents with floaters and visual loss. The exam demonstrates retinitis marked by hemorrhages and whitish-yellowish infiltrates and may include perivascular exudation and a granular appearance to the retinitis. CMV retinitis occurs most commonly in the late-transplant period and is associated with lymphopenia and prolonged immunosuppression [2]. Systemic illness with CMV including viremia and manifestations in other organs, such as pneumonitis, may or may not be present. Treatment is typically with intravenous ganciclovir at induction doses for 2–3 weeks, followed by a longer duration of valganciclovir if gastrointestinal absorption is adequate. For site-threatening retinitis, intravitreal ganciclovir or foscarnet should be added to systemic therapy. Implantable sustained-release ganciclovir, previously available as an adjunctive treatment option for CMV retinitis, is no longer manufactured. Treatment should be monitored with serial exams and PCR for systemic viremia. Failure to respond to these agents should prompt genotyping for resistance mutations, in accordance for CMV management among transplant recipients, discussed elsewhere in this book and in expert guidelines [16].

Acute Retinal Necrosis: ARN refers to a characteristic clinical appearance of retinitis that is most classically characterized by peripheral confluent retinitis. The pattern is not specific to herpesviruses, although they are overwhelmingly the most common etiology. Toxoplasmosis in an immunocompromised host can also present with ARN. ARN typically presents with visual loss and necrotizing retinitis, often in association with pain. It progresses to retinal detachment in at least half of patients, but this does not typically occur until 2 months after the initial infection. VZV is the most common cause of this syndrome, which results from reactivation of latent virus and may involve one or both eyes. Available data suggest that this is a late complication of transplant, like CMV retinitis (which can also present with an ARN pattern). In the series of Ng et al., five cases of ARN were observed with a mean of 540 days posttransplant [1]. Treatment with intravenous acyclovir (for HSV and VZV) or ganciclovir (for suspicion of CMV) should begin immediately once a diagnosis of ARN is suspected, as appropriate treatment has been shown to limit damage to the affected eye and prevent injury to the fellow eye [17]. If ARN is sight-threatening, intravitreal ganciclovir or foscarnet should be added to systemic therapy. ARN usually is associated with intense inflammation of the uveal tract and/or optic nerve. When the inflammation is substantial, and/or contributes to visual impairment, glucocorticoids are frequently given as an adjunct to antivirals. While there are no high-quality clinical data to guide when to administer steroids, glucocorticoids do not appear to increase the risk of retinal detachment, at least in small series. Thus, glucocorticoids may be considered as an adjunctive therapy, particularly when inflammation is substantial. Glucocorticoids should not be started until there is clear improvement on an antiviral therapy. Once infection is controlled with IV antiviral therapy, transition to oral antiviral therapy is often possible. When

possible, or infections felt likely to be from HSV or VZV, we prefer the use of valacyclovir over acyclovir and famciclovir, given the better bioavailability. Typically valganciclovir is used for oral therapy for CMV. Antiviral therapy is continued for at least 6 weeks with an oral agent, and often longer, to control the primary syndrome as well as to prevent damage to the contralateral eye. On the basis of documented efficacy for prolonged acyclovir for ARN, and efficacy of suppressive acyclovir for other conditions such as recurrent genital HSV and prevention of VZV in transplant, long-term suppressive oral antivirals may be given in the hopes of decreasing the chances of recurrent vision-threatening infection. If there is no response to acyclovir, alternate causes such as CMV or acyclovir-resistant VZV or HSV should be considered (discussed below in "Therapeutic Failure"), and additional ocular and systemic diagnostic testing may be indicated.

Progressive Outer Retinal Necrosis: A related necrotizing retinitis preferentially involving the outer retina and forming coalescent patches with minimal vitreal inflammation is known as progressive outer variant (PORN). While initially considered a variant of ARN, PORN is now recognized as a distinct clinical entity, more aggressive than ARN and with a worse visual prognosis [18]. Despite these clinical differences, PORN is caused by the same herpesviruses that cause ARN, and diagnosis and treatment are similar. PORN occurs exclusively in immunocompromised hosts, whereas ARN can occur in immunocompetent or immunocompromised individuals.

Zoster Ophthalmicus: Zoster most commonly presents as a peripheral or cranial neuritis. When this involves the ophthalmic branch of the trigeminal nerve, ocular structures can be affected, including the cornea, conjunctiva, and uvea. Vesicles on the tip of the nose (Hutchinson's sign) are suggestive of ocular involvement, as such vesicles indicate involvement of the nasociliary nerve that also innervates multiple ocular structures [19].

Keratitis: Herpesviruses may also cause a keratitis. The clinical syndrome is marked by severe pain, and HSV is the most common cause. The exam is notable for dendritic ulceration of the cornea, particularly visible with fluorescein staining. Treatment is with oral or intravenous acyclovir.

PTLD: Rarely, ocular proliferation of EBV-transformed lymphocytes may occur as a result of a systemic or localized lymphoproliferative disorder. This rare disorder requires multidisciplinary treatment that may include reduction of immunosuppression, radiation, and other therapies.

Therapeutic Failure: Treatment of ARN with acyclovir or ganciclovir usually leads to improvement or stabilization within 48–72 h even in cases of severe immunocompromised hosts [20], and the lack of such a response should raise the possibility of treatment failure. Therapeutic failure is not uncommon among herpesvirus infections, and a number of factors should be considered including suboptimal drug levels, drug resistance, or an incorrect diagnosis. *Drug levels:* Intravenous acyclovir and ganciclovir reach relatively high levels in the vitreous and retina, and oral absorption of the valylated derivatives valacyclovir and valganciclovir is greatly

improved compared with earlier agents. However, intravenous therapy remains the most reliable way to achieve consistent drug levels and should be strongly considered in treatment failure, particularly if oral absorption may vary as a result of gastrointestinal disease such as GVHD. *Drug resistance:* CMV can develop resistance to ganciclovir; in some cases, this resistance is dose-dependent and can be overcome by increasing drug doses and/or switching to intravenous therapy [21–23]. Adjunctive therapies such as CMV immunoglobulin and reduction of immunosuppressive therapy may be considered [16]. Alternate agents may also be considered, including foscarnet and letermovir, and some experts might use experimental agents such as maribavir, brincidofovir, and leflunomide. Cellular immunotherapy has been used experimentally [24]. Rarely, HSV can develop resistance to acyclovir and derivatives, in which case fewer alternative agents are available and treatment typically involves foscarnet [25, 26]. Since the genetic mutations that confer drug resistance to these agents are well understood, in most cases resistance can be evaluated by sending a genotype, which should be pursued in the case of treatment failure for a microbiologically confirmed diagnosis with adequate drug levels. *Incorrect diagnosis:* Herpesvirus syndromes overlap to a great degree, particularly in immunosuppressed transplant recipients, and other organisms including viruses, fungi, parasites, and bacteria, as well as a wide array of noninfectious causes, can mimic Herpesvirus retinitis [6, 15, 27]. Coinfection is also possible. In the case of non-response on appropriate antivirals, alternate diagnoses should be considered, and invasive procedures such as anterior chamber paracentesis, diagnostic vitrectomy, and/or retinal biopsy may be necessary to establish a diagnosis. Effective treatment is available for herpesvirus retinal infection, so treatment failure in the presence of adequate drug delivery and in the absence of drug resistance should prompt an aggressive search for another diagnosis.

Summary: Herpesviruses are the most commonly identified cause of ocular infection in transplant recipients in most large series. Herpesvirus infections may occur at any point during the posttransplant period but most commonly occur late and in the setting of heavy immunosuppression or poor immune reconstitution. Herpesviruses cause a variety of overlapping clinical syndromes, most commonly CMV retinitis but also vitritis, necrotizing retinitis, keratitis, and neuritis. While individual viruses are associated with particular syndromes, there is substantial clinical and microbiological overlap, particularly in transplant recipients, where the overlapping clinical manifestations among herpesviruses are magnified by the propensity of immunocompromised hosts to present with atypical clinical manifestations. Therefore, in the case of non-response, the initial diagnosis may need to be reassessed with additional testing. In addition to herpesviruses, retinitis and related syndromes can be caused by a wide variety of organisms including other viruses, fungi, bacteria, and parasites. Management of most cases begins with intravenous antivirals and intravitreal antivirals, followed by a switch to oral valacyclovir or valganciclovir to complete a course, as detailed in Table 1.

Table 1 Clinical syndromes, causative pathogens, and treatment strategies for infections caused by herpesviruses (medication doses assume normal renal function)

Syndromes	Pathogens	Exam features	Diagnostic tests	Treatment
Retinitis	CMV	Hemorrhages, retinal infiltrate, and may include perivascular exudates and a granular appearance to the retinitis	Serum/ ocular fluid PCR, serologies	2–3 weeks induction with ganciclovir 5 mg/kg IV q12 hr.; followed by suppressive valganciclovir 900 mg PO daily thereafter, prolonged, e.g., 3–6 months Intravitreal ganciclovir and/ or foscarnet
Acute retinal necrosis	VZV	Confluent areas of retinitis, most often in the periphery; necrosis	Serum PCR, serologies	Acyclovir 10–15 mg IV q8 hrs induction, then valacyclovir 1–2 g PO TID, at least 6 weeks; lifelong suppression is often given with acyclovir 400 mg bid particularly if immune suppression cannot be weaned. Systemic steroids are commonly used as adjunctive therapy Intravitreal foscarnet
Zoster ophthalmicus	VZV	Vesicles, conjunctivitis, iritis		Acyclovir 10 mg/kg IV q8 hrs, then valacyclovir 1000 mg PO TID 7-14d
Keratitis	HSV	Dendritic ulceration of fluorescein staining		Acyclovir 10 mg/kg IV q8 hrs, then valacyclovir 1000 mg PO TID 7–14d. Topical agents also effective, including trifluridine 1% q1–2 hr × 14 days, ganciclovir 0.15% five times daily until epithelial healing then TID × 1 week

Fungal Infections

Fungal infections are the second most common cause of ocular infections in transplant patients [28, 29]. There are several key distinctions between fungal infections and herpesvirus infections. First, in contrast to herpesvirus infections that occur commonly in both immunocompetent and immunocompromised hosts, endogenous fungal infections occur almost exclusively in immunosuppressed hosts. Second, in contrast to herpesvirus infections that tend to occur later in the transplant course and are associated with chronic immunosuppression, fungal infections most commonly present within the first few months after transplantation, around the time of greatest

Table 2 Clinical syndromes, causative pathogens, and treatment strategies for infections caused by fungi (medication doses assume normal renal function)

	Pathogens	Exam features	Diagnostic test	Treatment
Candida endophthalmitis	*Candida* spp.	Fluffy yellow, possibly vitreal infiltrates	Candida blood cultures, vitreous samples for stains/cultures/PCR; vitrectomy provides a more substantial amount of material for diagnostic studies and is associated with higher diagnostic yield than "tap and inject" samples	Systemic fluconazole; systemic (800 mg loading dose then 600 mg daily) if susceptible; otherwise voriconazole or amphotericin (echinocandins have poor ocular penetration and limited clinical efficacy for ocular infection); intravitreal voriconazole or amphotericin. Vitrectomy is often needed for debridement
Mold endophthalmitis				
	Aspergillus spp.	Fluffy yellow, possibly vitreal infiltrates	Blood cultures, vitreous samples for stains/culture/PCR; vitrectomy provides a more substantial amount of material for diagnostic studies and is associated with higher yield than "tap and inject" samples	Voriconazole or isavuconazole although there is limited clinical experience with isavuconazole for ocular infection; systemic + intravitreal. Vitrectomy is often needed for debridement
	Pseudallescheria boydii	Fluffy yellow, possibly vitreal infiltrates	Blood cultures, vitreous samples for stains/culture/PCR; vitrectomy provides a more substantial amount of material for diagnostic studies and is associated with higher yield than "tap and inject" samples	Voriconazole; systemic + intravitreal Vitrectomy is often needed for debridement

(continued)

Table 2 (continued)

	Pathogens	Exam features	Diagnostic test	Treatment
Rhinocerebral mucormycosis	Genera *Rhizopus* *Mucor* *Rhizomucor* *Cunninghamella* *Apophysomyces* *Absidia* species (now *Lichtheimia*) *Saksenaea* species	Necrotic eschar in sinuses, proptosis	Fungal stains and culture	Liposomal amphotericin 5 mg/kg systemic (some add posaconazole, isavuconazole, or micafungin) +/− emergent surgical debridement

immunosuppression; however, they may also occur late, particularly in the setting of heavy, chronic immunosuppression such as for GVHD. Third, invasive fungal infections in transplant recipients are rarely limited to the eye; typically ocular infection is a complication from a disseminated process. Aggressive treatment, including both medical and surgical treatment, may be necessary. Despite these efforts, mortality remains high in published series [28–30].

Endophthalmitis: Endogenous endophthalmitis is the most common presentation of ocular fungal infection seen in transplant recipients [4, 5, 31]. Clinical symptoms include blurred vision, pain, and redness. Ocular examination typically demonstrates white, fluffy infiltrates in the vitreous or retina. Among transplant recipients, fungal endophthalmitis is almost always endogenous endophthalmitis as a complication of a disseminated infection involving the bloodstream. Both yeasts and molds may cause endophthalmitis. Among yeasts, *Candida* spp. are the most common cause of endophthalmitis (Table 2). Among molds, *Aspergillus* spp. and *Pseudallescheria* (*Scedosporium*) spp. are reported most often in transplant patients [32]. Endophthalmitis caused by molds usually progresses rapidly and carries a graver prognosis than infection caused by yeasts. Rarely, endophthalmitis can occur without frank ocular symptoms; some cases are diagnosed at autopsy [28].

In all cases of endophthalmitis, close attention must be paid to the systemic complications and other sites of metastatic involvement. *Candida* may be associated with hepatosplenic abscesses. Abscesses in the brain, skin, and lungs are common among invasive molds [27, 33, 34]. The eye may serve as the sentinel site that signals a disseminated invasive fungal infection. The finding of endophthalmitis should trigger a detailed search for symptoms and complete physical exam including the head and neck, oral cavity, lungs, abdomen, skin, and central nervous system along with blood cultures. Conversely, findings of fungemia or evidence of disseminated fungal infection should prompt consideration of a dilated ophthalmological exam, as early fungal endophthalmitis may be asymptomatic, and some patients may not report symptoms due to medical comorbidities or diminished consciousness [28].

In some cases, surveillance examinations are warranted, especially with neutropenia, in accordance with IDSA guidelines [35].

Treatment of endogenous endophthalmitis is multidisciplinary, often medical and surgical, and depends on the causative organism and involvement of other sites [34, 36, 37]. Typically, systemic therapy is required, but intraocular therapy may also be necessary. Some cases require vitrectomy not only for diagnosis but also for treatment to debulk infectious material. Serial assessment of clinical status, including repeated fundoscopic exams, is important early in the course of therapy to evaluate the response to therapy and guide need for more alternative management strategies. With the possible exception of *Candida* endophthalmitis, oral therapy is typically reserved for step-down therapy once control of the infection has been achieved through a combination of parenteral antifungals, intraocular antifungal infections, and vitrectomy, as indicated. Ocular penetration of liposomal amphotericin B is poor, while most azoles achieve better intraocular levels, especially when the eye is inflamed [38]. Among the azoles, fluconazole and voriconazole have been used most extensively for treating endophthalmitis [39–41]. Posaconazole appears to have low vitreous concentrations, but successful use has been reported [42]. There is accumulating experience with isavuconazole [43, 44].

Therapeutic Failure: Therapeutic failure unfortunately is common in fungal endophthalmitis. Combined medical and surgical therapy is often required, and vitrectomy should be considered in all cases of fungal endophthalmitis, both for diagnostic purposes on initial evaluation and for therapeutic purposes to remove fungal debris and microabscesses [45–47]. As in the cases of therapeutic failure for herpesvirus infections, the possibility of suboptimal drug levels, drug resistance, an incorrect diagnosis, and the need for adjunctive therapies should be considered. The use of sensitivities to guide the selection of antifungal therapy and therapeutic drug monitoring can be helpful if an organism has been cultured, and therapeutic drug levels are known to correlate with improved outcome [48–50]; however, culture is not always available. Unfortunately, for some fungi such as *Scedosporium* spp., existing therapies are not fungicidal, and there is little clinical data from controlled human trials to guide antifungal agent selection, although retrospective clinical series have identified voriconazole and surgical management as associated with improved outcomes [33, 51].

Rhinocerebral Mucormycosis: Mucormycosis is a rare but devastating infectious complication of transplant that may involve the eye through direct invasion or metastatic spread of systemic infection [52]. Clinical exam may identify an eschar on mucosal surfaces such as the palate or nasal passages and/or proptosis [53, 54]. Imaging may demonstrate sinus disease extending into the orbit and/or skull base [55]. Management decisions must be made promptly as mucormycosis progresses rapidly. Treatment usually involves amphotericin-based antifungal therapy along with surgical evaluation that may include debridement with potential orbital exenteration [52, 56]. Histopathology may demonstrate thrombosis with angioinvasion. Other fungi, including *Aspergillus* spp., can cause a similar syndrome.

Summary: Endogenous fungal endophthalmitis is the second most common ocular infection among transplant recipients. In contrast to herpesvirus infections

that are typically a late complication of transplant, fungal endophthalmitis usually occurs during periods of profound immunosuppression and neutropenia. Most cases occur early, particularly around engraftment for HSCT, but may also occur late, particularly in patients with GVHD when heavier immunosuppression is required. Rhinocerebral mucormycosis is a rare but dreaded fungal infection. Fungal infections among transplant recipients are typically severe and represent a metastatic complication of a disseminated process involving the bloodstream. These infections may portend a grave prognosis; however, medical and surgical treatment strategies can be effective, so urgent or emergent evaluation and management are important to limit morbidity and mortality. For additional information on the extraocular and systemic manifestations of invasive fungal infections, please see Chaps. ▶ 44, "*Candida* Infections in Solid Organ Transplantation," ▶ 45, "*Aspergillus* in Solid Organ and Stem Cell Transplant Patients: Emerging Options for Diagnosis and Management," ▶ 46, "Management of Mucorales Infections in Transplant Patients," ▶ 47, "Non-*Aspergillus* Fungal Infections in Transplant Patients," ▶ 48, "Management of Cryptococcosis in Transplant Candidates and Recipients," ▶ 50, "Coccidioidomycosis in Transplantation," ▶ 51, "Emerging Epidemics in Transplant: Histoplasmosis, Blastomycosis, and Paracoccidioidomycosis," in this volume.

Bacterial Infections

Transplant patients are also at risk for bacterial infections. This includes Gram-positive and Gram-negative bacteria as well as atypical infections such as those caused by mycobacteria, intracellular bacteria, and spirochetes.

"Typical" bacteria: For Gram-positive and Gram-negative bacterial infections (here referred to as "typical" bacteria), the spectrum of disease resembles that of the community-acquired ocular infections that are caused by such bacteria. Ocular manifestations of bacterial infection include abscess, dacryocystitis, preseptal cellulitis, and orbital cellulitis. These infections are commonly caused by pyogenic bacteria such as *Staphylococcus* and *Streptococcus* spp. Diagnosis is guided by clinical features. Preseptal and orbital cellulitis both present with inflammation in periorbital skin and soft tissues; however, in orbital cellulitis, there are associated "orbital" signs that may include proptosis, restricted extraocular movements, and impaired visual acuity. These infections often originate in the sinuses, which should be evaluated carefully by specialists in otolaryngology as well as with imaging (typically CT) in affected patients. Abscess can occur in any of the soft tissues of the eye and face. In these cases, incision and drainage procedures are both diagnostic and therapeutic, and a course of antibiotics targeting causative pathogens should be provided to complete therapy. Empiric treatment usually targets polymicrobial oral flora using a beta-lactam plus beta-lactamase inhibitor and may also include agents active against MRSA, depending on patient and epidemiologic risk factors. Infectious dacryocystitis can result from lacrimal duct obstruction and bacterial proliferation and can be managed similar to an abscess. Dacryocystorhinostomy is often

considered following resolution of dacryocystitis. Bacterial endophthalmitis is managed similarly in immunocompetent and immunocompromised hosts.

"**Atypical" Bacterial Infections** *Mycobacterial Infections:* Tuberculosis (TB) and non-tuberculous mycobacteria (NTM) can cause serious ocular infections affecting all ocular structures [57–59]. Ocular TB is an underreported manifestation of TB and most commonly presents as choroidal nodules or scars, with or without granulomatous features; however, the clinical spectrum is broad and includes papillitis, macular lesions, vitritis, choroidal tuberculoma, dacryoadenitis, posterior scleritis, retinal vasculitis, anterior uveitis, and retinal detachment [58]. A confirmed diagnosis rests upon microbiologic or histologic identification of organism in a compatible clinical syndrome; a probable diagnosis may be made on the basis of immunoreactivity with TB antigen (positive purified protein derivative or interferon-gamma release assay) and/or chest x-ray showing granulomatous disease in the presence of compatible ocular manifestations and exclusion of other etiologies. While a thorough pretransplant screening should facilitate appropriate treatment for latent tuberculosis prior to transplant-associated immune suppression, the clinician should be aware that ocular symptoms may sometimes be the first clinical manifestation of systemic TB, particularly in transplant recipients and other immunosuppressed individuals [9]. In these cases, other manifestations of TB are frequently identified when sought, as ocular manifestations are rare in the absence of a systemic syndrome in this population. NTMs are less commonly found in ocular infections but have recently been found to cause a wide spectrum of ocular disease [59], including endophthalmitis among transplant recipients [8, 60]. Although data are limited on management of ocular TB, it is treated with the same regimens as used for active TB, typically for 9–12 months. A fluoroquinolone is sometimes substituted for ethambutol to reduce the likelihood diagnostic of confusion between infectious and drug-induced optic neuritis, which is a potential side effect of ethambutol but also a rare complication of ocular TB. For additional information on TB screening and treatment in transplant recipients, please (see ▶ Chap. 18, "*Mycobacterium tuberculosis* in Transplantation").

Fastidious bacteria: Fastidious bacteria such as spirochetes and intracellular bacteria are an increasingly recognized and likely underappreciated cause of ocular infection in the non-transplant population. For example, Bodaghi et al. studied 1321 European patients with uveitis of unknown etiology and identified infections with fastidious bacteria in 78 cases with serologies and advanced molecular techniques [61]. This included 21 cases of *Bartonella* spp., 9 cases of *Treponema pallidum,* 9 cases of *Coxiella burnetii* (agent of Q fever), and 14 cases of *Borrelia burgdorferi* (the agent of Lyme disease), as well as a number of other agents in smaller numbers. Case series and reports from the transplant literature also suggest that fastidious organisms are important in transplant patients. For example, *B. henselae* was reported as a cause of chorioretinitis in a renal transplant patient in 2008 [62] and has since been recognized in two additional cases [10]. *Tropheryma whipplei* (the causative agent of Whipple's disease) has been reported in a single case of uveitis in a renal transplant recipient. In light of these emerging data, infection with fastidious bacteria should be considered in the differential diagnosis for unexplained ocular infection in transplant recipients.

Parasitic Infections

Toxoplasma chorioretinitis: The Apicomplexan parasite *Toxoplasma gondii* is an important cause of chorioretinitis in the general population and among transplant recipients [6, 7, 63]. *T. gondii* can cause a severe retinochoroiditis that is easily and commonly confused with herpetic retinitis, fungal endophthalmitis, and non-infectious retinopathies [7, 64]. In immunocompetent hosts, the retinitis is often focal and in cases of reactivation, adjacent to a pre-existing retinal scar. In immunocompromised hosts, atypical presentations with larger areas of retinitis can be seen [64]. Clinicians should consider *T. gondii* as a potential cause of necrotizing retinitis, particularly if risk factors such as immigration from an endemic area, cat exposure, or consumption of raw meat are present [65, 66]. The diagnosis of ocular toxoplasmosis is made on the basis of clinical, serological, microbiological, and histopathological grounds, although it is important to recognize that the clinical presentation and immune response allowing serodiagnosis may be atypical or absent in immunocompromised transplant recipients. When the diagnosis is in question, direct microbiological assessment of anterior chamber and vitreous fluid by PCR should be performed [67]. Treatment is effective using a variety of agents, including sulfadiazine-pyrimethamine, trimethoprim-sulfamethoxazole, clindamycin, azithromycin, and atovaquone [68, 69]. For hard-to-treat cases, intravitreal injections of antiparasitic agents such as clindamycin or trimethoprim-sulfamethoxazole may be considered, although there are limited data to guide therapy in refractory cases.

Other parasites: A variety of other less-common parasitic infections have the potential to cause ocular disease among transplant recipients. *Leishmania* parasites can reactivate during immunosuppression, causing systemic illness which may include ocular manifestations [11]. Free-living *Acanthamoeba* spp. cause disease in immunocompetent contact lens wearers and have been reported as a cause of endophthalmitis following transplant as a part of a disseminated infection [70].

Summary and Key Points

Ophthalmologic infections are an uncommon but serious complication after transplant. Historical series estimate the rate of symptomatic ocular infection among transplant recipients at approximately 1–2% [1, 4, 5]. We have reviewed the epidemiology, presentation, and management of the most common causes of ocular infection in transplant recipients. In doing so, some general themes emerge: First, the epidemiology of ocular infections in this patient population indicates that fungal and viral opportunists cause the majority of infectious pathology in this group. Herpesviruses tend to involve the retina and surrounding structures and affect patients late in the transplant course, particularly those with chronic, more intense immunosuppression. In contrast, fungal infections preferentially seed the vitreous and adjacent structures via the bloodstream, a process which tends to occur early in the posttransplant course during the period of maximal immunosuppression. Nevertheless, there is a long list of other infections that may present with overlapping

clinical manifestations and must also be considered, particularly if initial or empiric treatment fails. Second, the nature of these infections requires multidisciplinary collaboration between ophthalmologists, transplant specialists, and infectious disease specialists. Finally, ocular infection is frequently a complication of systemic infection and should trigger an evaluation for systemic disease and other metastatic foci of infection.

As with all infections in the immunocompromised population, ocular infections should trigger careful workup and antimicrobial treatment as well as consideration about whether immune function can be improved by weaning immunosuppressive agents. More research is needed to define further the spectrum of ocular pathology among transplant recipients and to create new therapies that improve unsatisfactory rates of cure, particularly for fungal infections. Despite these challenges, effective treatment is available for a great many of the ocular infections encountered in transplant recipients. An understanding of the epidemiological and clinical patterns of disease along with a considered, timely, and multidisciplinary approach to diagnosis and treatment will limit the morbidity and mortality of this potentially devastating complication of organ transplantation.

Acknowledgments The authors gratefully acknowledge Ramy Elshaboury, PharmD, for guidance with pharmacotherapy administration and dosing.

References

1. Ng P, McCluskey P, McCaughan G, Glanville A, MacDonald P, Keogh A. Ocular complications of heart, lung, and liver transplantation. Br J Ophthalmol. 1998;82(4):423–8. PMCID: PMC1722568
2. Papanicolaou GA, Meyers BR, Fuchs WS, Guillory SL, Mendelson MH, Sheiner P, Emre S, Miller C. Infectious ocular complications in orthotopic liver transplant patients. Clin Infect Dis. 1997;24(6):1172–7. PMID: 9195078
3. Tarabishy AB, Khatib OF, Nocero JR, Budev M, Kaiser PK. Ocular complications in patients with lung transplants. Br J Ophthalmol. 2011;95(9):1295–8. PMID: 21131376
4. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, Ayas M, Chaudhri N, Al-Sharif F, Al-Zahrani H, Mohammed SY, Nassar A, Aljurf M. Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology. 2009;116(9):1624–9. PMID: 19729097
5. Chung H, Kim K-H, Kim J-G, Lee SY, Yoon YH. Retinal complications in patients with solid organ or bone marrow transplantations. Transplantation. 2007;83(6):694–9. PMID: 17414700
6. Conrath J, Mouly-Bandini A, Collart F, Ridings B. Toxoplasma gondii retinochoroiditis after cardiac transplantation. Graefes Arch Clin Exp Ophthalmol. 2003;241(4):334–8. PMID: 12719997
7. Pauleikhoff D, Messmer E, Beelen DW, Foerster M, Wessing A. Bone-marrow transplantation and toxoplasmic retinochoroiditis. Graefes Arch Clin Exp Ophthalmol. 1987;225(3):239–43. PMID: 3301551
8. Modi D, Pyatetsky D, Edward DP, Ulanski LJ, Pursell KJ, Tessler HH, Goldstein DA. Mycobacterium haemophilum: a rare cause of endophthalmitis. Retina. 2007;27(8):1148–51. PMID: 18040262
9. Mehta S, Suratkal L. Ophthalmoscopy in the early diagnosis of opportunistic tuberculosis following renal transplant. Indian J Ophthalmol. 2007;55(5):389–91. PMCID: PMC2636020

10. Lee RA, Ray M, Kasuga DT, Kumar V, Witherspoon CD, Baddley JW. Ocular bartonellosis in transplant recipients: two case reports and review of the literature. Transpl Infect Dis. 2015;17(5):723–7. PMID: 26146758
11. Gontijo CMF, Pacheco RS, Orefice F, Lasmar E, Silva ES, Melo MN. Concurrent cutaneous, visceral and ocular leishmaniasis caused by Leishmania (Viannia) braziliensis in a kidney transplant patient. Mem Inst Oswaldo Cruz. 2002;97(5):751–3. PMID: 12219147
12. Holland GN. The progressive outer retinal necrosis syndrome [Internet]. Int Ophthalmol. 1994:163–165. Available from: https://doi.org/10.1007/bf00915966
13. PMK T, Hooper CY, Lightman S. Antiviral selection in the management of acute retinal necrosis. Clin Ophthalmol. 2010;4:11–20. PMCID: PMC2819764
14. Ganatra JB, Chandler D, Santos C, Kuppermann B, Margolis TP. Viral causes of the acute retinal necrosis syndrome. Am J Ophthalmol. 2000;129(2):166–72. PMID: 10682968
15. Balansard B, Bodaghi B, Cassoux N, Fardeau C, Romand S, Rozenberg F, Rao NA, Lehoang P. Necrotising retinopathies simulating acute retinal necrosis syndrome. Br J Ophthalmol. 2005;89(1):96–101. PMCID: PMC1772458
16. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, Humar A, The Transplantation Society International CMV Consensus Group. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102(6):900–31. PMID: 29596116
17. Palay DA, Sternberg P Jr, Davis J, Lewis H, Holland GN, Mieler WF, Jabs DA, Drews C. Decrease in the risk of bilateral acute retinal necrosis by acyclovir therapy. Am J Ophthalmol. 1991;112(3):250–5. PMID: 1882936
18. Austin RB. Progressive outer retinal necrosis syndrome: a comprehensive review of its clinical presentation, relationship to immune system status, and management. Clin Eye Vis Care. 2000;12(3–4):119–29. PMID: 11137426
19. Zaal MJW, Völker-Dieben HJ, D'Amaro J. Prognostic value of Hutchinson's sign in acute herpes zoster ophthalmicus. Graefes Arch Clin Exp Ophthalmol. 2003;241(3):187–91. PMID: 12644941
20. Ormerod LD, Larkin JA, Margo CA, Pavan PR, Menosky MM, Haight DO, Nadler JP, Yangco BG, Friedman SM, Schwartz R, Sinnott JT. Rapidly progressive herpetic retinal necrosis: a blinding disease characteristic of advanced AIDS. Clin Infect Dis. 1998;26(1):34–45; discussion 46–7. PMID: 9455507
21. Chou SW. Cytomegalovirus drug resistance and clinical implications. Transpl Infect Dis. 2001;3(Suppl 2):20–4. PMID: 11926745
22. Jabs DA, Martin BK, Forman MS, Ricks MO, Cytomegalovirus Retinitis and Viral Resistance Research Group. Cytomegalovirus (CMV) blood DNA load, CMV retinitis progression, and occurrence of resistant CMV in patients with CMV retinitis [Internet]. J Infect Dis. 2005:640–649. Available from: https://doi.org/10.1086/432012
23. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev. 2010;23(4):689–712. PMCID: PMC2952978
24. Einsele H, Roosnek E, Rufer N, Sinzger C, Riegler S, Löffler J, Grigoleit U, Moris A, Rammensee H-G, Kanz L, Kleihauer A, Frank F, Jahn G, Hebart H. Infusion of cytomegalovirus (CMV)–specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood. 2002;99(11):3916–22. American Society of Hematology. PMID: 12010789
25. Frobert E, Burrel S, Ducastelle-Lepretre S, Billaud G, Ader F, Casalegno J-S, Nave V, Boutolleau D, Michallet M, Lina B, Morfin F. Resistance of herpes simplex viruses to acyclovir: an update from a ten-year survey in France. Antivir Res. 2014;111:36–41. PMID: 25218782
26. Morfin F, Thouvenot D. Herpes simplex virus resistance to antiviral drugs. J Clin Virol. 2003;26(1):29–37. PMID: 12589832
27. McKelvie PA, Wong EY, Chow LP, Hall AJ. Scedosporium endophthalmitis: two fatal disseminated cases of Scedosporium infection presenting with endophthalmitis. Clin Exp Ophthalmol. 2001;29(5):330–4. PMID: 11720162
28. Hunt KE, Glasgow BJ. Aspergillus endophthalmitis. An unrecognized endemic disease in orthotopic liver transplantation. Ophthalmology. 1996;103(5):757–67. PMID: 8637684

29. Vergoulidou M, Krause L, Foerster MH, Thiel E, Schwartz S. Endogenous filamentous fungal endophthalmitis–single-centre survey in patients with acute leukaemia or postallogeneic stem cell transplantation and review of the literature. Mycoses. 2011;54(6):e704–11. Wiley Online Library
30. Sahi H, Avery RK, Minai OA, Hall G, Mehta AC, Raina P, Budev M. Scedosporium apiospermum (Pseudallescheria boydii) infection in lung transplant recipients. J Heart Lung Transplant. 2007;26(4):350–6. PMID: 17403476
31. Porter R, Crombie AL, Gardner PS, Uldall RP. Incidence of ocular complications in patients undergoing renal transplantation. Br Med J. 1972;3(5819):133–6. PMCID: PMC1788625
32. Kalkanci A, Ozdek S. Ocular fungal infections. Curr Eye Res. 2011;36(3):179–89. PMID: 21158591
33. Panackal AA, Marr KA. Scedosporium/Pseudallescheria infections. Semin Respir Crit Care Med. 2004;25(2):171–81. PMID: 16088460
34. Trief D, Gray ST, Jakobiec FA, Durand ML, Fay A, Freitag SK, Lee NG, Lefebvre DR, Holbrook E, Bleier B, Sadow P, Rashid A, Chhabra N, Yoon MK. Invasive fungal disease of the sinus and orbit: a comparison between mucormycosis and Aspergillus. Br J Ophthalmol. 2016;100(2):184–8. PMID: 26112869
35. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1–50. PMCID: PMC4725385
36. Cornely OA. Aspergillus to zygomycetes: causes, risk factors, prevention, and treatment of invasive fungal infections [Internet]. Infection. 2008:296–313. Available from: https://doi.org/10.1007/s15010-008-7357-z
37. Peterson KL, Wang M, Canalis RF, Abemayor E. Rhinocerebral mucormycosis: evolution of the disease and treatment options. Laryngoscope. 1997;107(7):855–62. PMID: 9217119
38. Felton T, Troke PF, Hope WW. Tissue penetration of antifungal agents. Clin Microbiol Rev. 2014;27(1):68–88. PMCID: PMC3910906
39. Riddell J 4th, Comer GM, Kauffman CA. Treatment of endogenous fungal endophthalmitis: focus on new antifungal agents. Clin Infect Dis. 2011;52(5):648–53. PMID: 21239843
40. Hariprasad SM, Mieler WF, Lin TK, Sponsel WE, Graybill JR. Voriconazole in the treatment of fungal eye infections: a review of current literature. Br J Ophthalmol. 2008;92(7):871–8. PMID: 18577634
41. Eltoukhy NS, Crank CW. Antifungal distribution into cerebrospinal fluid, vitreous humor, bone, and other difficult sites. Curr Fungal Infect Rep. 2010;4(2):111–9.
42. Sponsel WE, Graybill JR, Nevarez HL, Dang D. Ocular and systemic posaconazole (SCH-56592) treatment of invasive Fusarium solani keratitis and endophthalmitis. Br J Ophthalmol. 2002;86(7):829–30. BMJ Publishing Group Ltd
43. Guest J, Singh PK, Revankar SG, Chandrasekar PH, Kumar A. Therapeutic efficacy of isavuconazole in experimental Aspergillus fumigatus endophthalmitis. Open Forum Infect Dis. 2017;4(Suppl 1):S282. Oxford University Press. PMCID: PMC5631991
44. Guest JM, Singh PK, Revankar SG, Chandrasekar PH, Kumar A. Isavuconazole for treatment of experimental fungal endophthalmitis caused by Aspergillus fumigatus. Antimicrob Agents Chemother [Internet]. 2018;62(11). Available from: https://doi.org/10.1128/AAC.01537-18 PMCID: PMC6201095
45. Essman TF, Flynn HW Jr, Smiddy WE, Brod RD, Murray TG, Davis JL, Rubsamen PE. Treatment outcomes in a 10-year study of endogenous fungal endophthalmitis. Ophthalmic Surg Lasers. 1997;28(3):185–94. PMID: 9076791
46. Celiker H, Kazokoglu H. The role of pars plana vitrectomy in the management of fungal endogenous endophthalmitis. Eur J Ophthalmol. 2018;26:1120672118815105. PMID: 30474397
47. Lingappan A, Wykoff CC, Albini TA, Miller D, Pathengay A, Davis JL, Flynn HW Jr. Endogenous fungal endophthalmitis: causative organisms, management strategies, and visual acuity outcomes. Am J Ophthalmol. 2012;153(1):162–6.e1. PMID: 21917234

48. Martin-Vicente A, Guarro J, González GM, Lass-Flörl C, Lackner M, Capilla J. Voriconazole MICs are predictive for the outcome of experimental disseminated scedosporiosis [Internet]. J Antimicrob Chemother. 2016:dkw532. Available from: https://doi.org/10.1093/jac/dkw532
49. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann J-W, Kern WV, Marr KA, Ribaud P, Lortholary O, Sylvester R, Rubin RH, Wingard JR, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar PH, Hodges MR, Schlamm HT, Troke PF, de Pauw B, Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med. 2002;347(6):408–15. PMID: 12167683
50. Tortorano AM, Richardson M, Roilides E, van Diepeningen A, Caira M, Munoz P, Johnson E, Meletiadis J, Pana Z-D, Lackner M, Verweij P, Freiberger T, Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, Lanternier F, Pagano L, Skiada A, Akova M, Arendrup MC, Boekhout T, Chowdhary A, Cuenca-Estrella M, Guinea J, Guarro J, de Hoog S, Hope W, Kathuria S, Lortholary O, Meis JF, Ullmann AJ, Petrikkos G, Lass-Flörl C, European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group, European Confederation of Medical Mycology. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: Fusarium spp., Scedosporium spp. and others. Clin Microbiol Infect. 2014;20(Suppl 3):27–46. PMID: 24548001
51. Musk M, Chambers D, Chin W, Murray R, Gabbay E. Successful treatment of disseminated scedosporium infection in 2 lung transplant recipients: review of the literature and recommendations for management. J Heart Lung Transplant. 2006;25(10):1268–72. PMID: 17045941
52. Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis. 2012;54(Suppl 1):S23–34. PMID: 22247442
53. Van der Westhuijzen AJ, Grotepass FW, Wyma G, Padayachee A. A rapidly fatal palatal ulcer: rhinocerebral mucormycosis. Oral Surg Oral Med Oral Pathol. 1989;68(1):32–6. PMID: 2666896
54. Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Kong DCM, Chen SC-A. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clin Microbiol Infect. 2019;25(1):26–34. PMID: 30036666
55. Therakathu J, Prabhu S, Irodi A, Sudhakar SV, Yadav VK, Rupa V. Imaging features of rhinocerebral mucormycosis: a study of 43 patients. Egypt J Radiol Nucl Med. 2018;49(2):447–52.
56. Jiang N, Zhao G, Yang S, Lin J, Hu L, Che C, Wang Q, Xu Q. A retrospective analysis of eleven cases of invasive rhino-orbito-cerebral mucormycosis presented with orbital apex syndrome initially. BMC Ophthalmol. 2016;16:10. PMCID: PMC4709997
57. Muñoz P, Rodríguez C, Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. Clin Infect Dis. 2005;40(4):581–7. PMID: 15712081
58. Bouza E, Merino P, Muñoz P, Sanchez-Carrillo C, Yáñez J, Cortés C. Ocular tuberculosis. A prospective study in a general hospital. Medicine. 1997;76(1):53–61. PMID: 9064488
59. Moorthy RS, Valluri S, Rao NA. Nontuberculous mycobacterial ocular and adnexal infections. Surv Ophthalmol. 2012;57(3):202–35. PMID: 22516536
60. Ambler JS, Meisler DM, Nicholas Zakov Z, Hall GS, Spech TJ. Endogenous Mycobacterium chelonae endophthalmitis [Internet]. Am J Ophthalmol. 1989:338–339. Available from: https://doi.org/10.1016/0002-9394(89)90136-0
61. Bodaghi B, Drancourt M, Cassoux N, Raoult D, Lehoang P. Uveitis caused by fastidious bacteria [Internet]. Acta Ophthalmologica. 2009:0–0. Available from: https://doi.org/10.1111/j.1755-3768.2009.4453.x
62. Patel SJ, Petrarca R, Shah SM, Zimmer-Galler I, Janjua KA, Do DV, Nguyen QD. Atypical Bartonella henselae chorioretinitis in an immunocompromised patient. Ocul Immunol Inflamm. 2008;16(1):45–9. PMID: 18379943

63. Roemer E, Blau IW, Basara N, Kiehl MG, Bischoff M, Günzelmann S, Kirsten D, Sanchez H, Wocker EL, Fauser AA. Toxoplasmosis, a severe complication in allogeneic hematopoietic stem cell transplantation: successful treatment strategies during a 5-year single-center experience. Clin Infect Dis. 2001;32(1):E1–8. PMID: 11118397
64. Elkins BS, Holland GN, Opremcak EM, Dunn JP Jr, Jabs DA, Johnston WH, Green WR. Ocular toxoplasmosis misdiagnosed as cytomegalovirus retinopathy in immunocompromised patients. Ophthalmology. 1994;101(3):499–507. PMID: 8127570
65. Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG. Risk factors for Toxoplasma gondii infection in the United States. Clin Infect Dis. 2009;49(6):878–84. PMID: 19663709
66. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. Toxoplasma gondii infection in the United States: seroprevalence and risk factors. Am J Epidemiol. 2001;154(4):357–65. PMID: 11495859
67. Robert-Gangneux F, Sterkers Y, Yera H, Accoceberry I, Menotti J, Cassaing S, Brenier-Pinchart M-P, Hennequin C, Delhaes L, Bonhomme J, Villena I, Scherer E, Dalle F, Touafek F, Filisetti D, Varlet-Marie E, Pelloux H, Bastien P. Molecular diagnosis of toxoplasmosis in immunocompromised patients: a 3-year multicenter retrospective study. J Clin Microbiol. 2015;53(5):1677–84. PMCID: PMC4400795
68. Casoy J, Nascimento H, Silva LMP, Fernández-Zamora Y, Muccioli C, de Dias JRO, Nóbrega MJ, HAJ N, Zummo J, Belfort R Jr. Effectiveness of treatments for ocular toxoplasmosis. Ocul Immunol Inflamm. 2019;26:1–7. PMID: 30806556
69. Joynson DHM, Wreghitt TG. Toxoplasmosis: a comprehensive clinical guide. Cambridge, UK: Cambridge University Press; 2005.
70. Kutner A, Aldrich M, Patel S, Kang JJ, Amin B, Mann R, Ali IKM, Martines RB, Cope JR, De Boccardo GO, Puius YA. Acanthamoeba endophthalmitis during treatment for cutaneous disease in a renal transplant patient. Transpl Infect Dis. 2018;20(2):e12843. PMCID: PMC5973505

Pulmonary Syndromes in Transplantation

65

Sonya Trinh and Alfred Luk

Contents

Introduction 1615
Solid Organ Transplantation 1616
Infectious Pulmonary Complications 1616
Noninfectious Pulmonary Complications 1619
Hematopoietic Stem Cell Transplantation 1622
Infectious Pulmonary Complications 1622
Noninfectious Pulmonary Complications 1625
Evaluation of Patient with Pulmonary Symptoms 1626
Recipient and Donor Epidemiologic Exposures 1626
Diagnostic Evaluation 1627
Imaging 1630
Empiric Antimicrobial Therapy 1631
Conclusions 1632
Key Points 1632
Cross-References 1633
References 1633

Introduction

Infectious and noninfectious pulmonary syndromes remain significant contributors to morbidity and mortality after solid organ and hematopoietic stem cell transplantation [1–3]. Multiple factors increase the risk pulmonary complications including

S. Trinh (✉)
Department of Infectious Diseases, Ochsner Health, New Orleans, LA, USA
e-mail: sonya.trinh@ochsner.org

A. Luk
Department of Medicine, Adult Infectious Diseases Section, Tulane University School of Medicine, New Orleans, LA, USA
e-mail: aluk@tulane.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_64

the surgical trauma from solid organ transplantation (SOT), chemotherapy and radiation therapy prior to hematopoietic stem cell transplantation (HSCT), the degree of immunosuppression, and the immune responses leading to organ rejection and graft-versus-host disease (GVHD) [1, 2]. The first section of the chapter reviews the timeline of pulmonary syndromes among SOT and HSCT patients. The second section provides an approach to the evaluation of a transplant patient presenting with pulmonary symptoms or infiltrates. Chapters on specific pulmonary pathogens should be referenced for additional information on clinical presentation, diagnostic evaluation, and management.

Solid Organ Transplantation

Infectious Pulmonary Complications

The spectrum of microorganisms responsible for pulmonary infections after SOT has increased with the introduction of new diagnostic assays, the emergence of antimicrobial-resistant organisms, and advances in surgical technique and immunosuppressive therapies leading to prolonged patient survival [4]. Pulmonary infections present in a predictable pattern related to the changing risk factors in the recipient over time: the time since surgery or transplant, degree of immunosuppression, prophylactic antibiotics, and hospital and community exposures [4]. The timeline of pulmonary infections after SOT is classified into three risk periods (Fig. 1):

Phase 1: Perioperative period to 30 days after SOT
Phase II: 1 to 6–12 months after SOT
Phase II: Beyond 6–12 months after SOT [4]

Variations in the incidence, timeline, and microbiology of pulmonary infections exist based on the type of organ transplanted. Lung transplant recipients have the highest incidence of pneumonia related to surgical trauma to the allograft, donor-derived infections, recipient airway colonization, and constant exposure of the allograft to the environment [5].

Phase I: Perioperative Period to 30 Days After SOT

During the first risk period after SOT, pulmonary infections are primarily related to surgery and hospitalization [6–10]. Early postoperative pneumonias are primarily caused by healthcare and ventilator-associated pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and antimicrobial-resistant gram-negative bacilli (*Pseudomonas* species, *Stenotrophomonas* species). Risk factors for pneumonia with multidrug-resistant organisms (MDRO) include prolonged hospitalization pretransplant, extended postoperative mechanical ventilation, and preexisting lung pathology (chronic obstructive pulmonary disease and bronchiectasis). Aspiration pneumonia may occur in patients who are unable to protect their airways

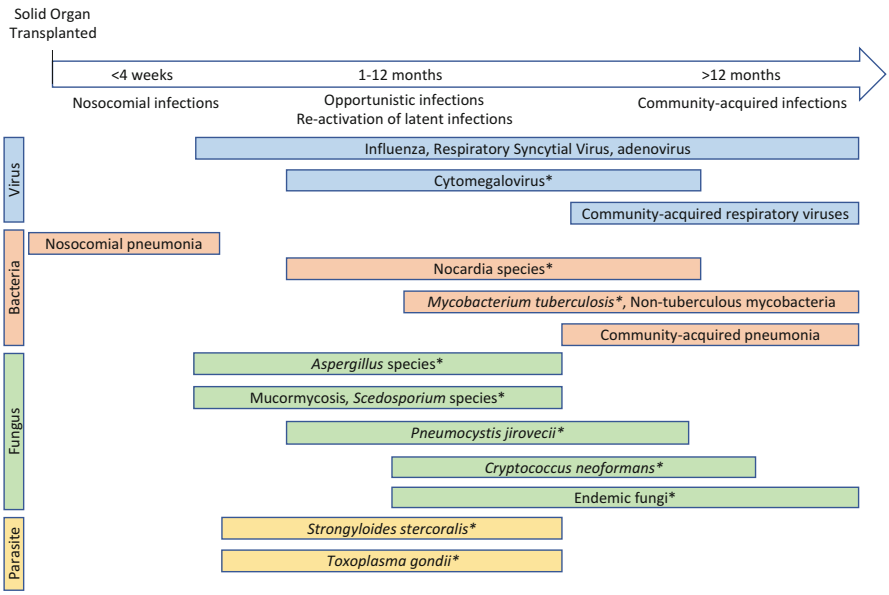


Fig. 1 Timeline of infectious pulmonary complications after solid organ transplant [3]. *Prophylaxis alters risk and the timeline

because of altered mental status, debilitation, or renal or hepatic dysfunction after transplant [6–10].

Lung transplant recipients are at increased risk of postoperative nosocomial pneumonia related to the surgical trauma of the transplanted lung, prolonged intubation, and preexisting colonization of donor and recipient lungs with MDRO [5, 11, 12]. Surgical disruption of the lung allograft vasculature and innervation compromises the anatomical and functional protections against pulmonary infection such as ciliary function, airway patency, and cough reflexes [5]. In the early postoperative period, the diminished vascular supply to the bronchial anastomosis leads to mucosal ischemia placing the area at increased risk of bacterial and fungal infections [13].

Phase II: 1 to 6–12 Months After SOT

The second risk period spans 1 to 6–12 months after SOT when the maximal sustained immunosuppression is administered, leading to the emergence of opportunistic infections and reactivation of latent infections [4]. Antimicrobial prophylaxis reduces the risk of opportunistic infections; however, excessive prolonged use is discouraged given adverse effects and selective pressure towards antimicrobial-resistant pathogens. Protocols for the indication and duration of prophylaxis vary by transplant center. Once antimicrobial prophylaxis is discontinued, opportunistic infections should be considered in the evaluation of pulmonary symptoms [4].

Trimethoprim-sulfamethoxazole (TMP-SMX) administered daily prevents pneumonia from *Pneumocystis jirovecii*, *Toxoplasma gondii*, and *Nocardia* species [14]. The risk for *P. jirovecii* pneumonia (PJP) is highest during the first 6 months

and declines after the first year [14]. Among lung transplant recipients, the risk remains elevated indefinitely, leading to the practice of lifelong prophylaxis with TMP-SMX [15]. Similarly, antifungal agents are used for prophylaxis in some lung transplant programs as recipients are at higher risk for fungal pneumonia; they are less commonly used with other organ transplants [16]. Valganciclovir prevents pneumonia from the herpes viruses cytomegalovirus (CMV), varicella zoster virus (VZV), and herpes simplex virus (HSV). The risk for CMV infection depends on the recipient and donor serostatus: seronegative recipients who receive an allograft from a seropositive donor (CMV D + R-) have the highest risk for CMV infection followed by CMV D + R+, CMV D-R+, and CMV D-R- patients [17]. CMV pneumonitis presents most frequently among lung transplant recipients since the lungs are a large reservoir for latent CMV [5].

During the second risk period, causes of viral pneumonia include community-acquired viruses (adenovirus, influenza, parainfluenza, respiratory syncytial virus [RSV], metapneumovirus) and herpes viruses (CMV, HSV, VZV). Community-acquired viruses present in the general population as a mild self-limited upper respiratory tract illness but tend to cause a more severe lower respiratory tract infection among SOT recipients [18–21]. Respiratory viruses may increase the risk of chronic allograft rejection among lung transplant recipients related to the stimulation of alloimmune processes targeting the bronchial epithelium [22].

Invasive fungal infections in the second risk period may present as a primary infection or invasion of a previously colonizing organism. The most common fungal pneumonias during the second risk period include *Aspergillus* species, *Cryptococcus neoformans*, and *P. jirovecii* [23, 24]. Lung transplant recipients are at increased risk for endobronchial aspergillosis where devitalized cartilage at the bronchial anastomosis provides a hospitable environment [13]. Other causes of fungal pneumonia to consider include mucormycosis, *Scedosporium*, *Fusarium*, and endemic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*) [23, 25].

In the second risk period, pneumonia may be caused by reactivation of latent infections including *Strongyloides stercoralis*, *T. gondii*, and *Mycobacterium tuberculosis. Strongyloides* hyperinfection syndrome occurs when filariform larvae reactivate in the setting of immunosuppression, penetrate the intestinal wall, and disseminate to the lungs causing pulmonary hemorrhage, pneumonitis, and edema [26]. Patients at the highest risk of *T. gondii* infection are seronegative heart and lung transplant recipients who receive an allograft from a seropositive donor [27]. Tuberculosis is a relatively uncommon infection in developed countries after transplant, but the rate of infection among SOT recipients is 30- to 100-fold higher than the general population [28]. While reactivation of latent infection is the primary mechanism for the development of active tuberculosis, donor-derived infection and primary infection after transplant have also been described [28]. The treatment of tuberculosis in SOT recipients is challenging because of the drug interactions between combination therapy and immunosuppression: rifampin is an inducer of the hepatic P-450 enzyme system and increases clearance of cyclosporine and tacrolimus [28].

Phase III: Beyond 6–12 Months After SOT

At 6–12 months after SOT, the allograft function stabilizes, and immunologic tolerance develops, allowing for a reduction in maintenance immunosuppression [4]. During the third risk period, the most common causes of pneumonia are community-acquired pathogens including *S. pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*. The differential diagnosis should remain broad depending on the patient's occupational, environmental, and travel exposures (see section "Donor and Recipient Epidemiologic Exposures"). SOT recipients with prolonged hospitalizations and recurrent courses of antimicrobial therapy remain at risk for antimicrobial-resistant bacterial pneumonias. Opportunistic infections should be considered during any period of augmented immunosuppression for the treatment of acute or chronic rejection [6–10].

Noninfectious Pulmonary Complications

Lung Transplant Complications

Airway Complications

Airway complications after lung transplantation result from decreases in systemic blood supply to the anastomosis between the donor and recipient bronchus during surgery [13]. In the first month posttransplant, the ischemic injury places the airway at risk for multiple complications ranging from focal necrosis with mucosal sloughing to partial or complete dehiscence of the anastomosis [13]. In the several months after transplant, excessive granulation tissue deposited in ischemic areas of the bronchial anastomosis can lead to airway stenosis or bronchomalacia (narrowing of the airway lumen by more than 50% during expiration) [13]. Patients with airway complications present with dyspnea, stridor, wheezing, recurrent episodes of postobstructive pneumonia, and progressive airflow obstruction on spirometry [13]. Diagnosis is confirmed with direct visualization on bronchoscopy [13]. Bronchial anastomotic narrowing is addressed with a variety bronchoscopic techniques: balloon dilatation, forceps debridement, laser therapy, cryosurgery, and endobronchial stenting [13]. Surgical management options include sleeve resection of stenotic area, lobectomy, and re-transplantation [13]. Bronchopleural, bronchomediastinal, and bronchovascular fistulas are rare airway complications that can lead to abscesses requiring immediate drainage and antimicrobial therapy [13].

Primary Lung Graft Dysfunction

After lung transplantation, a mild transient pulmonary edema develops related to multiple factors including ischemia-reperfusion injury, increases in microvascular permeability, surgical trauma, and lymphatic disruption [29]. Progression of lung allograft injury results in a type of acute respiratory distress syndrome (ARDS) termed primary graft dysfunction [29]. A diagnosis of primary graft dysfunction is defined as impaired oxygenation within the first 72 h of transplantations with

widespread radiographic infiltrates and exclusion of other causes of early graft dysfunction such as volume overload, pneumonia, and rejection [29]. Additional clinical findings include decreased pulmonary compliance, increased pulmonary vascular resistance, and intrapulmonary shunting [29]. Treatment is supportive with lung-protective ventilation using low tidal volumes and optimal fluid management [30]. The administration of inhaled nitric oxide or epoprostenol can decrease pulmonary artery pressures and improve oxygenation [30].

Acute Lung Rejection

Acute cellular rejection is the predominant form of acute lung rejection affecting more than a third of lung transplant recipients within the first year of transplant [31]. Acute cellular rejection is mediated by reactive T lymphocytes against donor human leukocyte antigens (HLA) and other antigens [31]. Patients with acute cellular rejection may be asymptomatic or present with nonspecific pulmonary symptoms (low-grade fever, dyspnea, productive or nonproductive cough) [31]. Abnormalities on pulmonary function testing (PFT) include airflow obstruction with decreased forced expiratory volume in 1 s (FEV_1) [31]. Chest imaging findings are nonspecific including ground-glass opacities, septal thickening, volume loss, and pleural effusion [31]. Diagnosis is confirmed with transbronchial biopsy demonstrating a lymphocyte inflammatory response around blood vessels and/or airways [32]. A histologic classification system developed by the International Society for Heart and Lung Transplantation (ISHLT) categorizes acute cellular rejection based on the severity of the vascular and/or airway abnormalities [32]. Treatment of acute cellular rejection consists of a course of high-dose steroids [31].

Antibody-mediated rejection (AMR) of lung transplant is a less commonly encountered form of acute rejection mediated by antibodies against donor HLA and other antigens [33]. Hyperacute rejection is a rare form of AMR mediated by preexisting donor-specific antibodies (DSA) that causes profound hypoxemia, diffuse noncardiogenic pulmonary edema, and alveolar hemorrhage within the first minutes to hours following lung allograft reperfusion [31]. Screening for HLA incompatibility by identifying HLA antibodies to donor HLA epitopes prior to lung transplant prevents episodes of hyperacute rejection [31]. Acute AMR presents weeks to months after lung transplant with nonspecific symptoms including fever, dyspnea, cough, and hemoptysis [33]. The ISHLT diagnostic criteria for acute AMR include the presence of DSA, acute lung injury on pathology, complement deposition in alveolar capillaries, and allograft dysfunction with the exclusion of other causes of dysfunction [33]. Treatment for hyperacute rejection and acute AMR includes therapies to deplete antibodies including immunoglobulin, rituximab, bortezomib, and therapeutic plasma exchange [33].

Chronic Lung Rejection

Chronic lung allograft rejection affects about half of the lung transplant recipients at 5 years after transplant and threatens the long-term survival of the allograft and the patient [34]. Bronchiolitis obliterans syndrome, the predominant form of chronic lung allograft rejection, presents as a progressive and irreversible airflow obstruction

characterized by chronic inflammation and fibrosis of the bronchiolar walls on pathology (bronchiolitis obliterans) [34, 35]. Chronic vascular rejection may present concurrently with atherosclerosis in the pulmonary vasculature [34, 35]. Risk factors for BOS include recurrent episodes of acute lung rejection, primary graft dysfunction, gastroesophageal reflux with aspiration, and CMV and respiratory viral infections [34, 35]. Patients with BOS present with dyspnea, cough, and recurrent episodes of purulent tracheobronchitis. Diagnostic findings include expiratory air trapping on imaging and progressive airflow obstruction on spirometry with declines in FEV_1 [34, 35]. Multiple strategies are employed to slow the progression of BOS including macrolide antibiotics, alteration of immunosuppressive therapy, anti-lymphocyte antibodies, extracorporeal photopheresis, plasmapheresis, and total lymphocyte irradiation; however, the only definitive treatment for BOS is lung re-transplantation [36].

Drug-Induced Lung Toxicity

Pulmonary toxicity is associated with the mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus used for induction and maintenance immunosuppression in SOT [37]. Although the pathogenesis is not completely understood, evidence suggests mTOR inhibitors cause direct lung injury and stimulate immune-mediated injury to the interstitial and alveolar space [37]. Patients typically present with nonspecific pulmonary symptoms (dry cough, progressive dyspnea) within the first 6 months of drug initiation, but later presentations have been described. Radiographic abnormalities include bilateral interstitial infiltrates, alveolar consolidation, and nodular opacities [37]. Diagnosis is confirmed with transbronchial lung biopsy; histopathological findings include interstitial lymphocytic infiltrates, bronchiolitis obliterans with organizing pneumonia, non-necrotizing granulomas, and diffuse alveolar hemorrhage [37]. Discontinuation of the mTOR inhibitor leads to resolution of symptoms, but radiographic abnormalities may take several months to resolve [37].

Malignancy

Malignancy should be considered in the evaluation of SOT recipients presenting with pulmonary symptoms as the comorbidities leading to end-stage organ disease also place the patient at risk for cancer. Liver transplant patients with a pretransplant history of hepatocellular carcinoma are at risk for recurrence with the lung being the most common site of recurrence [38]. Among lung transplant recipients, lung cancer is the most common de novo malignancy, and certain underlying disease etiologies (idiopathic pulmonary fibrosis, interstitial lung disease, and cystic fibrosis) are predictors for malignancy [39].

Posttransplant lymphoproliferative disorder (PTLD) is the abnormal proliferation of B-cells stimulated by Epstein-Barr virus (EBV) in the setting of a decreased cytotoxic T-cell response [40]. Risk factors for PTLD include primary infection with EBV at the time of transplant, higher intensity immunosuppression, and receipt of anti-lymphocyte antibody [40]. The incidence is highest among lung and heart transplant recipients related to the receipt of higher-intensity induction

immunosuppression compared to other SOT recipients [40]. Heart and lung transplant recipients are also more likely to present with intrathoracic PTLD including pulmonary nodules, regional adenopathy, and pleural effusions [40]. Treatment involves the reduction of immunosuppression and immunotherapy with rituximab (anti-CD20 monoclonal antibody) [40].

Hematopoietic Stem Cell Transplantation

Infectious Pulmonary Complications

Infectious pneumonia is a common complication following hematopoietic stem cell transplantation. The spectrum of pulmonary infections varies depending on the donor source of stem cells, conditioning regimen, and the degree and type of immunodeficiency the patient encounters at any particular time. The timeline of pulmonary infections after HSCT can be divided into three risk periods (Fig. 2):

Phase I: Pre-engraftment neutropenia
Phase II: Early post-engraftment (engraftment until day +100)
Phase III: Late post-engraftment (beyond day +100) [41, 42]

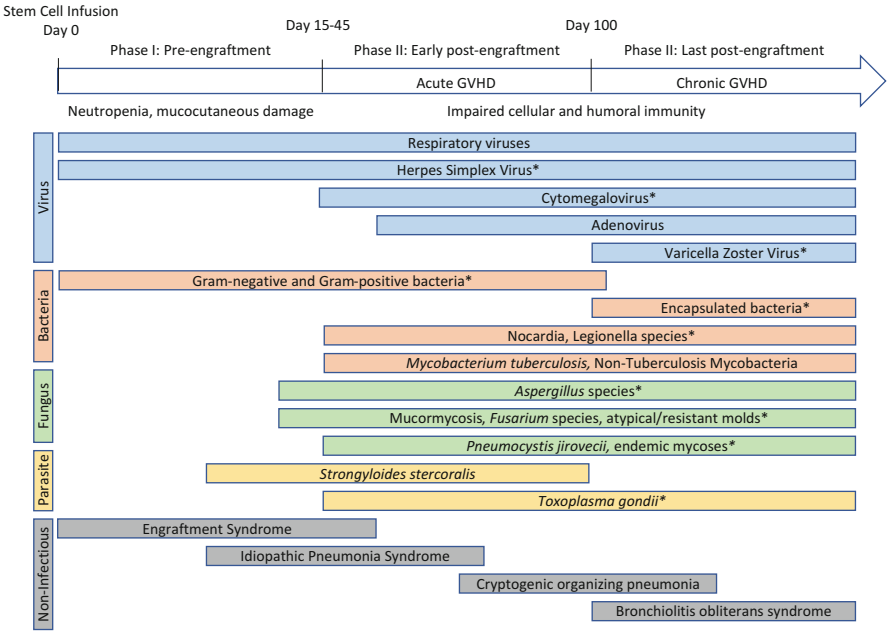


Fig. 2 Timeline of infectious and non-infectious pulmonary complications after hematopoietic stem cell transplant. *GVHD* graft-versus-host-disease, *ARDS* acute respiratory distress syndrome, *TRALI* transfusion related lung injury. *Prophylaxis alters risk and the timeline [2]

Phase I: Pre-engraftment Neutropenia

In the early pre-engraftment period, the majority of pulmonary infections are secondary to prolonged neutropenia and mucositis following conditioning chemotherapy. The duration of neutropenia is less prolonged in recipients of autologous stem cell transplants and those receiving non-myeloablative conditioning regimens prior to allogeneic stem cell transplants.

Bacterial pathogens causing pneumonia in the pre-engraftment period are similar to those encountered in patients with chemotherapy-induced neutropenia and mucositis including *Enterobacteriaceae*, *Pseudomonas* species, and MRSA [43]. MDROs need to be considered depending on the local epidemiology, the length of hospital stay, and the recipient's antibiotic, infection, and colonization history [44]. Viridans streptococcal sepsis (VSS) is a unique cause of ARDS among HSCT recipients caused primarily by *Streptococcus mitis* and *Streptococcus oralis* [45]. Risk factors for VSS include age, mucositis, cytarabine exposure, and prophylaxis with fluoroquinolones or TMP-SMX [45].

Aspergillosis is the most common cause of invasive fungal pneumonia secondary to prolonged neutropenia. There are two periods of increased incidence of invasive aspergillosis: the first peak occurs during the prolonged neutropenic phase, and the second occurs later in the context of GVHD and corticosteroids [42]. Additional risk factors include myeloablative conditioning, leukemia, delayed engraftment, marrow failure, prior history of fungal pneumonia, and marijuana use [46]. The incidence of invasive pulmonary aspergillosis in the early period can be reduced by decreasing the duration of neutropenia and initiating voriconazole prophylaxis [47].

Phase II: Early Post-Engraftment

The early post-engraftment phase encompasses the period following engraftment until day +100 when neutropenia has resolved but cell-mediated immunity has yet to normalize. T-cell function is suppressed due to lingering effects from conditioning chemotherapy as well as immunosuppressive therapy for GVHD prophylaxis, such as tacrolimus or cyclosporine. Viral (CMV, adenovirus, respiratory viruses) and fungal pathogens predominate during the early post-engraftment period [2, 41, 48].

Although the incidence of CMV pneumonitis has reduced in recent decades, it remains a significant cause of morbidity and mortality post-HSCT [49, 50]. The risk for CMV infection is highest among recipients with D−/R+ mismatch and can be reduced with antiviral prophylaxis or a preemptive therapy approach with regular viral polymerase chain reaction (PCR) monitoring. Letermovir is a novel antiviral agent approved for prophylaxis post-allogeneic HSCT in seropositive recipients [51]. The risk of CMV infections remains elevated in patients with acute GVHD and on immunosuppressive therapies.

Seasonal respiratory viruses, such as influenza, parainfluenza, RSV, human metapneumovirus, and adenovirus, can migrate from the upper to lower respiratory tract leading to a more severe clinical presentation among HSCT recipients [48]. Adenovirus can also present as a reactivation disease causing a spectrum of illnesses ranging from a limited upper respiratory tract infection to disseminated disease with hemorrhagic cystitis and colitis [52].

The second risk period for aspergillus occurs during the early post-engraftment period related to the development of GVHD and administration of high-dose corticosteroid therapy [42]. CT imaging of the lung may demonstrate consolidations, tree-in-bud opacities, and nodular infiltrates [42]. The "halo sign" (a dense area of consolidation surrounded by ground glass representing a region of alveolar hemorrhage) is associated with invasive pulmonary aspergillosis; however, it can be seen with other infectious and noninfectious etiologies [53, 54].

Phase III: Late Post-Engraftment

Immunosuppressive agents used to prevent GVHD, such as cyclosporine and tacrolimus, are usually tapered at day +100 after HSCT. At day +100 after transplant, allogeneic stem cell recipients remain at high risk of pulmonary infections related to functional hyposplenism and immunosuppressive therapy for the treatment of chronic GVHD [47]. Although bacterial pneumonias are less common during this period, the risk for encapsulated bacterial pneumonia such as *S. pneumoniae* and *H. influenzae* persists as many patients remain on immunosuppressive therapy and are unable to mount an appropriate response to vaccination. Strategies to reduce the incidence of infection related to encapsulated organisms include antibiotic prophylaxis and vaccination when clinically appropriate [47].

Patients with persistent immune deficits from chronic GVHD or other transplant-related conditions remain at increased risk for recurrent respiratory viral, resistant bacterial, and breakthrough fungal pneumonias. Late CMV infection can present in association with GVHD and after discontinuation of preventative and preemptive strategies. The use of agents such as letermovir for prolonged chemoprophylaxis is being studied [55]. Breakthrough fungal infections, including mucormycosis, *Fusarium*, and *Scedosporium*, should be considered in patients on antifungal prophylaxis [47]. While aspergillus and mucormycosis infections have similar risk factors among HSCT recipients, mucormycosis should be suspected among patients presenting with pulmonary nodules on voriconazole prophylaxis with negative serum and BAL galactomannan on evaluation. A "reverse halo sign" (a ground-glass opacity surrounded by a peripheral ring of consolidation) may be seen on chest imaging [53, 54]. *Fusarium* infections present as nodular pulmonary infiltrates and may be accompanied by cutaneous lesions and fungemia in the setting of disseminated disease. Among HSCT recipients, mortality rates for mucormycosis and *Fusarium* infections remain high related to the inability to reverse underlying immunosuppression and toxicities related to induction antifungal therapy liposomal amphotericin B [56].

The risk for PJP, highest during the first 6 months following HSCT, is significantly reduced with standardized chemoprophylaxis regimens, particularly with TMP-SMX. Many cases of PJP occur late after prophylaxis has been administered and completed per institutional protocol or discontinued early due to adverse effects despite ongoing immunosuppression. Breakthrough infections can be seen in those receiving alternative agents such as dapsone, atovaquone, or pentamidine [57].

Reactivation of *T. gondii* is a rare cause of pneumonia after HSCT, usually occurring during the first year in seropositive recipients who cannot tolerate or have discontinued prophylaxis with TMP-SMX. Toxoplasmosis pneumonia presents with diffuse interstitial lung infiltrates, and ring-enhancing brain lesions similar to those typically seen in HIV/AIDS may be present [58].

Noninfectious Pulmonary Complications

Noninfectious pulmonary complications are a frequent cause of pulmonary infiltrates in stem cell transplant recipients and may present concurrently with pulmonary infections [2]. Many common pulmonary syndromes, such as pulmonary embolism, pulmonary edema, pleural effusions, ARDS, and transfusion-related lung injuries, can occur at any period during the posttransplant period. These syndromes usually occur in the earlier phases of transplant, and the clinical presentation is not unique to transplant recipients. The two most common noninfectious pulmonary syndromes post-HSCT are idiopathic pneumonia syndrome and bronchiolitis obliterans syndrome.

Early

Idiopathic Pneumonia Syndrome

Idiopathic pneumonia syndrome (IPS) describes a spectrum of conditions causing acute lung injury early after HSCT. The median time of onset is 21 days post-transplant [59, 60]. IPS is defined by signs and symptoms of pneumonia, abnormal pulmonary physiology, the presence of diffuse interstitial infiltrates on chest imaging, the absence of lower respiratory tract infection on BAL, and no other identifiable alternative causes such as cardiac/renal failure or iatrogenic fluid overload [60]. Two clinically relevant IPS subtypes include diffuse alveolar hemorrhage and engraftment syndrome [59].

Diffuse alveolar hemorrhage (DAH) is a subtype of IPS occurring in 2–6% of patients after transplant. DAH is diagnosed with a progressive bloody return on BAL. Alternatively it can also be diagnosed in the presence of $\geq$20% siderophages among alveolar macrophages on BAL. Treatments vary by institution and include steroids, platelet transfusions, antifibrinolytic therapies [59, 60].

Engraftment syndrome (ES) occurs before or soon after neutrophil return usually within 5 days of engraftment [59]. ES is characterized by fever, rash, and/or acute respiratory failure with the exclusion of other infectious and noninfectious pulmonary syndromes. The pathogenesis is related to degranulating neutrophils causing capillary leakage and subsequent acute lung injury. Chest imaging reveals diffuse pulmonary infiltrates and pulmonary edema. ES is seen more frequently in autologous compared to allogeneic transplant recipients, and the syndrome is associated with higher doses of infused mononuclear cells and the use of granulocyte colony-stimulating factor [61]. Treatment is usually supportive with steroids reserved for severe cases.

Late

Bronchiolitis obliterans syndrome (BOS), a common late pulmonary complication following allogeneic HSCT, is considered the pulmonary manifestation of chronic GVHD. The onset of BOS is usually from 6 months to 2 years after HSCT and presents in 10% of patients with chronic GVHD [62]. The exact cause is unknown, but an alloimmune mechanism is suspected as BOS after HSCT can be prevented with T-cell depletion of the graft and its histology shares similarities with BOS in lung allograft recipients [62]. Patients usually develop respiratory symptoms insidiously and demonstrate new-onset airflow obstruction on PFTs with a progressive decline in FEV_1 on spirometry. The diagnosis is often delayed due to its nonspecific presentation and can often be masked by concomitant pulmonary conditions such as infection. The main goal of therapy is to delay further decline in pulmonary function. Treatment consists of prompt recognition and control of underlying chronic GVHD with immunomodulators and supportive care, such as intravenous immunoglobulin replacement, antimicrobial prophylaxis, and macrolide antibiotics in combination with inhaled therapies such as bronchodilators and steroids [63]. Prevention strategies are limited and mainly consist of surveillance using PFTs to monitor pulmonary function.

Cryptogenic organizing pneumonia (COP) is an interstitial lung disease that can present early after engraftment but more frequently occurs in the late period. Unlike BOS, the hallmark is a restrictive airway disease and is usually responsive to steroid therapy [42, 60].

Evaluation of Patient with Pulmonary Symptoms

The evaluation of pulmonary symptoms is challenging as transplant patients present with atypical signs and symptoms related to a blunted immune response from exogenous immunosuppression [4]. A broad differential diagnosis should be established as pulmonary infectious and noninfectious processes frequently occur simultaneously or sequentially [4]. Factors to consider include the timing after transplantation, the degree of immunosuppression, donor and recipient exposures, and hospital and regional epidemiology [4]. Given the high morbidity and mortality associated with pulmonary infections, an aggressive approach is required including the early use of diagnostics and prompt initiation of empiric antimicrobial therapy [1–3]. Establishing a specific diagnosis is essential for de-escalation to targeted antimicrobial therapy with avoidance of adverse effects (including the development of antimicrobial resistance) from broad-spectrum antibiotics.

Recipient and Donor Epidemiologic Exposures

A thorough assessment of the recipient and donor epidemiologic exposures is essential to guide diagnostic testing and initiation of empiric antimicrobial therapy. A detailed

medical and social history should include recent hospitalizations, prior infections, travel history, occupation, sexual practices, hobbies, and pets. Certain pulmonary pathogens are associated with geographical and environmental exposures as follows:

- Geography and travel:
  - Ohio and Mississippi River Valleys in the United States of America: *Histoplasma capsulatum*, *Blastomyces dermatitidis* [25]
  - Southwestern United States of America, Central and South America: *Coccidioides* and *Paracoccidioides* species [25, 65]
  - Southeast Asia: *Burkholderia pseudomallei* and *Talaromyces (Penicillium) marneffei* [65]
  - Endemic countries: *Strongyloides stercoralis* [26], *Mycobacterium tuberculosis* [28]
- Contaminated water supply – *Legionella pneumophila*, *Pseudomonas aeruginosa*, nontuberculous mycobacteria [4]
- Gardening and soil – *Aspergillus* species, *Nocardia* species, hantavirus [4]
- Animals – *Chlamydophila psittaci*, *Rhodococcus equi*, *Francisella tularensis*, *Coxiella burnetii*, *Pasteurella multocida*, *Cryptococcus neoformans*, *Toxoplasma gondii*, *Echinococcus granulosus* [66]
- Seasonal – winter (influenza, RSV, human metapneumovirus), spring-fall (rhinovirus), all seasons (adenovirus, parainfluenza, SARS-CoV-2) [20, 67]
- Nosocomial – MRSA, vancomycin-resistant enterococci (VRE), multidrug-resistant gram-negative bacteria [68]

Diagnostic Evaluation

The diagnostic evaluation is tailored based on the patient's medical history, degree of immunosuppression, exposures, and clinical presentation. The initial evaluation includes blood testing, sampling of the respiratory tract, and radiographic imaging [3]:

- The complete blood cell count with differential, electrolyte chemistries and liver function tests assist with the diagnosis and identify any toxicities to guide empiric antimicrobial therapy [3].
- Blood cultures obtained prior to initiation of empiric antimicrobial therapy are recommended. The yield of blood cultures is low in the setting of pneumonia; however, positive culture results provide useful information for tailoring antimicrobial therapy [69].
- Although urine *Legionella* antigen testing has low sensitivity for *L. pneumophila* and fails to detect other *Legionella* species, testing is recommended as positive results establish a diagnosis for appropriate de-escalation to antibiotic therapy for atypical pneumonia [70].
- Nasopharyngeal influenza molecular testing is recommended in seasonally appropriate times for initiation of antiviral therapy [20, 71]. Nasopharyngeal respiratory viral panels screen for multiple viruses simultaneously. In a patient with clinical

presentation consistent with viral pneumonia, a positive result may encourage early discontinuation of antibiotic therapy [67].
- Noninvasive lower respiratory tract testing includes sputum samples sent for gram stain and bacterial cultures. The yield of sputum cultures is generally low, but positive results allow for adjustments in antimicrobial therapy [68, 71].
- Chest X-ray is recommended for immunocompromised patients presenting with pulmonary symptoms. Chest computed tomography (CT) may be indicated in the initial evaluation based on patients with prolonged respiratory symptoms, high acuity clinical presentations, and a high net state of immunosuppression [1, 3].

Additional diagnostic evaluation is indicated if the patient does not improve clinically after the first 24–48 h after empiric antimicrobial therapy and a diagnosis has not been established with the initial work-up [3]:

- If not performed during the initial evaluation, CT chest is recommended as the location and characteristics of radiographic abnormalities narrow the differential diagnosis and identify the best diagnostic procedure for lower respiratory tract sampling (see "Imaging" section) [1, 3].
- Fungal antigen testing is indicated if patient exposures, net state of immunosuppression, clinical presentation, and radiographic findings are suggestive of fungal pneumonia. Serum testing includes *Cryptococcus* antigen, *Histoplasma* antigen, *Blastomyces* antigen, galactomannan (*Aspergillus*) antigen, and (1,3)-β-D-glucan. Urine antigen tests include *H. capsulatum* and *B. dermatitidis* [72].

Invasive diagnostic testing is recommended if the patient fails to improve clinically on empiric antimicrobial therapy and a diagnosis has not been established [1–3]. Diagnostic procedures used to sample the lower respiratory tract include bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsy, percutaneous needle biopsy, video-assisted thoracoscopic biopsy, and open lung excision [3]. The benefits of establishing a diagnosis must be weighed against the risks of invasive diagnostic testing. The initial BAL fluid and transbronchial biopsy evaluation include gram potassium hydroxide (KOH) and acid-fast bacilli (AFB) stains; bacterial, AFB, and fungal cultures; cytology; and histology with hematoxylin and eosin (H&E) and methenamine silver stains. Based on the clinical suspicion, additional testing can be performed on the BAL fluid including antigen (*Histoplasma*, *Blastomyces*, *Aspergillus* galactomannan), direct fluorescent antibody (DFA) (HSV, VZV, PJP, *Legionella*), and molecular testing (respiratory viruses, herpes viruses, atypical bacteria, *Legionella*, *M. tuberculosis*, *P. jirovecii*, *Aspergillus)* [72] (Table 1). Lung biopsy establishes the diagnosis of infectious pulmonary syndromes based on the pattern of inflammation on histology along with viral, bacterial, and fungal stains. Lung biopsy is essential for the diagnosis of noninfectious pulmonary syndromes including malignancy, rejection, and graft-versus-host disease.

Table 1 Studies for bronchoalveolar lavage fluid in the evaluation of pulmonary infection in transplant patients [3]

Infection	Stain	Culture	Antigen	PCR	DFA
Bacteria					
Gram-positive /gram-negative	Gram	Aerobic/anaerobic		*Bordetella pertussis* Methicillin-resistant *Staphylococcus aureus*	
Atypical				*Chlamydia pneumonia* *Mycoplasma pneumoniae*	
Legionella	Modified Gimenez	*Legionella*	*Legionella*	*Legionella*	*Legionella*
Nocardia	Modified AFB	Aerobic			
Mycobacteria	AFB	Mycobacterial		*Mycobacterium tuberculosis*	
Fungi					
Aspergillus	Silver	Fungal	Galactomannan	*Aspergillus*	
Pneumocystis jirovecii	Silver			*P. jirovecii*	*P. Jirovecii*
Viruses					
Respiratory		Viral	Influenza RSV	Influenza Multiplex	
Herpes viruses		Viral		HSV, VZV, EBV	HSV, VZV
CMV		Shell vial	pp65 staining	CMV	

PCR polymerase chain reaction, *DFA* direct fluorescent antibody, *AFB* acid-fast bacilli.

Imaging

Chest imaging is an essential component of the diagnostic evaluation as many pulmonary syndromes present with characteristic radiographic abnormalities with predictable rates of progression (Table 2). Acute abnormalities (less than 48 h) are suggestive of viral or bacterial pneumonias, while subacute and chronic findings (greater than 48 h) typically represent mycobacterial or fungal infections [4]. Radiographic abnormalities include consolidation, interstitial disease, nodules, cavitation, and lymphadenopathy [3]. Consolidations with air bronchograms represent alveolar air replaced with tissue density material with superimposed airways [3]. Interstitial infiltrates are suggestive of CMV, respiratory viruses, *Mycoplasma*, *Legionella*, PJP,

Table 2 Differential diagnosis of pulmonary syndromes based on radiographic appearance on chest radiograph or computed tomography by rate of disease progression [4]

Radiographic abnormality	Rate of disease progression	
	Acute	Subacute or chronic
Consolidation	Any organism	*Nocardia* species, *Actinomyces* species Mycobacteria Fungi Bronchiolitis obliterans syndrome
Bronchopneumonia	Community-acquired viruses Atypical bacteria (*Mycoplasma*, *Chlamydophila*)	Mycobacteria Bronchiolitis obliterans syndrome
Diffuse interstitial infiltrates	Community-acquired viruses CMV, EBV, HSV *Pneumocystis jirovecii* Pulmonary edema, pulmonary hemorrhage Acute respiratory distress syndrome	Mycobacteria Drug toxicity, radiation toxicity Metastatic cancer Pulmonary alveolar proteinosis
Nodular infiltrates	CMV Bacteria, septic emboli *P. Jirovecii*	*Nocardia* species, *Actinomyces* species Mycobacteria Fungi Kaposi sarcoma, Castleman disease Lung cancer
Adenopathy	Mycobacterium tuberculosis *Cryptococcus neoformans*	Mycobacterium tuberculosis PTLD Kaposi sarcoma, Castleman disease Lung cancer
Pleural effusion	Bacteria Tuberculosis Postoperative Heart, kidney, or liver failure	Non-Hodgkin's lymphoma Primary effusion lymphoma

CMV cytomegalovirus, *EBV* Epstein-Barr Virus, *HSV* Herpes Simplex Virus, *PTLD* posttransplant lymphoproliferative disorder.

fluid overload, and lung transplant rejection. Nodules are space-occupying, non-anatomic defects with well-defined rounded edges typically associated with *Nocardia*, mycobacteria, invasive mold infection, endemic fungal infections, and malignancy. Cavitation represents a necrotizing infection caused by gram-negative bacilli, anaerobic bacteria, mycobacteria, and fungi. Lymphadenopathy has a broad differential including pneumonia (viral, mycobacterial, fungal), sarcoidosis, PTLD, and lymphoma [3].

Chest radiograph is an inexpensive imaging modality with the ability to delineate many radiographic abnormalities; however, the image quality may be limited by suboptimal patient positioning. Chest CT provides more details on the features and location of the radiographic abnormality. Identifying the precise location of lung pathology helps determine the best procedure for tissue sampling [3].

Empiric Antimicrobial Therapy

The regimen chosen for empiric antimicrobial therapy should consider a variety of factors including the degree of immunosuppression, the rate of disease progression, epidemiological exposures, and prior microbial colonization history. Antibacterial therapy is the main component of empiric antimicrobial therapy in patients after transplant. During the influenza season, empiric antiviral therapy against influenza should be initiated while awaiting results of influenza-specific testing. Empiric antifungal therapy is rarely initiated unless the clinical and radiographic presentation is strongly suggestive of a fungal pathogen.

In patients without any risk factors for opportunistic or hospital-acquired pneumonia, empiric antimicrobial treatment should be initiated against community-acquired respiratory pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and atypical intracellular pathogens (*Mycoplasma* species, *Legionella* species). Recommended regimens for community-acquired pneumonia include (1) combination therapy with a beta-lactam and a macrolide or (2) respiratory fluoroquinolone monotherapy [71]. Adverse drug effects and local resistance patterns should be considered when choosing between empiric regimens. Macrolides lead to increases in the levels of immunosuppressive agents. Fluoroquinolones are active against penicillin-resistant *Streptococcus pneumoniae* and macrolide-resistant *Mycoplasma* species, but its use may be limited due to its association with altered glucose metabolism, QTc prolongation, aortic aneurysm rupture, and *Clostridioides difficile* colitis [71].

Empiric antimicrobial therapy with coverage of MRSA and *Pseudomonas* species is recommended for patients with chronic bronchiectasis, airway colonization with these pathogens, hospital-acquired pneumonia, or ventilator-associated pneumonia [68]. Patients who fail to improve clinically on empiric community-acquired treatment may also benefit from broadening antimicrobial therapy. Antibiotics with MRSA coverage include vancomycin and linezolid; daptomycin is not recommended for primary pneumonia since it is inactivated by a pulmonary surfactant [68]. Anti-pseudomonal beta-lactam agents include cefepime, ceftazidime, aztreonam, piperacillin-tazobactam, imipenem, and meropenem [68]. Double coverage for

Pseudomonas species with fluoroquinolones, aminoglycosides, and polymyxins (and sometimes novel antibiotics) is recommended for patients at risk and hospital units with a high prevalence of MDRO [68].

Conclusions

Despite advances in surgical techniques, immunosuppressive regimens, and antibiotic prophylaxis, pulmonary syndromes remain significant contributors to morbidity and mortality in transplant recipients. A predictable timeline of pulmonary infections presents after transplant related to the patient's changing risk factors over time including the time after transplant, degree of immunosuppression, prophylactic antibiotics, and hospital and community exposures. Evaluation of transplant patients presenting with pulmonary symptoms begins with performing a detailed medical and social history to guide diagnostic testing and initiation of empiric antimicrobial therapy. Diagnostic assessment includes serum and urinary antigen assays, molecular assays, microbiology cultures, chest imaging, and lung tissue sampling. An aggressive approach to establishing a specific diagnosis is recommended for the initiation of targeted antimicrobial therapy and avoidance of adverse effects from broad-spectrum antibiotics.

Key Points

- Infectious and noninfectious pulmonary syndromes remain significant contributors to morbidity and mortality after solid organ and hematopoietic stem cell transplantation.
- The timeline of pulmonary infections after SOT can be divided into three risk periods related to the changing risk factors in the recipient: the time since surgery, degree of immunosuppression, prophylactic antibiotics, and hospital and community exposures.
- Noninfectious pulmonary syndromes related to lung transplantation include airway complications, primary graft failure, acute allograft rejection, and bronchiolitis obliterans syndrome.
- Medications and malignancy are noninfectious pulmonary syndromes to consider after SOT.
- Factors to consider in the differential diagnosis of pulmonary syndromes include the timing after transplantation, the degree of immunosuppression, donor and recipient exposures, and hospital and regional epidemiology.
- Given the high morbidity and mortality associated with pulmonary infections, an aggressive approach is required including the early use of diagnostics and prompt initiation of empiric antimicrobial therapy.
- Establishing a specific diagnosis is essential for de-escalation to targeted antimicrobial therapy with avoidance of adverse effects from broad-spectrum antibiotics.

Cross-References

- ▶ *Aspergillus* in Solid Organ and Stem Cell Transplant Patients: Emerging Options for Diagnosis and Management
- ▶ Coccidioidomycosis in Transplantation
- ▶ Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment
- ▶ Cytomegalovirus in Solid Organ Transplant Recipients: Prevention, Diagnosis, and Treatment
- ▶ Emerging Epidemics in Transplant: Histoplasmosis, Blastomycosis, and Paracoccidioidomycosis
- ▶ Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorders
- ▶ Herpes Simplex and Varicella Zoster in Transplant Candidates and Recipients
- ▶ Influenza Infections in Solid Organ and Stem Cell Transplant Recipients
- ▶ Management of Cryptococcosis in Transplant Candidates and Recipients
- ▶ Management of Mucorales Infections in Transplant Patients
- ▶ *Mycobacterium tuberculosis* in Transplantation
- ▶ *Nocardia* Infections in Transplantation: Diagnosis, Management, and Impact on Outcomes
- ▶ Non-*Aspergillus* Fungal Infections in Transplant Patients
- ▶ *Pneumocystis jiroveci* in Transplant: Recognizing Risk, Understanding Prevention, and Implementing Treatment
- ▶ *Pseudomonas aeruginosa* Infections in Transplant: Epidemiology and Emerging Treatment Options
- ▶ Rapidly Growing Mycobacterial Infections in Transplant: Evolving Epidemiology and Treatment Options
- ▶ Respiratory Syncytial Virus in Transplant: Stopping the Short-Term Spread, Solving the Longer-Term Problems
- ▶ Respiratory Viral Pathogens in Solid Organ and Hematopoietic Stem Cell Transplant Recipients
- ▶ Slow-Growing Nontuberculous Mycobacteria in Transplant
- ▶ Strongyloides: Emerging Challenges and Prevention
- ▶ *Toxoplasma gondii* in Solid Organ and Stem Cell Transplant: Prevention and Treatment

References

1. Dulek DE, Mueller NJ, Practice ASTIDCo. Pneumonia in solid organ transplantation: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13545.
2. Diab M, ZazaDitYafawi J, Soubani AO. Major pulmonary complications after hematopoietic stem cell transplant. Exp Clin Transplant. 2016;14(3):259–70.
3. Azoulay E, Russell L, Van de Louw A, Metaxa V, Bauer P, Povoa P, et al. Diagnosis of severe respiratory infections in immunocompromised patients. Intensive Care Med. 2020;46(2):298–314.
4. Fishman JA. Infection in organ transplantation. Am J Transplant. 2017;17(4):856–79.

5. Remund KF, Best M, Egan JJ. Infections relevant to lung transplantation. Proc Am Thorac Soc. 2009;6(1):94–100.
6. Hoyo I, Linares L, Cervera C, Almela M, Marcos MA, Sanclemente G, et al. Epidemiology of pneumonia in kidney transplantation. Transplant Proc. 2010;42(8):2938–40.
7. Weiss E, Dahmani S, Bert F, Janny S, Sommacale D, Dondero F, et al. Early-onset pneumonia after liver transplantation: microbiological findings and therapeutic consequences. Liver Transpl. 2010;16(10):1178–85.
8. Kupeli E, Ulubay G, Colak T, Ozdemirel TS, Ozyurek BA, Akcay S, et al. Pulmonary complications in renal recipients after transplantation. Transplant Proc. 2011;43(2):551–3.
9. Ikegami T, Shirabe K, Matono R, Yoshizumi T, Soejima Y, Uchiyama H, et al. Etiologies, risk factors, and outcomes of bacterial pneumonia after living donor liver transplantation. Liver Transpl. 2012;18(9):1060–8.
10. Giannella M, Munoz P, Alarcon JM, Mularoni A, Grossi P, Bouza E. Pneumonia in solid organ transplant recipients: a prospective multicenter study. Transpl Infect Dis. 2014;16(2):232–41.
11. Ruiz I, Gavaldà J, Monforte V, Len O, Román A, Bravo C, et al. Donor-to-host transmission of bacterial and fungal infections in lung transplantation. Am J Transplant. 2006;6(1):178–82.
12. Aguilar-Guisado M, Givalda J, Ussetti P, Ramos A, Morales P, Blanes M, et al. Pneumonia after lung transplantation in the RESITRA cohort: a multicenter prospective study. Am J Transplant. 2007;7(8):1989–96.
13. Mahajan AK, Folch E, Khandhar SJ, Channick CL, Santacruz JF, Mehta AC, et al. The diagnosis and management of airway complications following lung transplantation. Chest. 2017;152(3):627–38.
14. Fishman JA, Gans H. *Pneumocystis jiroveci* in solid organ transplantation: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13587.
15. Wang EH, Partovi N, Levy RD, Shapiro RJ, Yoshida EM, Greanya ED. Pneumocystis pneumonia in solid organ transplant recipients: not yet an infection of the past. Transpl Infect Dis. 2012;14(5):519–25.
16. Patel TS, Eschenauer GA, Stuckey LJ, Carver PL. Antifungal prophylaxis in lung transplant recipients. Transplantation. 2016;100(9):1815–26.
17. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102(6):900–31.
18. Pilie P, Werbel WA, Riddell J, Shu X, Schaubel D, Gregg KS. Adult patients with respiratory syncytial virus infection: impact of solid organ and hematopoietic stem cell transplantation on outcomes. Transpl Infect Dis. 2015;17(4):551–7.
19. Koo HJ, Lee HN, Choi SH, Sung H, Oh SY, Shin SY, et al. Human metapneumovirus infection: pneumonia risk factors in patients with solid organ transplantation and computed tomography findings. Transplantation. 2018;102(4):699–706.
20. Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P, et al. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67(9):1322–9.
21. Bruminhent J, Worawichawong S, Tongsook C, Pasomsub E, Boongird S, Watcharananan SP. Epidemiology and outcomes of early-onset and late-onset adenovirus infections in kidney transplant recipients. Open Forum Infect Dis. 2019;6(12), ofz489, https://doi.org/10.1093/ofid/ofz489.
22. Peghin M, Hirsch HH, Len O, Codina G, Berastegui C, Saez B, et al. Epidemiology and immediate indirect effects of respiratory viruses in lung transplant recipients: a 5-year prospective study. Am J Transplant. 2017;17(5):1304–12.
23. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the transplant-associated infection surveillance network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–11.
24. Gassiep I, McDougall D, Douglas J, Francis R, Playford EG. Cryptococcal infections in solid organ transplant recipients over a 15-year period at a state transplant center. Transpl Infect Dis. 2017;19(1). https://doi.org/10.1111/tid.12639.

25. Kauffman CA, Freifeld AG, Andes DR, Baddley JW, Herwaldt L, Walker RC, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the transplant-associated infection surveillance network (TRANSNET). Transpl Infect Dis. 2014;16(2):213–24.
26. Le M, Ravin K, Hasan A, Clauss H, Muchant DG, Pasko JK, et al. Single donor-derived strongyloidiasis in three solid organ transplant recipients: case series and review of the literature. Am J Transplant. 2014;14(5):1199–206.
27. Patrat-Delon S, Gangneux JP, Lavoue S, Lelong B, Guiguen C, le Tulzo Y, et al. Correlation of parasite load determined by quantitative PCR to clinical outcome in a heart transplant patient with disseminated toxoplasmosis. J Clin Microbiol. 2010;48(7):2541–5.
28. Abad CLR, Razonable RR. Mycobacterium tuberculosis after solid organ transplantation: a review of more than 2000 cases. Clin Transpl. 2018;32(6):e13259.
29. Snell GI, Yusen RD, Weill D, Strueber M, Garrity E, Reed A, et al. Report of the ISHLT working group on primary lung graft dysfunction, part I: definition and grading-A 2016 consensus group statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2017;36(10):1097–103.
30. Van Raemdonck D, Hartwig MG, Hertz MI, Davis RD, Cypel M, Hayes D Jr, et al. Report of the ISHLT working group on primary lung graft dysfunction part IV: prevention and treatment: a 2016 consensus group statement of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2017;36(10):1121–36.
31. Benzimra M, Calligaro GL, Glanville AR. Acute rejection. J Thorac Dis. 2017;9(12):5440–57.
32. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant. 2007;26(12):1229–42.
33. Levine DJ, Glanville AR, Aboyoun C, Belperio J, Benden C, Berry GJ, et al. Antibody-mediated rejection of the lung: a consensus report of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2016;35(4):397–406.
34. Verleden GM, Vos R, Vanaudenaerde B, Dupont L, Yserbyt J, Van Raemdonck D, et al. Current views on chronic rejection after lung transplantation. Transpl Int. 2015;28(10):1131–9.
35. Meyer KC, Raghu G, Verleden GM, Corris PA, Aurora P, Wilson KC, et al. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J. 2014;44(6):1479–503.
36. Benden C, Haughton M, Leonard S, Huber LC. Therapy options for chronic lung allograft dysfunction-bronchiolitis obliterans syndrome following first-line immunosuppressive strategies: a systematic review. J Heart Lung Transplant. 2017;36(9):921–33.
37. Lopez P, Kohler S, Dimri S. Interstitial lung disease associated with mTOR inhibitors in solid organ transplant recipients: results from a large phase III clinical trial program of Everolimus and review of the literature. J Transp Secur. 2014;2014:305931.
38. Fernandez-Sevilla E, Allard MA, Selten J, Golse N, Vibert E, Sa Cunha A, et al. Recurrence of hepatocellular carcinoma after liver transplantation: is there a place for resection? Liver Transpl. 2017;23(4):440–7.
39. Magruder JT, Crawford TC, Grimm JC, Kim B, Shah AS, Bush EL, et al. Risk factors for De Novo malignancy following lung transplantation. Am J Transplant. 2017;17(1):227–38.
40. Kumarasinghe G, Lavee O, Parker A, Nivison-Smith I, Milliken S, Dodds A, et al. Post-transplant lymphoproliferative disease in heart and lung transplantation: defining risk and prognostic factors. J Heart Lung Transplant. 2015;34(11):1406–14.
41. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143–238.
42. Bondeelle L, Bergeron A. Managing pulmonary complications in allogeneic hematopoietic stem cell transplantation. Expert Rev Respir Med. 2019;13(1):105–19.
43. Averbuch D, Orasch C, Cordonnier C, Livermore DM, Mikulska M, Viscoli C, et al. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European conference on infections in leukemia. Haematologica. 2013;98(12):1826–35.

44. Averbuch D, Cordonnier C, Livermore DM, Mikulska M, Orasch C, Viscoli C, et al. Targeted therapy against multi-resistant bacteria in leukemic and hematopoietic stem cell transplant recipients: guidelines of the 4th European conference on infections in leukemia (ECIL-4, 2011). Haematologica. 2013;98(12):1836–47.
45. Freifeld AG, Razonable RR. Viridans group streptococci in febrile neutropenic cancer patients: what should we fear? Clin Infect Dis. 2014;59(2):231–3.
46. Benedict K, Thompson GR, Jackson BR. Cannabis use and fungal infections in a commercially insured population, United States, 2016. Emerg Infect Dis. 2020;26(6):1308–10.
47. Baden LR, Swaminathan S, Angarone M, Blouin G, Camins BC, Casper C, et al. Prevention and treatment of cancer-related infections, version 2.2016, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2016;14(7):882–913.
48. Paulsen GC, Danziger-Isakov L. Respiratory viral infections in solid organ and hematopoietic stem cell transplantation. Clin Chest Med. 2017;38(4):707–26.
49. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. Hematol Oncol Clin North Am. 2011;25(1):151–69.
50. Erard V, Guthrie KA, Seo S, Smith J, Huang M, Chien J, et al. Reduced mortality of cytomegalovirus pneumonia after hematopoietic cell transplantation due to antiviral therapy and changes in transplantation practices. Clin Infect Dis. 2015;61(1):31–9.
51. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med. 2017;377(25):2433–44.
52. Sedláček P, Petterson T, Robin M, Sivaprakasam P, Vainorius E, Brundage T, et al. Incidence of adenovirus infection in hematopoietic stem cell transplantation recipients: findings from the AdVance study. Biol Blood Marrow Transplant. 2019;25(4):810–8.
53. Walker CM, Abbott GF, Greene RE, Shepard JA, Vummidi D, Digumarthy SR. Imaging pulmonary infection: classic signs and patterns. AJR Am J Roentgenol. 2014;202(3):479–92.
54. Raju S, Ghosh S, Mehta AC. Chest CT signs in pulmonary disease: a pictorial review. Chest. 2017;151(6):1356–74.
55. Chen K, Cheng MP, Hammond SP, Einsele H, Marty FM. Antiviral prophylaxis for cytomegalovirus infection in allogeneic hematopoietic cell transplantation. Blood Adv. 2018;2(16):2159–75.
56. Riches ML, Trifilio S, Chen M, Ahn KW, Langston A, Lazarus HM, et al. Risk factors and impact of non-Aspergillus mold infections following allogeneic HCT: a CIBMTR infection and immune reconstitution analysis. Bone Marrow Transplant. 2016;51(2):277–82.
57. De Castro N, Neuville S, Sarfati C, Ribaud P, Derouin F, Gluckman E, et al. Occurrence of *Pneumocystis jiroveci* pneumonia after allogeneic stem cell transplantation: a 6-year retrospective study. Bone Marrow Transplant. 2005;36(10):879–83.
58. Gajurel K, Dhakal R, Montoya JG. Toxoplasmosis in hematopoietic cell transplant recipients. Transpl Infect Dis. 2017;19(5). https://doi.org/10.1111/tid.12734.
59. Vande Vusse LK, Madtes DK. Early onset noninfectious pulmonary syndromes after hematopoietic cell transplantation. Clin Chest Med. 2017;38(2):233–48.
60. Ahya VN. Noninfectious acute lung injury syndromes early after hematopoietic stem cell transplantation. Clin Chest Med. 2017;38(4):595–606.
61. Cornell RF, Hari P, Drobyski WR. Engraftment syndrome after autologous stem cell transplantation: an update unifying the definition and management approach. Biol Blood Marrow Transplant. 2015;21(12):2061–8.
62. Bergeron A, Cheng GS. Bronchiolitis obliterans syndrome and other late pulmonary complications after allogeneic hematopoietic stem cell transplantation. Clin Chest Med. 2017;38(4):607–21.
63. Williams KM. How I treat bronchiolitis obliterans syndrome after hematopoietic stem cell transplantation. Blood. 2017;129(4):448–55.
64. Bergeron A, Chevret S, Granata A, Chevallier P, Vincent L, Huynh A, et al. Effect of azithromycin on airflow decline-free survival after allogeneic hematopoietic stem cell transplant: the ALLOZITHRO randomized clinical trial. JAMA. 2017;318(6):557–66.
65. Franco-Paredes C, Jacob JT, Hidron A, Rodriguez-Morales AJ, Kuhar D, Caliendo AM. Transplantation and tropical infectious diseases. Int J Infect Dis. 2010;14(3):e189–96.

66. Kotton CN. Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. Clin Infect Dis. 2007;44(6):857–66.
67. Walter JM, Wunderink RG. Testing for respiratory viruses in adults with severe lower respiratory infection. Chest. 2018;154(5):1213–22.
68. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016;63(5):e61–e111.
69. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. N Engl J Med. 2015;373(5):415–27.
70. Sivagnanam S, Podczervinski S, Butler-Wu SM, Hawkins V, Stednick Z, Helbert LA, et al. Legionnaires' disease in transplant recipients: a 15-year retrospective study in a tertiary referral center. Transpl Infect Dis. 2017;19(5). https://doi.org/10.1111/tid.12745.
71. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45–67.
72. Hage CA, Carmona EM, Epelbaum O, Evans SE, Gabe LM, Haydour Q, et al. Microbiological laboratory testing in the diagnosis of fungal infections in pulmonary and critical care practice. An official American Thoracic Society clinical practice guideline. Am J Respir Crit Care Med. 2019;200(5):535–50.

Diarrhea in Transplant: Diagnosis and Management

66

Diana F. Florescu and Andrea Zimmer

Contents

Introduction 1639
Etiology of Diarrhea in Transplant Recipients 1640
Emerging Infections 1642
Diagnostic Evaluation 1643
Enteric Bacterial Pathogens (*Salmonella*, *Shigella, Campylobacter*, *E. coli*, *Yersinia*, *Vibrio* sp.) 1646
Colonoscopy 1650
Management of Transplant Recipients with Diarrhea 1651
Conclusion 1657
Key Points 1657
References 1658

Introduction

Diarrhea, affecting 20–52% of solid organ transplant (SOT) recipients and up to 40–80% of hematopoietic stem cell transplant (HSCT) recipients, can lead to dehydration, malabsorption, electrolyte imbalance, drug toxicity, hospitalization, rejection, and adverse impact on patients' quality of life [1–4]. Diarrhea is defined by $\geq$3 bowel movements/day associated with a change in the consistency of stool [1]. In intestinal transplant recipients with chronic diarrhea or with an ostomy, diarrhea is defined as an increase in stool output by 50%. Depending on the

D. F. Florescu (✉)
Division of Infectious Diseases, Transplant Infectious Diseases Program, Transplant Surgery Division, University of Nebraska Medical Center, Omaha, NE, USA
e-mail: dflorescu@unmc.edu

A. Zimmer
Division of Infectious Diseases, University of Nebraska Medical Center, Omaha, NE, USA
e-mail: andreaj.zimmer@unmc.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_65

chronicity of the symptoms, diarrhea is classified by the duration of symptoms as acute (<14 days), persistent (14–29 days), and chronic (>30 days) [1].

Etiology of Diarrhea in Transplant Recipients

The causes of posttransplant diarrhea are presented in Table 1. It is estimated that community-onset diarrhea is more common than hospital-onset diarrhea (80% vs. 20%) [2].

The etiology of diarrhea in SOT recipients can be identified in about 80% of the patients [5]. The noninfectious causes of diarrhea should be considered after all infectious etiologies are excluded. Medications (i.e., immunosuppressant agents, antibacterial agents, laxatives, proton-pump inhibitors, antidiabetic drugs) are the most common culprits of diarrhea in SOT, followed by infections [1]. Mycophenolate mofetil (MMF) and its active metabolite, mycophenolic acid, inhibit the cell cycle of rapidly dividing cells causing diarrhea in up to 36% of SOT recipients [2, 6]. MMF-related diarrhea has been described mainly during the first 6 months of treatment, but it can occur at any point after transplantation [5, 7]. Calcineurin inhibitors affect gastrointestinal motility through a macrolide effect and are less frequently associated with diarrhea than MMF [6]. Dose reduction or change in the putative immunosuppressant leads to resolution of diarrhea but increases the risk of acute rejection and graft loss. Graft-versus-host disease (GVHD) in solid organ transplantation is infrequent and more commonly occurs in intestinal transplant recipients (6–9%) compared to recipients of other allografts [6]. Posttransplant lymphoproliferative disorder (PTLD) of the gastrointestinal tract may manifest with diarrhea, but commonly extraintestinal involvement (lymphadenopathy, cytopenia, masses in the liver or lung) is also seen at the same time [1]. A significant number of diarrheal episodes are self-limited and have no identifiable etiology, mainly due to the presence of other concurrent illnesses (i.e., peritonitis) or medical interventions (i.e., discontinuation of antibiotics or laxatives).

The etiology of diarrhea in HSCT recipients can be multifactorial and varies according to the cell source and timing posttransplantation. The highest incidence of diarrhea occurs within the first several weeks posttransplant and is most commonly related to the enteral mucosal injury due to the conditioning chemotherapy and/or radiotherapy [4]. Diarrhea can also be a manifestation of neutropenic colitis in the pre-engraftment period, a serious consequence of the chemotherapy-induced cytotoxic injury in the setting of prolonged neutropenia; the cellular injury allows bacterial organisms colonizing the gastrointestinal tract to invade the bowel wall or even to translocate into the bloodstream [8]. Acute graft-versus-host disease (aGVHD) is another common cause of diarrhea, responsible for up to 30% of diarrheal episodes in allogeneic transplant recipients, and typically occurs between engraftment and the first 100 days posttransplantation [9]. Cord colitis, a relatively newly recognized syndrome in umbilical cord blood transplant recipients, is reported to cause non-bloody diarrhea in the first 3–11 months after transplant and responds to treatment with metronidazole [10]. Engraftment syndrome occurs within a week of engraftment and encompasses a constellation of symptoms, including diarrhea,

Table 1 Etiologies of posttransplant diarrhea

Medications
Antiarrhythmics
Antibiotics
Conditioning chemotherapy[a]
Diabetic medications
Laxatives
Magnesium salts
Maintenance immunosuppression
Mycophenolate mofetil
Tacrolimus
Cyclosporine
Sirolimus
Protease inhibitors
Proton-pump inhibitors
Promotility agents
Viruses
Adenovirus
Astrovirus
Cytomegalovirus
Norovirus
Rotavirus
Sapovirus
Bacteria
Aeromonas spp.
Bacterial overgrowth[b]
Campylobacter spp.
Clostridium difficile
Escherichia coli
Salmonella spp.
Shigella spp.
Yersinia enterocolitica
Parasites
Blastocystis hominis
Cryptosporidium
Cyclospora
Entamoeba
Giardia
Isospora
Microspora
Strongyloides
Miscellaneous
Cord colitis[a]
Engraftment syndrome[a]
Gastroparesis
Graft-versus-host disease[a]
Inflammatory bowel disease
Malabsorption
Mucosal injury from conditioning chemotherapy or radiotherapy[a]
Neutropenic enterocolitis[a]
Posttransplant lymphoproliferative disease

[a]More common in hematopoietic stem cell transplantation
[b]More common in solid organ transplantation

fever, pulmonary edema, and rash [11]. Infections account for 6–40% of diarrheal illnesses in HSCT recipients [3, 12].

Emerging Infections

Astroviridae (astroviruses), *Picornaviridae* (bocavirus, cosavirus), and *Caliciviridae* (noroviruses, sapoviruses) are emerging infectious agents of enteritis in transplantation. Other viruses such as parvovirus, Epstein-Barr virus, human herpesvirus 6, and lymphocytic choriomeningitis virus can cause diarrhea as part of a systemic illness [5]. Viruses such as cosavirus, bocavirus, astrovirus, and sapovirus have been identified by conventional real-time PCR or nested reverse transcription PCR in transplant patients with diarrhea [5, 13].

Astroviruses causing protracted enteritis with prolonged viral shedding and even disseminated disease have been reported mainly in stem cell transplant recipients. HAstV-4, HAstV-VA1/HMO-C/PS, and MBL2 astroviruses have been associated with more severe disease such as progressive encephalitis, meningitis, and even death; headache, sensorineural hearing loss, motor incoordination, ataxia, brainstem dysfunction, progressive cognitive impairment, and seizures are the most commonly reported manifestations [14, 15].

Human bocavirus (HboV) usually causes respiratory symptoms, fever, and sometimes diarrhea. HBoV was described to cause severe diarrhea with profound hypovolemia and disseminated disease in a pediatric liver and stem cell transplant recipient, followed by prolonged shedding of the virus [16]. HboV can be detected at a high rate in the stool of pediatric patients, commonly as coinfection with other intestinal pathogens; its role as a primary cause of gastrointestinal infections remains to be determined [16]. Human cosavirus (HCoSV) has been detected in fecal samples from healthy children and adults and from children with acute non-polio flaccid paralysis. HCoSV infection was reported in a pediatric liver and an adult lung transplant recipient both of whom presented with persistent diarrhea, nausea, vomiting, fever, and abdominal pain [5, 13].

Torovirus in the family *Tobaniviridae* has been reported to cause nosocomial gastroenteritis in immunocompromised children. Although nonspecific, bloody diarrhea is the hallmark feature of torovirus infection [17]. Corona-like viral particles can be detected in the stool; the final diagnosis is made by electron microscopy in correlation with enzyme immunoassay with Breda virus reference antiserum [17].

Food poisoning with vomiting from ingestion of preformed *S. aureus* toxin is a well-described gastrointestinal syndrome, but a rare entity, staphylococcal enterocolitis, has also been published [18]. *S. aureus* can affect both the small and the large intestine, causing edema and ulcerations of the mucosa [18].

Leclercia adecarboxylata, a Gram-negative bacterium, has been described as an opportunistic pathogen causing mainly bacteremia and wound infections; a case of diarrhea, urinary tract infection, and pneumonia in a lung transplant recipient has been reported [19]. The prevalence of *L. adecarboxylata* infections is not known and probably underestimated given its close resemblance to *Escherichia* spp. [19]. The

identification of the bacteria can be done based on morphological and metabolic properties or with MALDI-TOF [19]. The bacterium tends to be sensitive to most of the antibiotics routinely used to treat *Enterobacteriaceae* but sometimes displays resistance to ampicillin and fosfomycin [19].

Laribacter hongkongensis, a Gram-negative facultative anaerobic bacterium, has been isolated from patients with community-acquired diarrhea and can cause prolonged non-bloody gastroenteritis with potential for invasive disease in transplant recipients [20]. *L. hongkongensis* infections should be suspected in patients who have recently traveled or consumed freshwater fish [20]. The diagnosis can be confirmed with MALDI-TOF or culture on special media [20]. Based on susceptibility testing and information that beta-lactamases (sometimes ampC and ESBL) have been previously detected, ampicillin and cephalosporin should not be used [20].

Diagnostic Evaluation

Broad stool testing for infectious pathogens in immunocompromised patients has a low yield; a more targeted approach based on epidemiological factors and clinical syndrome should be used instead [1]. Febrile patients might have a viral illness (i.e., cytomegalovirus, adenovirus), bacterial infection (i.e., *C. difficile*, *E. coli*), or a parasitic infection (i.e., *Cryptosporidium*). Detection of blood in the stool, abdominal pain, and cramps would suggest certain viral infections (i.e., cytomegalovirus), invasive bacterial infections (i.e., *E coli*, *Shigella*, *Salmonella*), parasitic infection (*Entamoeba*), or GVHD. Coinfections should always be taken in consideration; hence, a tiered approach to testing should be used in immunocompromised patients with diarrhea (see Fig. 1). In cases of prolonged diarrhea, stool culture on different special media should be given consideration, especially if the patients screen negative on multiplex PCR. Infectious etiologies should be assessed before modifying the immunosuppressive regimens, to avoid allograft rejection or uncontrolled progression of infection.

Multiplex PCR. Traditionally, the diagnosis of gastrointestinal infections has relied on a combination of several antigen detection tests, microscopy, culture, and several individual PCR assays. These test methods have good performance, allow targeted approach for diagnosis of diarrhea, but overall they are time-consuming and labor-intensive [21]. Multiplex molecular diagnostic panels seem to be better testing modalities, especially in critically ill patients and immunocompromised hosts, providing faster and more reliable results and allowing for detection of coinfections. Rapid identification of the etiology of diarrhea would allow adequate infection control and epidemiologic measures and reduce further testing, invasive procedures, and unnecessary medication changes [21]. There are laboratory-developed multiplex PCR assays and commercially available multiplex assays, of which only three assays are approved by the US Food and Drug Administration (FDA): Luminex xTAG gastrointestinal panel (Lumitex GPP, Lumitex Corp.), BioFire FilmArray gastrointestinal panel (BioFire GIP, BioFire Diagnostics, LLC), and Verigene Enteric Pathogens (Verigene EP) (see Table 2) [21, 22]. These tests have excellent sensitivity and

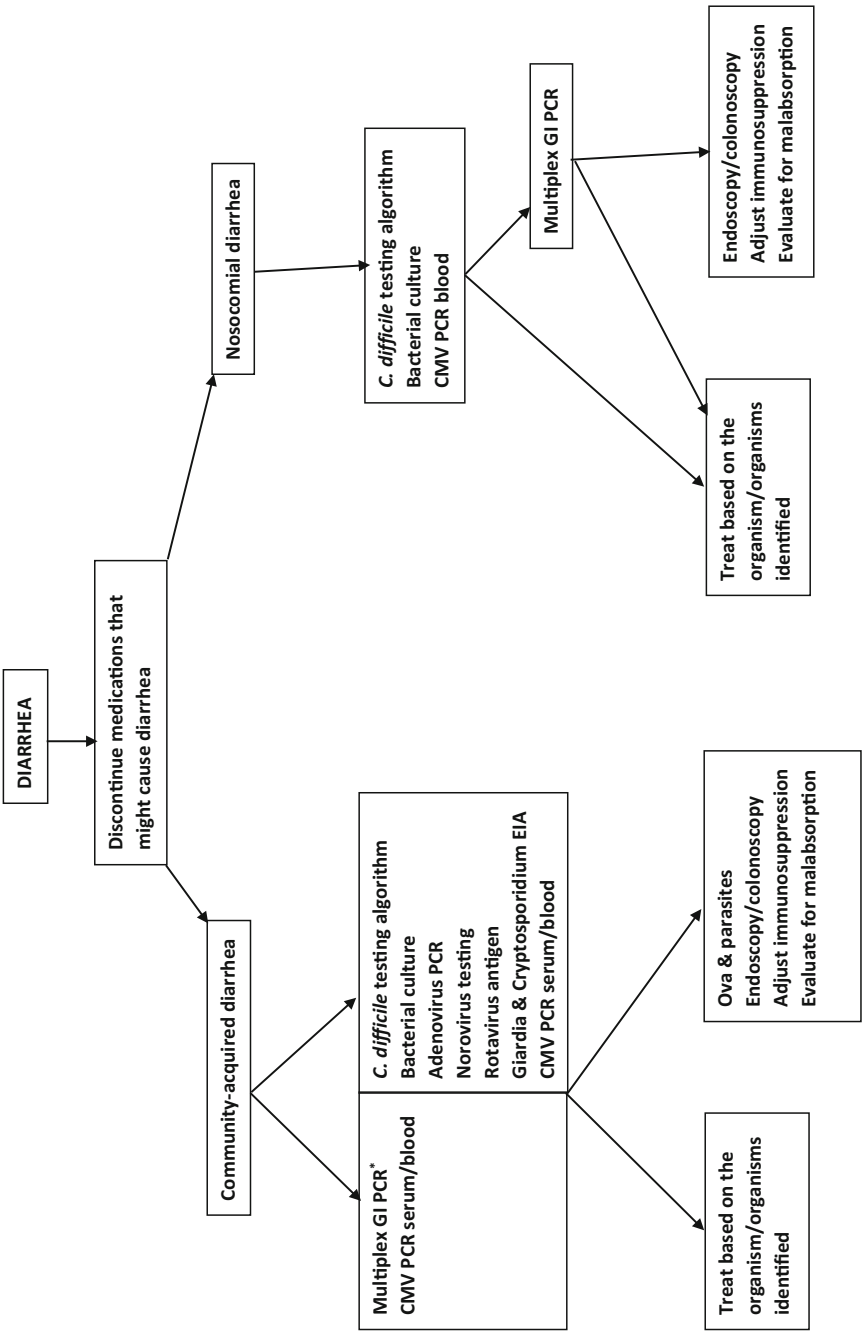


Fig. 1 Algorithm for diarrhea work-up

specificity and very high negative predictive value [22]. In many centers, syndromic testing through multiplex gastrointestinal panel is becoming the preferred approach, replacing less sensitive and slower methods (stool culture, viral antigen detection). However, multiplex gastrointestinal panels, by targeting microbial nucleic acid, cannot differentiate between viable and nonviable pathogens, cannot differentiate between shedding and active infection (e.g., in cases when *Salmonella*, norovirus, or adenovirus are detected) or between colonization with *C. difficile* and true infection [21]. The use of the gastrointestinal panels significantly increases the detection of pathogens by two- to fourfold compared to conventional methods and enables detection of new pathogens (i.e., sapovirus, EPEC). The multiplex PCR assays also lead to a higher reported rate of coinfections [21, 23]. Limited available data has demonstrated that multiplex gastrointestinal panels might be cost-effective by decreasing downstream expenses, despite increased laboratory cost [21].

CMV PCR serum/blood. When transplant recipients have diarrhea, CMV enteritis and colitis are always considered in the differential diagnosis, as CMV is the most common viral infection after transplantation. No single test can identify all cases of CMV colitis. Qualitative CMV PCR seems to have good sensitivity (up to 85%) and excellent specificity (95%) for diagnosis of the gastrointestinal tract infections in transplant recipients [24, 25]. The quantitative assay is preferred over the qualitative assay since it reflects CMV replication (associated with high viral load) [26]. The sensitivity and specificity of the test seems to be influenced by CMV donor/recipient serostatus (sensitivity 100% in D+/R− and 72% in D+/R+) and the timing after transplantation [24–26]. In CMV D+/R+ and D−/R+ solid organ transplant recipients more than 6 months after transplantation, endoscopy should be performed, especially in the absence of viremia when CMV colitis is considered. In D+/R− solid organ transplant recipients, the absence of viremia would argue against CMV colitis [25].

Clostridium difficile. Numerous diagnostic tests for *Clostridium difficile* infection (CDI) are now available. The historic gold standard is the cell culture cytotoxicity assay, which is both sensitive and specific, but as it requires cell cultures to demonstrate the cytopathic effect, it is a time-consuming and resource-intensive test and therefore not commonly used in the clinical setting. Glutamate dehydrogenase (GDH) is an enzyme present in all *C. difficile* isolates, and a GDH immunoassay (EIA) can detect this antigen. However, as this test cannot distinguish between toxigenic and nontoxigenic strains, it is not adequately specific to be used as a stand-alone test. Detection of toxins A and B produced by *C. difficile* can be performed using EIA, and while these tests are very specific, sensitivity varies. Nucleic acid amplification testing (NAAT) detects gene targets specific to toxigenic strains but cannot differentiate between clinical infection with toxin production and asymptomatic carriage. The optimal modality for *Clostridium difficile* diagnostic testing remains controversial, and expert guidelines recommend utilizing a multistep algorithm to increase sensitivity and specificity of testing [27]. Options for initial testing include (1) GDH plus toxin assay; (2) GDH plus toxin assay arbitrated by NAAT; or (3) NAAT plus toxin assay. If NAAT alone is performed, guidelines recommend placing criteria on when the testing for *C. difficile* should be performed

Table 2 Comparison between the targets of the FDA-approved multiplex gastrointestinal assays

Parameter	Luminex GPP	BioFire GIP	Verigene EP
Number of targets	14	22	9
Bacteria detected			
Campylobacter spp.	X	X	X
Clostridium difficile (toxins A and B)	X	X	X
Escherichia coli O157	X	X	
Enterotoxigenic *Escherichia coli*	X	X	
Enteropathogenic *Escherichia coli*		X	
Enteroaggregative *Escherichia coli*		X	
Enteroinvasive *Escherichia coli/Shigella*	X	X	X
Salmonella spp.	X	X	X
Shiga toxin-producing *Escherichia coli* (stx_1, stx_2)	X	X	X
Plesiomonas shigelloides		X	
Vibrio spp.		X	X
Vibrio cholerae	X	X	
Yersinia enterocolitica		X	X
Viruses detected			
Adenovirus 40/41	X	X	
Astrovirus		X	
Norovirus GI/GII	X	X	X
Rotavirus A	X	X	X
Sapovirus		X	
Parasites detected			
Cyclospora cayetanensis		X	
Cryptosporidium spp.	X	X	
Entamoeba histolytica	X	X	
Giardia lamblia	X	X	

GPP gastrointestinal pathogen panel, *GIP* gastrointestinal panel

[27–29]. Such criteria include a strong clinical suspicion for CDI in patients with new onset diarrhea with at least three or more episodes of unformed stools within a 24-h period, including patients who are not receiving laxatives and have no other explanation for diarrhea [27]. The presentation of CDI may be atypical in transplant recipients; the diagnosis may also be considered in patients with unexplained abdominal pain, leukocytosis, or fevers in a patient with an ileus [28, 29].

Enteric Bacterial Pathogens (*Salmonella, Shigella, Campylobacter, E. coli, Yersinia, Vibrio* sp.)

Given the rapid turnaround time, high sensitivity, and relative ease of testing, the multiplex panels are widely utilized in the diagnosis of bacterial enteric pathogens

[30, 31]. Most commercial multiplex tests include *Campylobacter, Salmonella, Yersinia enterocolitica, Vibrio* spp., *Shigella*, and significant pathogenic *E. coli* stains, such as Shiga-like toxin producing *E. coli* (STEC) and enteroinvasive *E. coli* (EIEC) on their panels (see Table 2) [21]. Some of the panels also include enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC) (see Table 2) [21]. However, multiplex PCRs cannot distinguish between active infection and nonviable microbes and cannot test for antibiotic susceptibilities. Therefore, sometimes stool cultures are indicated, and if planned, the specimens should be collected early in the illness, preferably prior to initiation of antibiotics. Some bacterial organisms require specialized media. If a clinician is concerned for a particular pathogen, they should alert their microbiology lab.

Adenovirus. Qualitative and quantitative adenovirus polymerase chain reaction (PCR) assays are the most commonly used tests for detection of adenovirus in blood, respiratory secretions, stool, and tissue specimens, replacing older diagnostic methods such as viral culture [32]. PCR assays are rapid and highly sensitive. PCR assays specifically targeting adenovirus are more sensitive and specific than multiplex stool or respiratory PCR assays that include adenovirus, and also they detect all adenovirus serotypes, while multiplex assays test for a selected number of serotypes [32]. PCR assays can also be performed serially, helping to decide if and when to initiate therapy and to monitor response to therapy. In HSCT recipients, detection of adenovirus in the stool often precedes the detection of adenovirus in blood [33]. Persistently high viral loads or a 0.5–1.0 log increase might suggest the need for treatment, especially in HSCT recipients. In contrast, decreasing viral loads would suggest that therapy is not necessary or that there is clinical improvement [33]. PCR results should always be correlated with clinical presentation and histopathology, when available, in an attempt to differentiate between asymptomatic infection and disease. Histopathologic evaluation remains the gold standard to diagnose adenovirus invasive disease by identification of “smudge cells” (cells with large nuclei with basophilic inclusions and a thin rim of cytoplasm and positive immune-peroxidase and in situ hybridization staining) [34].

Culture, serology, and electron microscopy are available, but not routinely used in clinical practice [34]. All adenovirus serotypes (except serotypes 40 and 41) can be grown in human epithelial cells, but this diagnostic method is not practical [34]. The rapid antigen detection kits are commercially available and provide rapid and specific results. Immunofluorescence assays are often used for respiratory specimens, while enzyme immunoassays can be used for stool samples; most of the assays detect only the common adenovirus serotypes [34].

Norovirus. Noroviruses cannot be grown in cell culture; therefore, diagnosis is based on detection of norovirus antigens or RNA in stool [5]. Several enzyme immunoassays (EIAs) are commercially available for the detection of noroviruses GI and GII antigens in stool specimens with 31.6–80% sensitivity and 65.3–100% specificity. EIAs are more sensitive in the outbreak setting compared to sporadic gastroenteritis [35]. Several commercial rapid antigen assays via lateral flow immunochromatography are alternative stool testing for noroviruses, with good sensitivity (57.1–90.2%) and specificity (93.7–100%) [35]. EIAs and antigen

assays do not require special diagnostic facilities, are simple to perform, and have a short turnaround time. Their sensitivity and specificity depend on the norovirus viral load and the viral genotypes present in the stool specimens. RT-PCR is now considered the gold standard for the detection and typing of noroviruses and can be performed in stool, vomitus, food, and environmental specimens [5]. There are several conventional and real-time norovirus RT-PCR assays that have the advantage of detection of GI and GII noroviruses in a single reaction. However, norovirus genotyping (or genogrouping) does not impact clinical management, but it is useful in outbreak settings to track individual strains. Of the commercially PCRs currently available, some allow for qualitative detection of GI and GII noroviruses, while others allow a broader viral detection (e.g., the RT-PCR identifies caliciviruses and astroviruses, but does not distinguish between noroviruses and sapoviruses) [35]. The newer assays have a short turnaround time (a few hours).

Rotavirus. Historically a disease of young children, rotavirus is becoming a more well-recognized pathogen in transplant recipients, with highest rates in the intestinal, liver, and HSCT populations. The test most commonly used to diagnose rotavirus in children is the enzyme-linked immunosorbent assay (ELISA), which detects rotavirus antigens in stool. Other options include electron microscopy (EM), latex agglutination, immunochromatography, and nucleic acid testing via polymerase chain reactive (PCR), which is the most sensitive and specific modality [36]. Rotavirus testing is included as part of all three of the commercially available multiplex stool assays (see Table 2) [21].

Sapovirus. Enzyme-linked immunosorbent assays (ELISAs) for sapovirus are not commercially available and not used for diagnosis due to antigenic diversity of the strains [37]. Reverse transcription-PCR (RT-PCR) is the preferred method of diagnosis for sapovirus detection from stool samples, with good specificity, sensitivity, and broad reactivity. RT-PCR can also be used for genotyping [37].

Astrovirus. The diagnosis of astrovirus disseminated disease, including encephalitis, requires a high index of suspicion, even when HAsV PCR is negative. Deep sequencing needs to be performed to have a more definite diagnosis, to detect HAstV-4, HAstV-VA1/HMO-C/PS, and MBL2 astroviruses [15].

Giardia. Fecal microscopy had been the traditional gold standard for diagnosing parasitic infections, including *Giardia*, but compared to other available testing, it has lower sensitivity (approximately 46%) due to intermittent excretion of parasites. This, combined with the need for repeated testing and variability in technician expertise, has resulted in decreased utilization of this modality. The preferred diagnostic procedures are enzyme immunoassays and nucleic acid amplification tests performed in stool specimen [31]. There are a number of available immunoassays to detect *Giardia* antigens or antibodies in fecal specimens with sensitivities and specificities of up to 100% and 98%, respectively; these assays are more valuable in clinical practice [38, 39]. Molecular testing via PCR for giardiasis can be performed either as part of the multiplex panel or as a stand-alone test. PCR can also be performed on environmental and zoonotic specimens as part of source investigation [38].

Cryptosporidium. Stool microscopy, the cheapest diagnostic method, is labor-intensive and has low sensitivity even with Ziehl-Neelsen staining or fluorescent techniques (auramine-rhodamine) [40, 41]. Direct immunofluorescence can be a stand-alone test or a combined *Cryptosporidium*/*Giardia* diagnostic kit [40, 41]. Enzyme-linked immunosorbent assays (ELISAs) are more automated and have 66–100% sensitivity with very good specificity [41, 42]. Immunochromatographic tests provide results in minutes, are easy to perform, but are sensitive to detect mainly *C. parvum* and *C. hominis* [41, 42]. Molecular methods described previously (multiplex PCR) became the preferred testing since they have the highest sensitivity and specificity. Direct fluorescent immunoassay, ELISA, and nucleic acid amplification tests are the preferred methods for diagnosis [31]. Histopathology is a useful method for diagnosis, especially when stool testing has been negative but suspicion for cryptosporidiosis or coinfection or other underlying disease persists. Parasites can be seen with hematoxylin staining lining the epithelial surfaces or in the lumen [41, 42].

Cyclospora. Diagnosis of cyclosporiasis can be established utilizing direct microscopy and either modified acid-fast staining or light microscopy as the oocysts auto-fluoresce. To increase sensitivity, microscopy should be performed on multiple fecal samples taken in 2–3 day intervals [43]. Molecular testing using PCR is more sensitive in diagnosing cyclosporiasis and is becoming more widely used particularly as part of the multiplex GI panels [21, 43].

Microsporidia. Spores can be detected using light microscopy after trichome staining on a concentrated specimen with the fungi appearing pink against a blue-green background [31, 44]. Sometimes microsporida can be seen when Giemsa stain is performed on fluid or tissue, but this is not the preferred modality to diagnosis microsporidiosis as it is not very sensitive, and cellular debris may be confused for microsporidia spores [44]. Tissue examination by electron microscopy is considered to be the preferred approach to diagnosis, but this testing is not available at all labs, is time intensive, and cannot reliably identify organisms to the species level. The use of immunofluorescence antibodies has been successful in detecting *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* species [45]. Certain species can also be detected using PCR, and this testing is available through several reference laboratories and the CDC [46].

Entamoeba. It is important to distinguish amebiasis due to *Entamoeba histolytica* from colonization due to one of the nonpathogenic *Entamoeba* species [47]. Stool microscopy can be performed but requires at least three stool samples, and concentration and fixation of specimens is typically recommended to increase diagnostic yield. Direct visualization of the parasite, either in the trophozoite or cyst form cannot differentiate *E. histolytica* from the nonpathogenic *E. dispar*. Therefore, stool microscopy is often performed in combination with serologic or antigen testing. Serology testing can distinguish *E. histolytica* from other *Entamoeba* species and is usually positive within a week of infection. However, serology testing cannot differentiate between previous and acute infection, so this test is best used in patients who do not reside in an endemic region. Antigen detection on stool utilizes monoclonal antibodies specific to antigens unique to *E. histolytica* and can therefore

differentiate this species from the others. The sensitivity and specificity of antigen detection is higher compared to stool microscopy and must be performed on fresh or frozen stool. Molecular methods that detect parasitic DNA in stool are the most sensitive and specific modalities to diagnosis amebiasis due to *E. histolytica*, but are not widely available and are expensive [47, 48].

Strongyloides. As strongyloidiasis is often a chronic parasitic infection, the recommended initial diagnostic test is the *Strongyloides* immunoglobulin G (IgG) ELISA antibody testing [49]. ELISA cannot distinguish between active and past infections and might be falsely negative in immunocompromised patients who are unable to mount an antibody response [50]. Stool examination for ova and parasites is less reliable, as larvae are passed infrequently and in small numbers. A single stool specimen has a sensitivity of only 15–30%, but consecutive daily sampling for a week increases the sensitivity to almost 100% [49, 50].

Colonoscopy

If stool testing or serologic testing is unrevealing for an infectious diagnosis, colonoscopy or sigmoidoscopy is typically the next step in establishing a diagnosis. While macroscopic findings may be nonspecific, histologic features can often help distinguish infectious from noninfectious etiologies. Once a diagnosis is established, a repeat scope to document improvement or resolution is usually not indicated as long as the patient is clinically improving. In cases where the diagnosis was initially established, but there is no improvement or even further clinical deterioration in spite of adequate therapy, endoscopy with ileum or colon biopsies would be recommended to diagnose possible coinfection.

While a high CMV viral load in the setting of diarrhea and colitis is suggestive of CMV colitis, histopathology remains the gold standard for diagnosing enteric CMV disease [26, 51]. On direct visual examination, CMV disease often appears as occasional erosions, ulcerations, or even mucosal hemorrhages. Less frequently it can cause confluent ulcerations, similar in appearance to inflammatory bowel disease, or even pseudomembranes [51]. Histopathologic features of enteric CMV disease are typically characterized by enlarged cells containing intranuclear inclusion bodies (known as "Cowdry bodies") surrounded by a halo [51]. Positive immunohistochemical staining for CMV antibodies confirms the diagnosis [26, 51].

Patients with MMF-associated colitis can have normal macroscopic findings (in 50% of the cases) or erythema, erosions, and ulcers; rectal involvement is not common [7]. Histologic findings consistent with MMF toxicity, more common in the right colon than in the left colon, are mucosal vascular injury, crypt architectural distortion, cryptitis, apoptosis, intraglandular lymphocytes, and increased neuroendocrine cells [7].

GVHD of the gastrointestinal tract is characterized by epithelial cell apoptosis and is most easily identified in areas of regenerating mucosa, particularly intestinal crypts in cases of lower tract involvement. In more severe cases, crypts have cystic dilation containing cellular debris or crypt destruction and loss [52]. Some of these

histologic features are nonspecific and can be seen in the setting of infection and medication toxicity, so staining for infectious etiologies and clinical response to immunosuppression needs to be considered in formulating a diagnosis [52].

Management of Transplant Recipients with Diarrhea

The initial step and the most important component of therapy in patient with diarrhea remains supportive care with oral or intravenous rehydration that should include potassium, magnesium, and bicarbonate in severe cases. Rehydration can be done orally in mild-to-moderate cases of dehydration and intravenously with normal saline or Ringer's lactate solution in severe cases, shock, altered mental status, ileus, or insufficient oral rehydration [1]. Antiemetic drugs (i.e., ondansetron) can be given for symptom control and to facilitate oral rehydration [31]. Antimotility and antisecretory agents (i.e., loperamide) decrease the duration of diarrhea but should be avoided when toxic megacolon is suspected [31]. Transplant recipients with IgG levels <350 mg/dl seem to be at high risk for opportunistic infections and might benefit from immunoglobulin administration (400 mg/kg) [33].

In severe cases, watery diarrhea after international travel, or when inflammatory diarrhea is suspected (due to symptoms such as fever, abdominal pain, blood in the stool), empiric therapy should be started early, with a quinolone or azithromycin for suspected bacterial pathogens [1, 31]. If clinical features of sepsis are identified, broad-spectrum antibiotic therapy should be chosen [31]. STEC O157 and non-O157 STEC infections present as inflammatory diarrhea, and treatment with antibiotics (beta-lactams, fluoroquinolones, trimethoprim/sulfamethoxazole) should be avoided [31]. Therapy should be targeted once the diagnosis is made to shorten the duration of illness and decrease the potential for allograft rejection [1]. Targeted antimicrobial therapy depends on reported resistance patterns in the location where the patient acquired the infection [31].

Ideally, in patients with infectious diarrhea, especially in severe cases, immunosuppression should be adjusted. Most likely, the severity of the disease is a reflection of the degree of immunosuppression, including low CD4 counts and lymphocyte dysfunction. The role of immune reconstitution in certain infections (i.e., cytomegalovirus and adenovirus infections) is highlighted by partial or total resolution of some infections after reduction of immunosuppression. Currently, there is no consensus on which immunosuppressive agent to reduce or discontinue first. Potential antimicrobial or antiparasitic properties of individual immunosuppressants should be taken in consideration when adjusting the regimen. Changes from calcineurin inhibitors (tacrolimus) to one of the mTOR inhibitors (sirolimus or everolimus) have been reported to influence recovery from infections [5].

Cytomegalovirus. The first-line antiviral drugs for treatment of gastrointestinal CMV disease are intravenous ganciclovir and oral valganciclovir, while foscarnet and cidofovir are second-line agents [26]. The dosing is described in Table 3. Virologic response should be monitored weekly with quantitative CMV PCR [26].

Table 3 Targeted treatment of posttransplant diarrhea

Etiologic agent	Treatment	Notes
Cytomegalovirus [1]	IV GCV 5 mg/kg every 12 h or oral VGC 900 mg twice daily, doses adjusted for creatinine clearance Secondary prophylaxis is not recommended** IVIG or CMV IG can be considered for CMV pneumonitis, life-threatening disease, drug-resistant CMV infections, and hypogammaglobulinemia Foscarnet 60 mg/kg IV every 8 h or 90 mg/kg every 12 h, doses adjusted for creatinine clearance Cidofovir 5 mg/kg once weekly for then every 2 weeks	IV GCV should be the initial treatment for severe or life-threatening CMV disease, patients with very high VL, and diarrhea that impairs absorption of VGC. CMV QPCR or pp65 antigenemia should be performed once weekly to assess virologic response Treatment should be continued until CMV VL or antigenemia are undetectable or below a predefined VL threshold Second-line agent used for UL54 and UL97-mutant ganciclovir-resistant CMV infection or disease Third-line agent may be used for UL97-mutant ganciclovir-resistant CMV infection or disease
Adenovirus [2]	Cidofovir 1 mg/kg three times weekly or 5 mg/kg/week induction for 2 weeks followed by 5 mg/kg every other week (see text) For creatinine clearance <50 ml/min (adult) and <0.3 ml/kg/min (children), the dose should be decreased 50% Probenecid (0.5–1.25 g/m^2) administered 3 h before, 2–3 h and 8 h after cidofovir Normal saline solution at 5 ml/kg/h administered the same time with probenecid A lipid conjugate of cidofovir (CMX001, Brincidofovir) was in development	5 mg/kg/week regimen might be more nephrotoxic, but it might be associated with less emergence of resistance and fewer breakthrough CMV and HSV infections
Rotavirus [3]	Rotavirus vaccine in all infants	
C. difficile [3, 4]	For first episode Oral vancomycin 125 mg QID × 10 days Fidaxomicin 200 mg BID × 10 days Fulminant colitis: oral or rectal vancomycin 500 mg QID + IV metronidazole 500 mg Q8H	Rectal vancomycin can be added if ileus is suspected

Salmonella enterica (typhi and paratyphi) [3, 4]	Ceftriaxone TMX/SMX Ciprofloxacin Amoxicillin	
Shigella spp. [3, 4]	Azithromycin Ciprofloxacin Ceftriaxone	
Campylobacter spp. [3, 4]	Azithromycin[a] Ciprofloxacin[b]	
Non-*Vibrio cholera* [3, 4]	Ceftriaxone+doxycycline TMX/SMX+aminoglycosides	Treat only invasive disease
Yersinia enterocolitica [4]	TMP/SMX Cefotaxime Ciprofloxacin	
Enteropathogenic *E. coli* [3, 4]	Fluoroquinolones Cephalosporins	
Enterohemorrhagic *E. coli* [3, 4]		Avoid antimotility agents Avoid antibacterial agents
Bacterial overgrowth [4]	Fluoroquinolones Amoxicillin-clavulanate	
Microsporidia		
Enterocytozoon bieneusi *Encephalitozoon* spp. [3, 4]	Fumagillin 20 mg TID × 14 days Albendazole 400 mg BID × 1–3 weeks	
Giardia [3, 4]	Tinidazole Metronidazole Nitazoxanide	
Cryptosporidium [3, 4]	Nitazoxanide Paromomycin	

(continued)

Table 3 (continued)

Etiologic agent	Treatment	Notes
Cyclospora [3, 4]	TMP/SMX Nitazoxanide	
Cystoisospora [3, 4]	TMP/SMX Pyrimethamine	
Trichinella spp. [3]	Albendazole Mebendazole	
Strongyloides [49, 50]	Ivermectin 200 mcg/kg PO for 2 days; repeat in 2 weeks Albendazole 400 mg PO BID for 10–14 days	In suspected or proven hyperinfection, repeat dosing daily until stools negative for O and P for 2 weeks Less effective; longer for hyperinfection

All antimicrobial doses should be adjusted for the patient's renal and hepatic function and potential interaction with concomitant medications as appropriate *BID* twice daily, *CMV* cytomegalovirus, *GCV* ganciclovir, *Ig* immunoglobulin, *IV* intravenous, *IVIg* intravenous immunoglobulin, *Q8H* every 8 hours, *QID* four times daily, *QPCR* quantitative polymerase chain reaction, *SOT* solid organ transplant recipients, *TMX/SMX* trimethoprim/sulfamethoxazole, *VGC* valganciclovir, *VL* viral load

Patients should be treated for a minimum of 2 weeks, but the length of therapy depends on resolution of clinical symptoms and clearance of viremia [26].

Adenovirus. High-risk allogeneic HSCT recipients (unrelated donor grafts, cord blood grafts, pediatric recipients of T-cell depleted grafts, severe graft-versus-host disease, severe lymphopenia, and treatment with alemtuzumab or antithymocyte globulin) should be screened and preemptively treated for adenovirus [53]. Serum adenovirus PCR should be monitored weekly starting at transplantation in high-risk HSCT recipients until immune reconstitution occurs. If patients develop viremia, immunosuppression should be reduced when possible; if viral loads increase above 1000 HAdV copies/ml or if the patient has persistent viremia, some centers may initiate preemptive therapy with cidofovir. If patients develop evidence of organ disease, such as colitis, or disseminated infection, treatment with cidofovir may be considered [53]. In SOT screening and preemptive therapy are not recommended [33]. Furthermore, no set threshold value or specific algorithm regarding when antiviral therapy should be started for adenovirus viremia in SOT has been developed [33]. Duration of therapy with cidofovir for adenovirus viremia or invasive disease in transplantation is not well defined. It has been suggested that cidofovir be continued until complete resolution of the signs and symptoms of disease and documentation of 1–3 negative adenovirus samples taken 1 week apart from sites that originally tested positive [33]. However, there are few caveats, the treatment should take in consideration the type of transplant and site of infection. In HSCT, hemorrhagic cystitis should be treated until hemorrhage resolves; adenovirus viremia would be a more reliable marker to follow rather the adenovirus viruria. In intestinal transplant recipients, the treatment should be determined based on the pathology results (finding of adenovirus inclusions) rather than adenovirus PCR in the tissue or stool. In SOT recipients, the treatment is usually reserved for severe cases, especially when immunosuppression cannot be adjusted. The cidofovir regimens are described in Table 3. The benefit of intravenous immunoglobulins for the treatment of adenovirus infections remains unclear [33].

Norovirus. No specific therapy for the treatment of norovirus enteritis is FDA approved. A Phase 2 study (NCT033395405) is currently ongoing to evaluate the safety and efficacy of nitazoxanide for the treatment of norovirus enteritis in HSCT and SOT recipients. Oral administration of human immunoglobulins (50 mg/kg four times/day) can be considered as an alternative treatment option for immunocompromised patients with prolonged norovirus diarrhea [54]. Although a favorable trend in resolution of diarrhea and a decreased stool output at 7 days after treatment was reported, this treatment did not shorten the length of hospital stay or decrease cost of hospitalization [1, 54].

Rotavirus. The role of orally administered human immunoglobulin and nitazoxanide for the treatment of rotavirus infections in immunocompromised patients has yet to be defined. Oral human immunoglobulins are immunologically active against rotavirus in the gastrointestinal tract, and their administration was associated with rapid improvement due to temporary neutralization of the virus, followed sometimes by recurrent symptoms. In a small study, nitazoxanide

administration was associated with shorter duration of clinical symptoms compared with treatment with oral human immunoglobulins or a combination of nitazoxanide and oral human immunoglobulin [55].

Astrovirus. No specific therapy is available for astrovirus infections. For disseminated cases (including encephalitis), treatment with ribavirin (30 mg/kg per day intravenously, then 15 mg/kg every 6 h for 4 days, followed by 8 mg/kg every 8 h for 3 days, then oral), intravenous immunoglobulin (1 g/kg), steroids (1 g/day for 3 days), and interferon alpha-2b (50 ug/kg) have been administered with unclear effectiveness [15].

Clostridium difficile. In SOT recipients, *C. difficile* is accountable for 3–31% of the diarrhea cases, with up to 33% rate of recurrence and up to 16% progression toward fulminant colitis [56]. First-line treatment for an initial episode is oral vancomycin with the addition of intravenous metronidazole in cases of fulminant CDI [27]. Vancomycin administered per rectum can also be considered in fulminant colitis or in patients with ileus. Recurrent episodes can be treated with either oral vancomycin followed by a tapered dosing regimen or with fidaxomicin. The use of proton-pump inhibitors and unnecessary antibiotics should be avoided if possible as they are associated with increased risk of recurrence. In patients with multiple recurrences, fecal microbiota transplantation (FMT) may be considered, but it is unclear if restoration of the microbiome would be associated with more infectious complications (related to the bacterial translocation or to the procedure) and changes in pharmacodynamics of the immunosuppressive drugs [27, 57]. In immunocompromised patients, most commonly reported adverse events – nausea, vomiting, abdominal pain, and procedure-related diarrhea – were self-limited. However, worsening of underlying inflammatory disease and reactivation of cytomegalovirus infections have been reported [56]. The cure rates with FMT for recurrent severe or fulminant *C. difficile* in SOT recipients are about 64% at 1 month, 59% at 3 months, and up to 95% after additional therapy (repeat procedure with or without antibiotics) [56]. Several factors were described as predictors of FMT failure: severe or fulminant colitis, presence of pseudomembranous colitis, and administration of antibiotics with no *C. difficile* activity at the time of FMT [56]. FMT, compared with different antibiotic regimens (metronidazole, vancomycin, and fidaxomicin), seems to be the most cost-effective treatment for recurrent *C. difficile* infections [57].

Enteric pathogens. Targeted antimicrobial therapy by pathogen is presented in Table 3. However, no specific dose or length of therapy in immunocompromised patients was recommended in the recently published guidelines [1, 31]. Immunocompromised patients might require more prolonged therapy compared with immunocompetent patients. The length of antimicrobial therapy must be carefully weighed against unintended and potentially harmful consequences such as antibiotic side effects, the prolongation of a carrier state (particularly in *Salmonella*), drug resistance, and the possibility of induction of disease-producing phages by antibiotics (such as Shiga toxin phage induced by quinolone antibiotics).

Cryptosporidium. When adjusting immunosuppression in cryptosporidiosis, the possibility that tacrolimus level might be increased due to enteritis and

that mycophenolate mofetil might have some antiparasitic activity against *Cryptosporidium* should be taken into consideration [41]. To date, no randomized clinical trial for the treatment of cryptosporidiosis with paromomycin, nitazoxanide, or azithromycin has been conducted in an immunocompromised population; most of the recommendations are from case series or extrapolated from patients with human immunodeficiency virus infection [41]. Treatment recommendations are presented in Table 3. Nitazoxanide is FDA approved for treatment of cryptosporidiosis. Nitazoxanide reduced the duration of diarrhea and eradicated *Cryptosporidium* cysts from stool [41]. Paromomycin, useful in reducing oocyst excretion, has not been proven to be efficacious as a stand-alone agent, though it may be useful as part of combination therapy with nitazoxanide and azithromycin for refractory cases [41]. Less data is available for azithromycin, clarithromycin, and spiramycin, and they also have significant drug-drug interactions with tacrolimus and cyclosporine. All three drugs reduce the duration of symptoms and oocyst shedding and are usually used in refractory cases [41]. Data on combination therapy relies on case reports and small case series, which may only reflect positive outcomes.

Conclusion

Diarrhea is a frequent complication of SOT and HSCT and can be associated with significant morbidity, healthcare costs, and adverse impacts on patient quality of life. Evaluation for infectious causes should be tailored based on clinical presentation, timing posttransplantation, and risk factors. While common etiologies should be high on the differential diagnosis, clinicians need to consider emerging infections, especially in patients with atypical presentations or where initial work-up is unrevealing.

Key Points

- Diarrhea complicates up to 50% of SOT, and most common causes in this population include infections; medications, particularly immunosuppressants (mycophenolate mofetil≫calcineurin inhibitors); GVHD; and PTLD. At least 20% of diarrheal episodes are self-limited and without a defined etiology.
- Up to 80% of HSCT recipients have diarrhea with the highest rates occurring in the first few weeks posttransplantation. Diarrhea is most commonly related to chemotherapy agents, neutropenic colitis, aGVHD, and infections.
- Viral agents, including *Astroviridae*, *Picornaviridae*, and *Caliciviridae* are important emerging infections in transplant recipients.
- Multiplex PCR testing of the stool is a practical, convenient modality to evaluate common infectious etiologies of diarrhea in posttransplant populations.

References

1. Angarone M, Snydman DR, AST ID Community of Practice. Diagnosis and management of diarrhea in solid-organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;26:e13550.
2. Echenique IA, Penugonda S, Stosor V, Ison MG, Angarone MP. Diagnostic yields in solid organ transplant recipients admitted with diarrhea. Clin Infect Dis. 2015;60(5):729–37.
3. van Kraaij MG, Dekker AW, Verdonck LF, van Loon AM, Vinje J, Koopmans MP, et al. Infectious gastro-enteritis: an uncommon cause of diarrhoea in adult allogeneic and autologous stem cell transplant recipients. Bone Marrow Transplant. 2000;26(3):299–303.
4. Hamdeh S, Abdelrahman AAM, Elsallabi O, Pathak R, Giri S, Mosalpuria K, et al. Clinical approach to diarrheal disorders in allogeneic hematopoietic stem cell transplant recipients. World J Hematol. 2016;5(1):23–30.
5. Lee LY, Ison MG. Diarrhea caused by viruses in transplant recipients. Transpl Infect Dis. 2014;16(3):347–58.
6. Angarone M, Ison MG. Diarrhea in solid organ transplant recipients. Curr Opin Infect Dis. 2015;28(4):308–16.
7. Calmet FH, Yarur AJ, Pukazhendhi G, Ahmad J, Bhamidimarri KR. Endoscopic and histological features of mycophenolate mofetil colitis in patients after solid organ transplantation. Ann Gastroenterol. 2015;28(3):366–73.
8. Nesher L, Rolston KV. Neutropenic enterocolitis, a growing concern in the era of widespread use of aggressive chemotherapy. Clin Infect Dis. 2013;56(5):711–7.
9. Yamasaki S, Miyagi-Maeshima A, Kakugawa Y, Matsuno Y, Ohara-Waki F, Fuji S, et al. Diagnosis and evaluation of intestinal graft-versus-host disease after allogeneic hematopoietic stem cell transplantation following reduced-intensity and myeloablative conditioning regimens. Int J Hematol. 2013;97(3):421–6.
10. Bhatt AS, Freeman SS, Herrera AF, Pedamallu CS, Gevers D, Duke F, et al. Sequence-based discovery of *Bradyrhizobium enterica* in cord colitis syndrome. N Engl J Med. 2013;369(6):517–28.
11. Cornell RF, Hari P, Drobyski WR. Engraftment syndrome after autologous stem cell transplantation: an update unifying the definition and management approach. Biol Blood Marrow Transplant. 2015;21(12):2061–8.
12. Robak K, Zambonelli J, Bilinski J, Basak GW. Diarrhea after allogeneic stem cell transplantation: beyond graft-versus-host disease. Eur J Gastroenterol Hepatol. 2017;29(5):495–502.
13. Campanini G, Rovida F, Meloni F, Cascina A, Ciccocioppo R, Piralla A, et al. Persistent human cosavirus infection in lung transplant recipient, Italy. Emerg Infect Dis. 2013;19(10):1667–9.
14. Cordey S, Vu DL, Schibler M, L'Huillier AG, Brito F, Docquier M, et al. Astrovirus MLB2, a new gastroenteric virus associated with meningitis and disseminated infection. Emerg Infect Dis. 2016;22(5):846–53.
15. Lum SH, Turner A, Guiver M, Bonney D, Martland T, Davies E, et al. An emerging opportunistic infection: fatal astrovirus (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient. Transpl Infect Dis. 2016;18(6):960–4.
16. de Vries JJ, Bredius RG, van Rheenen PF, Smiers FJ, Scholvinck EH, Vossen AC, et al. Human bocavirus in an immunocompromised child presenting with severe diarrhea. J Clin Microbiol. 2009;47(4):1241–3.
17. Jamieson FB, Wang EE, Bain C, Good J, Duckmanton L, Petric M. Human torovirus: a new nosocomial gastrointestinal pathogen. J Infect Dis. 1998;178(5):1263–9.
18. Nomura T, Tasaki Y, Hirata Y, Emoto A, Yamasaki M, Mimata H, et al. Intestinal perforation after cadaveric renal transplantation. Int J Urol. 2004;11(9):774–7.
19. Spiegelhauer MR, Andersen PF, Frandsen TH, Nordestgaard RLM, Andersen LP. Leclercia adecarboxylata: a case report and literature review of 74 cases demonstrating its pathogenicity in immunocompromised patients. Infect Dis (Lond). 2019;51(3):179–88.
20. Engsbro AL, Nielsen KL, Hornum M, Andersen LP. Laribacter hongkongensis: clinical presentation, epidemiology and treatment. A review of the literature and report of the first case in Denmark. Infect Dis (Lond). 2018;50(6):417–22.

21. Binnicker MJ. Multiplex molecular panels for diagnosis of gastrointestinal infection: performance, result interpretation, and cost-effectiveness. J Clin Microbiol. 2015;53(12):3723–8.
22. Zhang H, Morrison S, Tang YW. Multiplex polymerase chain reaction tests for detection of pathogens associated with gastroenteritis. Clin Lab Med. 2015;35(2):461–86.
23. Polage CR. Editorial commentary: microbiologic testing in post-solid organ transplant diarrhea. Clin Infect Dis. 2015;60(5):738–40.
24. Durand CM, Marr KA, Arnold CA, Tang L, Durand DJ, Avery RK, et al. Detection of cytomegalovirus DNA in plasma as an adjunct diagnostic for gastrointestinal tract disease in kidney and liver transplant recipients. Clin Infect Dis. 2013;57(11):1550–9.
25. Fisher CE, Alexander J, Bhattacharya R, Rakita RM, Kirby KA, Boeckh M, et al. Sensitivity of blood and tissue diagnostics for gastrointestinal cytomegalovirus disease in solid organ transplant recipients. Transpl Infect Dis. 2016;18(3):372–80.
26. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;28:e13512.
27. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):987–94.
28. Dubberke ER, Burdette SD, AST Infectious Diseases Community of Practice. *Clostridium difficile* infections in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):42–9.
29. Baden LR, Swaminathan S, Angarone M, Blouin G, Camins BC, Casper C, et al. Prevention and Treatment of Cancer-Related Infections, Version 2.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2016;14(7):882–913.
30. Dekker JP, Frank KM. *Salmonella*, *Shigella*, and *Yersinia*. Clin Lab Med. 2015;35(2):225–46.
31. Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis. 2017;65(12):1963–73.
32. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. 2014;27(3):441–62.
33. Florescu DF, Schaenman JM, AST Infectious Diseases Community of Practice. Adenovirus in solid organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;12:e13527.
34. Echavarria M. Adenoviruses in immunocompromised hosts. Clin Microbiol Rev. 2008;21(4):704–15.
35. Robilotti E, Deresinski S, Pinsky BA. Norovirus. Clin Microbiol Rev. 2015;28(1):134–64.
36. Yin Y, Metselaar HJ, Sprengers D, Peppelenbosch MP, Pan Q. Rotavirus in organ transplantation: drug-virus-host interactions. Am J Transplant. 2015;15(3):585–93.
37. Oka T, Wang Q, Katayama K, Saif LJ. Comprehensive review of human sapoviruses. Clin Microbiol Rev. 2015;28(1):32–53.
38. Heyworth MF. Diagnostic testing for *Giardia* infections. Trans R Soc Trop Med Hyg. 2014; 108(3):123–5.
39. Jahan N, Khatoon R, Ahmad S. A comparison of microscopy and enzyme linked immunosorbent assay for diagnosis of *Giardia lamblia* in human faecal specimens. J Clin Diagn Res. 2014;8(11):DC04–6.
40. Shirley DA, Moonah SN, Kotloff KL. Burden of disease from cryptosporidiosis. Curr Opin Infect Dis. 2012;25(5):555–63.
41. Florescu DF, Sandkovsky U. Cryptosporidium infection in solid organ transplantation. World J Transplant. 2016;6(3):460–71.
42. Chalmers RM, Katzer F. Looking for *Cryptosporidium*: the application of advances in detection and diagnosis. Trends Parasitol. 2013;29(5):237–51.
43. Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. Clin Microbiol Rev. 2010;23(1):218–34.
44. Garcia LS. Laboratory identification of the microsporidia. J Clin Microbiol. 2002;40(6): 1892–901.

45. Ghoshal U, Khanduja S, Pant P, Ghoshal UC. Evaluation of immunoflourescence antibody assay for the detection of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. Parasitol Res. 2016;115(10):3709–13.
46. Saigal K, Khurana S, Sharma A, Sehgal R, Malla N. Comparison of staining techniques and multiplex nested PCR for diagnosis of intestinal microsporidiosis. Diagn Microbiol Infect Dis. 2013;77(3):248–9.
47. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev. 2007;20(3):511–32, table of contents.
48. Van Lint P, Rossen JW, Vermeiren S, Ver Elst K, Weekx S, Van Schaeren J, et al. Detection of *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* in clinical stool samples by using multiplex real-time PCR after automated DNA isolation. Acta Clin Belg. 2013;68(3): 188–92.
49. Roxby AC, Gottlieb GS, Limaye AP. Strongyloidiasis in transplant patients. Clin Infect Dis. 2009;49(9):1411–23.
50. Mobley CM, Dhala A, Ghobrial RM. *Strongyloides stercoralis* in solid organ transplantation: early diagnosis gets the worm. Curr Opin Organ Transplant. 2017;22(4):336–44.
51. Baniak N, Kanthan R. Cytomegalovirus colitis: an uncommon mimicker of common colitides. Arch Pathol Lab Med. 2016;140(8):854–8.
52. Salomao M, Dorritie K, Mapara MY, Sepulveda A. Histopathology of graft-vs-host disease of gastrointestinal tract and liver: an update. Am J Clin Pathol. 2016;145(5):591–603.
53. Hiwarkar P, Kosulin K, Cesaro S, Mikulska M, Styczynski J, Wynn R, et al. Management of adenovirus infection in patients after haematopoietic stem cell transplantation: state-of-the-art and real-life current approach: a position statement on behalf of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation. Rev Med Virol. 2018;28(3):e1980.
54. Florescu DF, Hermsen ED, Kwon JY, Gumeel D, Grant WJ, Mercer DF, et al. Is there a role for oral human immunoglobulin in the treatment for norovirus enteritis in immunocompromised patients? Pediatr Transplant. 2011;15(7):718–21.
55. Flerlage T, Hayden R, Cross SJ, Dallas R, Srinivasan A, Tang L, et al. Rotavirus infection in pediatric allogeneic hematopoietic cell transplant recipients: clinical course and experience using nitazoxanide and enterally administered immunoglobulins. Pediatr Infect Dis J. 2018;37(2):176–81.
56. Cheng YW, Phelps E, Ganapini V, Khan N, Ouyang F, Xu H, et al. Fecal microbiota transplantation for the treatment of recurrent and severe *Clostridium difficile* infection in solid organ transplant recipients: a multicenter experience. Am J Transplant. 2019;19(2):501–11.
57. Pouch SM, Friedman-Moraco RJ. Prevention and treatment of *Clostridium difficile*-associated diarrhea in solid organ transplant recipients. Infect Dis Clin North Am. 2018;32(3):733–48.

Altered Mental Status in the Solid Organ Transplant Recipient

67

Dilek Ince

Contents

Introduction 1661
Timeline of Etiologies for Altered Mental Status 1662
Postoperative Period (<1 Month) 1662
Early Posttransplant Period (1–6 Months) 1664
Late Posttransplant Period (>6 Months) 1665
Organ-Specific Risk Factors and Complications Leading to Altered Mental Status 1666
Liver Transplantation 1666
Kidney Transplantation 1666
Heart Transplantation 1667
Lung Transplantation 1667
Intestinal and Multivisceral Transplantation 1667
Workup of Transplant Recipient with Altered Mental Status 1667
Management and Prevention 1669
Conclusions 1670
Key Points 1670
References 1670

Introduction

Altered mental status describes any change in a patient's baseline mental status and covers a broad range in acuity and severity, from confusion to delirium to stupor and coma. A broad differential diagnosis, such as primary neurologic, metabolic, infectious, and psychiatric diseases, should be considered in patients with altered mental status [1]. In addition to usual causes, factors exclusive to transplant populations, including pretransplant neurologic dysfunction, immunosuppressive regimen,

D. Ince (✉)
Department of Internal Medicine, Division of Infectious Diseases, University of Iowa Hospitals, Iowa City, IA, USA
e-mail: dilek-ince@uiowa.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_66

antimicrobial prophylaxis, epidemiology, as well as metabolic abnormalities and drug toxicities related to transplantation, contribute to altered mental status in the posttransplant setting [2, 3]. Many cases are multifactorial [4].

The spectrum of conditions leading to altered mental status in transplant recipients depends on the time elapse since transplantation as well as transplanted organ type [5, 6]. A systematic approach, taking into consideration the timeline after transplantation, enables early identification and treatment of these causes (Table 1).

Timeline of Etiologies for Altered Mental Status

Postoperative Period (<1 Month)

Postoperative delirium is common after liver, heart, and lung transplantation, occurring in 10–70% of cases [7, 8], while in a recent report, only 0.8% of kidney transplant patients developed delirium [9]. It is associated with adverse outcomes, including prolonged hospital stay, decline in functional status, graft rejection, mortality, and a major economic impact on healthcare systems [10, 11].

Infections due to surgical complications, such as bile leak, surgical site infections, nosocomial infections (catheter related bloodstream infections, *Clostridium difficile* infections, aspiration), pre-existing recipient infections, and donor-derived infections can cause altered mental status [12]. Donor-derived infections can be especially challenging to identify and fatal [13, 14]. Information from organ procurement organizations regarding information on other organ recipients from the same donor might be needed for diagnosis. Opportunistic infections are uncommon during this period in the absence of prior immunosuppressive therapies as full effect of transplant immunosuppression is not yet present [15] and usually occur only in the setting of intense epidemiologic exposures [2].

Anesthetics and perioperative pain medications play a significant role in mental status changes. Antibiotics used for surgical prophylaxis or for treatment of postoperative infections can also be implicated. Fluoroquinolones can be associated with confusion, seizures, and psychosis among their other side effects [16]. Beta-lactam antibiotics (especially cefepime) can lead to confusion, hallucinations, encephalopathy, or seizures [17]. Trimethoprim-sulfamethoxazole can lead to encephalopathy or aseptic meningitis [18]. Corticosteroid use and withdrawal of steroids following prolonged use can lead to metabolic derangements resulting in delirium. Calcineurin inhibitors (CNIs, i.e., tacrolimus, cyclosporine), especially with high serum levels, can lead to posterior reversible encephalopathy syndrome (PRES), a syndrome caused by vasoconstriction resulting in alterations in brain perfusion [19]. PRES can present with headache, visual abnormalities, focal neurologic signs, and altered mental status, seizure activity, or a combination of these symptoms. CNIs can also cause psychosis, hallucinations, seizures, and encephalopathy, especially at higher drug levels [8].

Metabolic derangements during organ recovery and fluid and electrolyte shifts can result in altered mental status. Patients with alcohol-related liver disease or

Table 1 Timeline of etiologies for altered mental status [2, 6, 20]

Postoperative (<1 month)
Infectious causes:
Nosocomial infections
Systemic infections (sepsis, catheter-related infections)
Pneumonia
Urinary tract infections
Infections due to technical complications (bile leak, wound infection)
CNS infections (donor, recipient, or transfusion derived)
Active or incubating infections in recipient prior to transplant
Noninfectious causes
Drugs
Antibiotics (fluoroquinolones), steroids, benzodiazepines, narcotics, anesthetics, CNI
DRESS syndrome
Epilepsy
Electrolyte imbalances
Osmotic demyelination
Liver failure
Hyperammonemia
Low oxygen
Anemia
Hypoxia
Poor cardiac output
Coagulopathy
Retention (urine and fecal)
Intracranial
Cerebrovascular accident
Increased intracranial pressure
Uremic encephalopathy
Metabolic/Wernicke's encephalopathy
Early posttransplant (1–6 months)
Infectious causes:
Opportunistic infections
Viral: Cytomegalovirus, Epstein-Barr virus, varicella zoster
Fungal: *Cryptococcus neoformans*, *Aspergillus* sp.
Parasitic: *Toxoplasma gondii*
Noninfectious causes:
Drugs, including PRES
Malignancy: PTLD, lymphoma
Rejection, IRIS
>6 months
Infectious causes:
Community-acquired infections
Opportunistic infections in the setting of rejections
PTLD

(continued)

Table 1 (continued)

Noninfectious causes:
Transplanted organ failure
Disease recurrence
Rejection episode
Renal failure due to immunosuppressives (e.g., CNI)
Malignancy
PTLD
Skin cancer with local or CNS metastasis (SCC, melanoma)
Unusual neurologic syndromes
Giant cell arteritis
Multiple sclerosis (optic neuritis)
Neuro-Whipple's disease
Sarcoidosis
NMDA receptor encephalitis

severe hepatic encephalopathy tend to have more prolonged encephalopathy in the posttransplant period [8].

Seizures occur in 5–10% of transplant patients and are commonly due to metabolic disorders (electrolyte disturbances), organ dysfunction, ischemic or hemorrhagic stroke, central nervous system infection, or PRES in the first month after transplant [5]. Alcohol withdrawal or Wernicke's encephalopathy can manifest in the perioperative period, especially as anesthetics are weaned off. Central pontine myelinolysis (CPM) can occur in 1–4% of liver transplant recipients, mainly in those who have had prolonged hyponatremia leading to fluctuations in consciousness [8].

Early Posttransplant Period (1–6 Months)

During this period of most intense immunosuppression, opportunistic infections and reactivation of latent infections peak. Bacterial infections such as *Nocardia* sp., *Listeria* sp., and *Mycobacterium* sp. can lead to central nervous system (CNS) infections and altered mental status. Herpesvirus infections (cytomegalovirus (CMV), herpes simplex (HSV), or varicella zoster (VZV)) are uncommon in patients on prophylaxis, but can occur upon discontinuation of prophylaxis or as breakthrough infections. Endemic mycoses, *Cryptococcus neoformans* infection, and mold infections can lead to severe disease, including CNS infection (Fig. 1). *Toxoplasma gondii* CNS infection is rare on patients on trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis [2].

Decrease in immunosuppression in the setting of infections can precipitate immune reconstitution syndrome (IRIS), which may present with encephalomyelitis [20]. CNS IRIS should be considered in patients with a previous diagnosis of invasive fungal infections, tuberculosis, toxoplasmosis, cryptococcal infection, CMV, or progressive multifocal leukoencephalopathy [20].

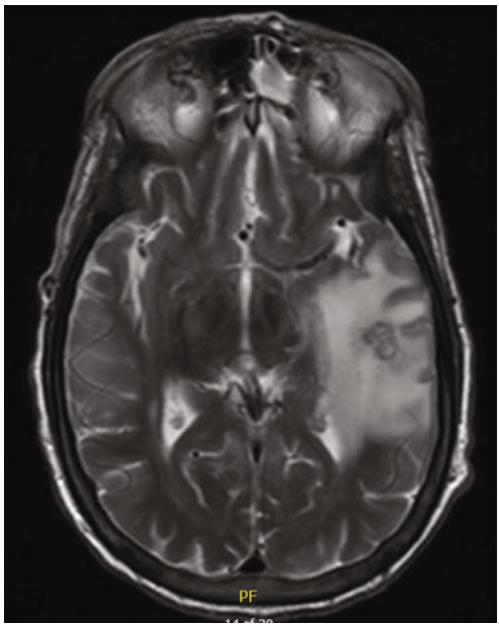


Fig. 1 T2-weighted magnetic resonance imaging of brain in a liver transplant recipient with altered mental status and left temporal lobe abscess, found to be *Scedosporium apiospermum/ Pseudallescheria boydii*. (Image: Courtesy of Dr. Camille Nelson Kotton)

Metabolic derangements, electrolyte abnormalities, electrolyte, and fluid shifts are uncommon during this period in the absence of an inciting event, such as rejection leading to graft dysfunction. Changes in medications or increased CNI levels due to graft dysfunction can lead to altered mental status.

Late Posttransplant Period (>6 Months)

Infection risk is usually decreased in the late posttransplant phase due to reduced maintenance immunosuppression and reflects the degree and duration of immunosuppression necessary to prevent rejection. Among infectious etiologies, community-based epidemiologic exposures including viruses, foodborne diseases, exposures related to hobbies (i.e., endemic mycoses, zoonotic illness, tick-borne disease), travel (i.e., malaria, dengue, ▶ Chap. 40, "West Nile Virus and Transplantation"), or residence and work (mold exposures) can lead to CNS infections or systemic infections resulting in altered mental status [15, 20]. Increase in immunosuppression for rejection therapy can set the timeline back and lead to mental status changes associated with opportunistic infections. Reactivation of herpesviruses leading to encephalitis can occur following discontinuation of prophylaxis. Infections with rare atypical organisms such as progressive multifocal leukoencephalopathy due to JC virus infection may also be encountered, mostly in the setting of prolonged intense immunosuppression [21].

A change in immunosuppressive regimen or other medications can suggest new drug-related encephalopathy and need to be reviewed carefully. Solid organ

transplant recipients have a fourfold increased risk of malignancy due to decreased immune surveillance in transplant recipients and increased susceptibility to oncogenic viruses due to immunosuppression [21]. Lymphoma is the most common brain tumor in transplant recipients, presenting as either primary CNS lymphoma or associated with posttransplant lymphoproliferative disorder (PTLD) [4]. Metastatic skin cancer (squamous cell cancer or melanoma) can also lead to altered mental status. CNS autoimmune diseases can be encountered despite ongoing immunosuppression and can be triggered by infections (such as NDMA receptor encephalitis) [6, 20].

Organ-Specific Risk Factors and Complications Leading to Altered Mental Status

Liver Transplantation

Postoperative delirium is more frequent among patients who had alcoholic cirrhosis as an indication for liver transplantation, especially those with increased pretransplant serum ammonia levels and shorter duration of abstinence [7, 8]. Other risk factors include metabolic liver disease, Model for End-Stage Liver Disease (MELD) scores above 15, and mechanical ventilation prior to transplantation [8]. Hepatic encephalopathy symptomatic at the time of transplantation was the strongest predictor of postoperative encephalopathy in one series [22].

Common complications following liver transplantation that can lead to altered mental status include cerebrovascular accidents (ischemic or hemorrhagic), osmotic demyelination, PRES, and seizures [22]. Seizure etiology is diverse and can vary from metabolic (hypoglycemia, hyponatremia, uremia), medications (CNIs), PRES, hemorrhage, and mitochondrial dysfunction secondary to acute hyperammonemia [20].

Kidney Transplantation

Major preoperative risk factors for delirium in kidney transplant patients included age greater than 65, frailty, and increasing comorbidities [9]. Postoperative complications specific to kidney transplantation leading to altered mental status include uremic encephalopathy and hypertensive stroke [20]. Cerebrovascular stroke or hemorrhage is seen frequently in kidney transplant recipients, with an incidence of stroke or transient ischemic attack of 5% in the first year and 9.5 in the second year [21]. A large proportion of these cases can be explained by comorbidities such as diabetes, age, prior stroke, polycystic kidney disease, or hypertension.

Risk of PTLD is lower following kidney transplant (1–2%) compared to other organs, with the highest risk factors being EBV serostatus and immunosuppression [21]. Incidence of PTLD is highest in the first year after transplant, with rates decreasing significantly afterward.

Heart Transplantation

Ischemic stroke is more frequent in heart transplant recipients than other organ transplants, occurring in up to 10% [23] and commonly leading to altered mental status. Heart transplant recipients also have the highest risk for toxoplasmosis, including cerebral toxoplasmosis among solid organ transplant recipients [20, 23], especially in those not on trimethoprim-sulfamethoxazole prophylaxis.

Lung Transplantation

In a retrospective series, the most common neurologic sequela following lung transplantation were postoperative delirium (40%) and PRES (4%), followed by stroke and TIA [24].

Idiopathic hyperammonemia syndrome is a rare but often fatal complication following solid organ transplantation and is predominantly seen in lung transplant recipients with an incidence of 0.5–4.1% in this patient population [25]. Patients present with encephalopathy and hyperammonemia in the absence of liver disease or congenital metabolic disorders, usually within 2 weeks of transplantation, though cases up to months after transplantation have been reported [26]. Infection with urease producing bacteria have been proposed as the etiology (i.e., ▶ Chap. 22, "*Mycoplasma* and *Ureaplasma* Infections in Transplantation: A Big Impact Despite the Lack of a Cell Wall"), though immunosuppressive therapy with tacrolimus has also been implicated [25].

Intestinal and Multivisceral Transplantation

Complex metabolic derangements, nutritional insufficiencies, and high immunosuppression requirements lead to relatively high neurologic complications following intestinal and multivisceral transplantation compared to other organ transplantations, with altered mental status (43%) and seizures (17%) being among the most common neurologic complications [27]. Liver failure commonly related to cholestatic TPN-related liver injury, but also hepatic allograft rejection, liver cirrhosis, bacterial overgrowth, and urea cycle enzyme abnormalities can occur resulting in hepatic encephalopathy. The more intense immunosuppression required in intestinal and multivisceral transplantation increases the risk of infections, including early invasive fungal infections [27]. Rejection leads to intestinal barrier defect and sepsis, which can result in altered mental status [20].

Workup of Transplant Recipient with Altered Mental Status

Timely diagnosis and treatment of altered mental status in a transplant recipient requires a thorough clinical assessment, followed by directed laboratory testing (sometimes including cerebrospinal fluid), and, if indicated, imaging, electrophysiology tests, and tissue sampling [28] (Table 2).

Table 2 Evaluation of altered mental status in solid organ transplant recipient [2, 6]

History
Prior neurologic symptoms
Graft function
Immunosuppression (length and degree)
Other drugs
Pace of illness
Intraoperative complications
Social history
Residence
Travel, place of birth, military service
Tuberculosis exposure
Sexual history
Substance abuse history
Pets and hobbies
Vaccination history and serologic data
Prior infection/colonization history
Physical examination
Vital signs (fever, tachycardia, tachypnea, hypotension)
Crackles, pleural effusion, heart murmur, sinus tenderness, skin lesions
Back and spine
Costovertebral angle tenderness
Spinal tenderness
Neurology
Focal or global deficits
Meningitis
Cranial nerve findings
Laboratory examination
Complete blood count and differential
Renal and liver, pancreatic chemistries, NH3
Lactate
Drug levels (calcineurin inhibitors)
Toxin screen
CMV, Epstein-Barr virus viral load
Antigen/antibody testing for endemic mycoses
Serum cryptococcal antigen
Cultures as appropriate (blood, urine, sputum)
Chest X-ray or chest CT
Neuroimaging
Lumbar puncture (cell count and differential, glucose, protein
Smears and culture for bacteria, fungi and mycobacteria
Molecular studies by PCR: HSV, VZV, CMV, HHV-6, EBV, JC virus, West Nile virus
▶ Chap. 40, "West Nile Virus and Transplantation" IgM
Cryptococcal antigen
Syphilis testing
Electroencephalography
Biopsy (brain, skin, lung, lymph node)

Initial evaluation should include detailed patient and donor demographics, prior infection/colonization data, comorbidities, underlying disease leading to transplantation, graft function, immunosuppressive regimen, and other medications. Detailed social history, including residence, employment, hobbies, travel history, sexual and substance abuse history, and pets, can give insights into prior exposures and emerging infectious diseases. Immunization records and serologic data should be reviewed, with the caveat that not all immunizations lead to protective immunity in the immunocompromised host [2].

Clinical presentations and physical examination findings can be delayed and atypical in transplant patients [29]. Fever can be absent in the setting of immunosuppression. Kernig and Brudzinski signs can be subtle or absent in transplant patients with meningitis [30]. A detailed physical exam can identify findings of common systemic or neurologic diseases that can lead to altered mental status [2].

Lab evaluation should include complete blood count with differential, kidney, and liver chemistries and drug level assays, such as for calcineurin inhibitors. Cultures including blood and if appropriate urine and sputum should be performed. Viral load assays for CMV and Epstein-Barr virus and antigen testing for endemic mycoses and cryptococcal infection can shorten time to diagnosis of infectious etiologies. Serologies can be delayed or negative in transplant patients depending on the intensity of immunosuppression.

Neuroimaging is recommended for most immunocompromised patients with altered mental status, and urgent brain imaging is needed for those with focal neurologic deficits. Cerebrospinal fluid evaluation should include cell count and differential, chemistry, routine bacterial, fungal and mycobacterial smears and cultures, meningoencephalitis panel, or at least HSV nucleic acid testing and cryptococcal antigen. Syphilis testing should be performed in the setting of fever or in those with no clear etiology of altered mental status. Additional specialized testing, such as viral nucleic acid testing, antigen and antibody, and 16S ribosomal RNA, may be required based on the clinical scenario [30].

Electroencephalography can identify seizures, including status epilepticus. Biopsies of focal lesions (skin, pulmonary, lymph node, brain) may be required to make a diagnosis [2, 30].

Management and Prevention

Prognosis and management for patients with altered mental status is largely dependent on the underlying cause. Patients with altered mental status due to drug toxicity or reversible metabolic causes usually do well, whereas patients with altered mental status due to CNS infections, hypoxic-ischemic encephalopathy, or CPM often do poorly [4].

In patients with toxic-metabolic encephalopathies, treatment is directed toward correcting the underlying cause (withdrawal of implicated drug, correction of metabolic derangement) while providing supportive care. If infection is suspected,

empiric antimicrobials should be started after appropriate samples for cultures are obtained.

Immunosuppression is managed individually, though reduction of immunosuppression is common for most infections. Immune reconstitution syndrome is usually treated with steroids.

Conclusions

Transplant recipients have a high prevalence of altered mental status, which is associated with poor outcomes. A systematic approach to evaluation is key to identify and manage the often coexisting causes of altered mental status in transplant recipients.

Key Points

- A variety of infectious and non-infectious etiologies can lead to altered mental status in solid organ transplant recipients.
- Organ transplanted and timeline after transplantation can give clues to the etiology.
- Urgent evaluation with physical examination, comprehensive laboratory evaluation and imaging can help identify etiology.
- Prognosis and management is dependent on the underlying cause.

References

1. Smith AT, Han JH. Altered mental status in the emergency department. Semin Neurol. 2019;39:5–19.
2. Wright AJ, Fishman JA. Central nervous system syndromes in solid organ transplant recipients. Clin Infect Dis. 2014;59:1001–11.
3. Fishman JA, Gonzalez RG, Branda JA. Case records of the Massachusetts General Hospital. Case 11–2008. A 45-year-old man with changes in mental status after liver transplantation. N Engl J Med. 2008;358:1604–13.
4. Dhar R, Human T. Central nervous system complications after transplantation. Neurol Clin. 2011;29:943–72.
5. Pedroso JL, et al. Neurological complications of solid organ transplantation. Arq Neuropsiquiatr. 2017;75:736–47.
6. Pruitt AA. Neurologic complications of transplantation. Continuum (Minneap Minn). 2017;23:802–21.
7. Sonneville R, Mariotte E, Brouwer MC. Cerebral complications of solid organ transplantation. Intensive Care Med. 2019;45:394–7.
8. Te HS. Altered mental status after liver transplant. Clin Liver Dis (Hoboken). 2017;10:36–41.
9. Haugen CE, et al. Incidence, risk factors, and sequelae of post-kidney transplant delirium. J Am Soc Nephrol. 2018;29:1752–9.

10. Beckmann S, Schubert M, Burkhalter H, Dutkowski P, De Geest S. Postoperative delirium after liver transplantation is associated with increased length of stay and lower survival in a prospective cohort. Prog Transplant. 2017;27:23–30.
11. Oliver N, et al. Post-liver transplant delirium increases mortality and length of stay. Ochsner J. 2017;17:25–30.
12. Fishman JA. Infection in organ transplantation. Am J Transplant. 2017;17:856–79.
13. Mathur G, et al. High clinical suspicion of donor-derived disease leads to timely recognition and early intervention to treat solid organ transplant-transmitted lymphocytic choriomeningitis virus. Transpl Infect Dis. 2017;19
14. Wolfe CR, Ison MG, the AST Infectious Diseases Community of Practice. Donor-derived infections: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13547.
15. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med. 2007;357:2601–14.
16. Mazzei D, Accardo J, Ferrari A, Primavera A. Levofloxacin neurotoxicity and non-convulsive status epilepticus (NCSE): a case report. Clin Neurol Neurosurg. 2012;114:1371–3.
17. Tchapyjnikov D, Luedke MW. Cefepime-induced encephalopathy and nonconvulsive status epilepticus: dispelling an artificial dichotomy. Neurohospitalist. 2019;9:100–4.
18. Bruner KE, Coop CA, White KM. Trimethoprim-sulfamethoxazole-induced aseptic meningitis-not just another sulfa allergy. Ann Allergy Asthma Immunol. 2014;113:520–6.
19. Song T, et al. Calcineurin inhibitors associated posterior reversible encephalopathy syndrome in solid organ transplantation: report of 2 cases and literature review. Medicine (Baltimore). 2016;95:e3173.
20. Pruitt AA, Graus F, Rosenfeld MR. Neurological complications of solid organ transplantation. Neurohospitalist. 2013;3:152–66.
21. Shoskes A, Wilson R. Neurologic complications of kidney transplantation. Transl Androl Urol. 2019;8:164–72.
22. Dhar R, Young GB, Marotta P. Perioperative neurological complications after liver transplantation are best predicted by pre-transplant hepatic encephalopathy. Neurocrit Care. 2008;8:253–8.
23. Munoz P, et al. Infectious and non-infectious neurologic complications in heart transplant recipients. Medicine (Baltimore). 2010;89:166–75.
24. Smith PJ, et al. Neurological sequelae and clinical outcomes after lung transplantation. Transplant Direct. 2018;4:e353.
25. Krutsinger D, et al. Idiopathic hyperammonemia after solid organ transplantation: primarily a lung problem? A single-center experience and systematic review. Clin Transpl. 2017;31
26. Nowbakht C, et al. Two cases of fatal hyperammonemia syndrome due to mycoplasma hominis and ureaplasma urealyticum in immunocompromised patients outside lung transplant recipients. Open Forum Infect Dis. 2019;6:ofz033.
27. Zivkovic SA, Eidelman BH, Bond G, Costa G, Abu-Elmagd KM. The clinical spectrum of neurologic disorders after intestinal and multivisceral transplantation. Clin Transpl. 2010;24:164–8.
28. Pustavoitau A, Bhardwaj A, Stevens R. Neurological complications of transplantation. J Intensive Care Med. 2011;26:209–22.
29. Zhong D, Liang SY. Approach to transplant infectious diseases in the emergency department. Emerg Med Clin North Am. 2018;36:811–22.
30. Guenette A, Husain S. Infectious complications following solid organ transplantation. Crit Care Clin. 2019;35:151–68.

Transplant Infectious Disease Evaluation of Cytopenias

68

Rachel Miller and Annette Schlueter

Contents

Introduction 1673
Overview of Cytopenias and Their Relevance in Transplantation 1674
Etiologies of Transplant-Associated Cytopenias 1678
Etiologies Impacting Both SOT and HSCT 1678
Etiologies Unique to SOT: Graft-Versus-Host Disease 1694
Etiologies Unique to HSCT: Graft Failure 1695
Approach to Diagnosis of Transplant-Associated Cytopenias 1696
General Approach to Management of Transplant-Associated Cytopenias 1698
Conclusion 1700
Key Points 1700
Cross-References 1700
References 1701

Introduction

Transplant recipients with cytopenias are commonly encountered in the clinical setting, and new cytopenias may occur throughout the post-transplant period. This chapter discusses the range of etiologies of transplant-associated cytopenias, followed by a general approach to diagnosis and management.

R. Miller (✉)
Division of Infectious Diseases, Department of Internal Medicine, Duke University, Durham, NC, USA
e-mail: rachel.a.miller@duke.edu

A. Schlueter
Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA, USA
e-mail: annette-schlueter@uiowa.edu

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_67

Overview of Cytopenias and Their Relevance in Transplantation

Various terminologies are used to define cytopenia and its severity. Using published reference standards [1], thrombocytopenia is defined as a platelet count of <150,000/uL and anemia as a hemoglobin of <12 g/dL. As neutrophils comprise the majority of leukocytes in the peripheral blood and bone marrow, most cases of leukopenia manifest as neutropenia. However, there is ongoing debate regarding the WHO definition of neutropenia based on the Absolute Neutrophil Count (ANC), calculated as the total WBC/uL x percent (neutrophils + bands)/100 [2]. The normal threshold level for ANC is currently defined as >1800 cells/mm^3, but this may vary based on age, sex, and race [1]. In the post-transplant setting, lymphopenia may also substantially contribute to overall leukopenia. Pancytopenia refers to depression of all three hematopoietic lineages. Bicytopenia refers to depression of two lineages. The Common Terminology Criteria for Adverse Events (CTCAE) provides further breakdown of these definitions by grading based on severity [3]. Though numerical cutoffs guide diagnostic and treatment approaches for clinical syndromes, actionable levels directed to cytopenia itself are not always clear-cut and depend on the clinical context.

Cytopenias, whether involving one or multiple cell lines, occur due to one or more general mechanisms: bone marrow infiltration, blood cell destruction/sequestration, and/or bone marrow dysplasia or in its most severe form, aplasia. The differential diagnosis within each category is broad (Table 1), with some conditions causing cytopenias by more than one mechanism. Mild cytopenias may go unnoticed by the patient or cause only mild symptoms. However, in more severe cases, the manifestations can be life-threatening, with sepsis and bleeding as the greatest concerns.

Solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients are particularly vulnerable to developing cytopenias because of their comorbidities, complex drug regimens, and altered immune systems. Accordingly, there are frequently more than one cytopenia mechanism at play simultaneously. Post-transplant timing is also an important factor to consider as potential etiologies may shift over time, with varying timelines depending on the type of transplant, post-transplant clinical course, and periodic adjustments in medication regimens. For example, leukopenia with associated neutropenia among SOT recipients occurs commonly. It is estimated that more than 50% of SOT recipients experience leukopenia at some time point during their post-transplant course, most commonly during the first year [4–7]. Thoracic organ transplantation portends the highest risk, likely related to the higher doses of immunosuppressive therapy needed to maintain allograft function [8]. Alternatively, as a result of the conditioning regimen, HSCT recipients are profoundly pancytopenic at the time of their transplant and remain so until engraftment. As such, the threshold to investigate and treat cytopenias is usually determined on an individual patient basis with consideration of the underlying transplant type, severity, and timing post-transplant. It is crucial to consider the diagnostic possibilities in the post-transplant setting, both infectious and non-infectious, in order to promptly intervene to avoid complications.

Table 1 Clinical syndromes (other than drug reactions) associated with post-transplant cytopenias, classified by the hematopoietic lineage(s) affected, typical timing post-transplant, clinical manifestations, and diagnostic approach

Etiology	Hematologic findings	Timing after transplant	Other clinical/lab features	Diagnosis confirmation
Infections				
Cytomegalovirus (CMV)	Pancytopenia, especially leukopenia Atypical lymphocytosis	Early Usually in first 6 mos or after withdrawal of prophylaxis Periods of enhanced immunosuppression	Constitutional symptoms End-organ involvement of GI tract, liver, lung, eye	Blood CMV PCR Tissue biopsy demonstrating cytopathic effect, CMV IHC Serology poor
Parvovirus B19	RBC aplasia May have accompanying TCP and leukopenia	Early Periods of enhanced immunosuppression	Constitutional symptoms Symptoms of profound anemia Occasional ↑ LFTs	Blood parvovirus PCR Serology to confirm prior infection Bone marrow biopsy demonstrating giant pronormoblasts
Adenovirus	Pancytopenia	Early Usually in first 3 mos	Constitutional symptoms End-organ involvement of GI tract, liver, lung, brain, urinary tract	Blood adenovirus PCR Adenovirus PCR of other body fluids Tissue biopsy with IHC Serology poor

(continued)

Table 1 (continued)

Etiology	Hematologic findings	Timing after transplant	Other clinical/lab features	Diagnosis confirmation
Human herpesvirus (HHV) 6, 7, 8	Pancytopenia Delayed engraftment	Early	Encephalitis Rare hepatitis	Compatible clinical syndrome Blood/CSF PCR Tissue biopsy (HHV8)
Histoplasmosis	Pancytopenia	Variable Most in first year	Subacute, multisystem illness End-organ involvement of GI tract, lung, skin, reticuloendothelial system	Serum/urine antigen EIA Blood/tissue biopsy and culture Serology poor
Other syndromes				
Hemophagocytic Lymphohistiocytosis syndrome (HLH)	≥2 cell lines	Most cases within 2 mos, but variable over years	↑↑ ferritin ↑ LFTs, LDH, triglycerides ↑↑ soluble IL-2 receptor alpha [sIL-2R]	Compatible clinical syndrome Bone marrow biopsy
Thrombotic microangiopathy	Anemia, TCP	Early Usually in first 3 mos	Hypertension, mental status changes, CNI use ↑ LDH, bilirubin, Cr, and urine free HB, ↓ haptoglobin Schistocytes on blood smear	Compatible clinical syndrome Kidney biopsy

Autoimmune cytopenias	Hemolytic anemia Anemia> TCP> all lineages	Variable	↑ LDH, bilirubin, and urine free HB, ↓ haptoglobin Symptoms are associated with lineage affected Multiple potential triggers	Positive DAT, spherocytes on PBS, reticulocytosis No direct diagnostic tests
Passenger lymphocyte syndrome	Hemolytic anemia	Early First 1–3 weeks	Primarily occurs in SOT Lab findings consistent with hemolysis	Compatible clinical syndrome Self-limiting within 3 mos
Post-transplant lymphoproliferative disease (PTLD)/EBV	Pancytopenia May present as HLH	Variable Early PTLD: usually polyclonal Late PTLD: usually monoclonal	Lymphadenopathy, visceral/soft tissue masses B symptoms	PET-CT scan Tissue biopsy Blood EBV viral load
Graft-versus-host disease (SOT)	Pancytopenia	Early	Rash, diarrhea, sparing of the allograft	Tissue biopsy, often skin, bowel Assessment for chimerism
Graft failure (HSCT)	≥2 cell lines	Early: primary graft failure Late: secondary graft failure	Symptoms are associated with the lineage(s) affected	Bone marrow biopsy Assessment for chimerism

PCR polymerase chain reaction, *IHC* immunohistochemistry, *TCP* thrombocytopenia, *LFTs* liver function tests, *CSF* cerebrospinal fluid, *EIA* enzyme immunoassay, *CNI* calcineurin inhibitor, *LDH* lactate dehydrogenase, *Cr* creatinine, *HB* hemoglobin, *DAT* direct antiglobulin testing, *PBS* peripheral blood smear, *PET-CT* positron emission tomography-computed tomography, *EBV* Epstein-Barr virus, *SOT* solid organ transplant, *HSCT* hematopoietic stem cell transplant, *HB* hemoglobin, *RBC* red blood cell

Etiologies of Transplant-Associated Cytopenias

Etiologies Impacting Both SOT and HSCT

Infections

Infectious etiologies are a major consideration in the evaluation of cytopenias. Transplant recipients are susceptible not only to common infections but also to opportunistic infections, which may manifest with one or more cytopenias by a variety of mechanisms. As a group, viral pathogens are the most frequently encountered, particularly those within the *Herpesviridae* family. While acute bacterial sepsis is often transiently associated with cytopenias, there are specific bacterial infections in which cytopenias manifest as a more prominent feature. Likewise, parasitic infections due to Leishmania, Toxoplasma, Babesia, and malaria may all cause cytopenias. Fungal infections, aside from histoplasmosis, infrequently manifest with cytopenias. The most common infections associated with cytopenias are discussed below, with a more comprehensive list of etiologies outlined in Table 2.

Cytomegalovirus

Cytomegalovirus (CMV) is a ubiquitous herpesvirus which infects the majority of humans, with an estimated seroprevalence of 30–97%. The seroprevalance varies based on geography and socioeconomic status and increases with age [9]. Primary infection typically manifests as asymptomatic infection or a self-limited febrile illness in immunocompetent host, after which the virus establishes latency in monocytes and various other cell types. These cells serve as a reservoir for reactivation infection or a vehicle to transmit CMV infection to susceptible hosts via blood transfusion, SOT, or HSCT. CMV infection is of particular importance as an opportunistic infection in the post HSCT and SOT transplant setting [10–12]. Pancytopenia, especially leukopenia, is a common finding, often associated with atypical lymphocytes on peripheral blood smear. Direct inhibition of hematopoiesis due to virus-induced damage to bone marrow stroma and reduced regeneration of marrow repopulating pluripotent stem cells has been implicated in the pathogenesis of cytopenias [13]. Associated clinical findings range from asymptomatic infection to a flu-like illness to life-threatening invasive tissue disease. CMV infection may also contribute to delayed engraftment in HSCT recipients. Broadly, risk factors for post-transplant CMV include donor/recipient sero-mismatch and increased depth of immunosuppression. CMV infection classically manifests in the early post-transplant period, specifically in the first 6 months. However, post-transplant antiviral prophylaxis regimens have become standard of care which result in postponement or prevention of CMV infection, altering the traditionally observed time course [11].

The diagnosis of post-transplant CMV infection/disease relies on detection of CMV viremia by polymerase chain reaction (PCR) assay and/or demonstration of cytopathic effect and viral inclusions on tissue biopsy [14, 15]. In most instances, the diagnosis of CMV-associated pancytopenia is inferred from identification of CMV viremia in the appropriate clinical context. In SOT-associated CMV infection, ganciclovir or valganciclovir are the initial treatments of choice. Though pancytopenia, particularly leukopenia, is a well-established side effect of this agent, blood

Table 2 Pathogens causing infections associated with cytopenias, categorized by hematopoietic lineage affected

	Lineage affected		
	WBCs	Hemoglobin	Platelets
Viruses			
Cytomegalovirus (CMV)	√√	√	√
Parvovirus B19	√	√√	√
Adenovirus	√	√	√
Human herpesvirus (HHV) 6, 7	√	√	√
HHV 8	√	√	√
Epstein-Barr virus (EBV)	√	√	√
Herpes simplex virus (HSV)	√√	√	√
(LCMV)	√	√	√
Enterovirus	√	√	√
Dengue virus	√	√	√√
Hepatitis A/B/C/E	√	√	√
Human immunodeficiency virus (HIV)	√	√	√
Parainfluenza	√	√	√
Fungi			
Histoplasma	√	√	√
Bacteria			
Mycobacterium tuberculosis	√	√	√
Mycobacterium avium complex	√	√	√
Ehrlichia/Anaplasma	√	√	√
Brucella	√	√	√
Leptospirosis	√	√	√√
Bartonella henselae	√	√	√
Typhoid fever	√	√	√
Parasites			
Leishmania	√	√	√
Toxoplasma	√	√	√
Babesia		√	√
Malaria		√	√

counts typically improve despite use of this agent in the setting of active CMV infection [11]. In HSCT recipients, foscarnet is more frequently used as first-line CMV therapy in the early post-transplant period to avoid ganciclovir-associated toxicity to the bone marrow graft [16].

For a more extensive discussion of CMV management, refer to ▶ Chaps. 24, "Cytomegalovirus in Solid Organ Transplant Recipients: Prevention, Diagnosis, and Treatment" and ▶ 25, "Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment."

Parvovirus

Human parvovirus B19 infection is a common illness of childhood, with approximately half of the population demonstrating seropositivity by age 15 [17, 18].

Infection in transplant recipients may be through primary infection, reactivation from latent virus, or donor transmission [19, 20]. Despite the high seropositivity among adults, the incidence of symptomatic infection among transplant recipients is estimated to be <5%. The virus has a particular tropism for human erythroid progenitor cells expressing the P antigen [21]. The P antigen facilitates viral entry into the cell with subsequent viral replication leading to cellular lysis and resultant red blood cell aplasia. The pathogenesis of this finding is speculated to be non-specific cytopathic effects in the bone marrow or restricted nonstructural protein expression in megakaryocytes, leading to cytotoxicity but not viral progeny.

The clinical manifestations of human parvovirus B19 in transplant recipients reflect this pathogenesis, in that the most common manifestation is severe and/or persistent anemia without accompanying reticulocytosis or response to erythropoietin. A subset of transplant recipients will manifest concomitant leukopenia (38%), thrombocytopenia (21%), or both with the anemia [22, 23]. Organ invasive diseases, including carditis, hepatitis, pneumonitis, glomerulonephropathy, vasculitis, and neurologic diseases, have been described among both HSCT and SOT recipients, though these are distinctly less common than the hematological manifestations [19, 23]. In the SOT setting, direct involvement of the allograft may lead to graft failure. Rash and arthropathy are seen commonly in parvovirus infection among immunocompetent children and adults, respectively. However, these findings are infrequent among transplant recipients, which are postulated to be due to impaired formation of antigen-antibody complexes. Clinically apparent infection is more common in the early months following transplant, typically during the period of more intense immunosuppression. However, relapsing or persistent anemia associated with viremia is a common occurrence, in some cases lasting months to years [19].

Published guidelines recommend the initial diagnostic approach for suspected active parvovirus B19 infection should be based around a serum/whole blood PCR assay and potentially also include serology [19]. The positive predictive value of parvovirus viremia is high in the transplant recipient with a compatible clinical syndrome, especially red blood cell aplasia. A higher viral load correlates with symptomatic disease. Serology can also be obtained, though the interpretation is problematic in this patient population and it should not serve as the sole diagnostic test. Serology may be unreliable to diagnose primary infection due to blunted antibody production in the setting of immunosuppression. Additionally, background parvovirus seroprevalence is estimated to be 60–90% in adults, and thus serologic distinction between primary, remote, or reactivation infection is challenging. Eid and colleagues detail the variation in parvovirus IgG and IgM serology results among 98 transplant recipients with confirmed parvovirus disease [22]. When serology and serum/blood PCR results are inconclusive, bone marrow examination with concomitant in situ hybridization or immunohistochemistry (IHC) staining should be performed [19]. Pathognomonic bone marrow biopsy findings include giant pronormoblasts with prominent intranuclear inclusions and cytoplasmic vacuolations and absent late normoblasts [22, 24]. Unlike many chronic viral infections, a strong humoral immune response is required to control and clear the infection. Accordingly, immunoglobulin (IVIG) therapy has historically been the primary approach to

parvovirus treatment in transplant recipients [19, 20]. However, the optimal dose and duration have not been established. Relapsed and recurrent infections occur in an estimated 23% of transplant recipients, with or without IVIG therapy [22]. There are no known effective antiviral drugs recommended for the treatment of parvovirus B19 infection. As such, a reduction in immunosuppression should be attempted if possible. Successful resolution of the clinical syndrome and viremia is prolonged, typically taking weeks to months.

Adenovirus

Adenoviruses remain an important clinical pathogen in the setting of transplantation. There are over 50 serotypes that cause human infection with worldwide endemicity [25]. The virus establishes latency primarily in T-lymphocyte populations in various tissues, including lung, intestine, CNS, tonsils, and adenoids [26]. These sites can harbor small quantities of replicating and nonreplicating viral DNA in the absence of symptoms [25]. During latency, the virus evades immune surveillance. Adenovirus infections are more frequently encountered in children than adults, both in the immunocompetent and immunocompromised patient populations. The highest incidence is in children under the age of 5 years and decreases toward adolescence. Among SOT recipients, the incidence depends on the allograft type and degree of immunosuppression: 11% for kidney recipients; 3.5–38% for liver recipients; 7–50% for heart, lung, and heart-lung recipients; and 4.3–57.1% for intestinal and multivisceral recipients [26]. The incidence of adenovirus infections in allogeneic HSCT recipient ranges from 2.5% to 20%, with higher rates reported among the pediatric and T cell-depleted HSCT recipients [20]. Most clinically significant infections occur within the first 3 months post-transplant, though given the ubiquitous nature of the virus, adenovirus infections may occur anytime in the post-transplant period [27]. Early adenovirus disease post-transplantation suggests either endogenous adenovirus reactivation of latent virus or donor-derived infection. Serologic testing for adenovirus in donors and recipients as a risk stratification strategy is not routinely performed in clinical practice, as this is unlikely to alter patient management. Sporadic adenovirus infections occurring later in the post-transplant period are generally presumed to be primary infections. In general, distinguishing between primary and reactivation infection is not thought to be clinically relevant.

Clinical manifestations among transplant recipients demonstrate a spectrum from asymptomatic viral shedding to severe, disseminated disease. The gastrointestinal tract is the most common site affected, typically manifesting with fever (53%) and diarrhea (68%) [20, 26]. End-organ involvement may also manifest as respiratory disease with pneumonitis (21%), hepatitis, encephalitis, and/or hemorrhagic cystitis. Disseminated disease is defined as the involvement of two or more organ systems. Pancytopenia, particularly leukopenia, and thrombocytopenia are common, though rarely manifest as an isolated clinical finding. In allogeneic HSCT recipients, with the exception of hemorrhagic cystitis and gastroenteritis, end-organ disease is associated with a mortality rate of 20–44% [28–30]. There are isolated case reports of mortality associated with adenovirus infections among SOT recipients, suggesting a lower mortality rate as compared to HSCT recipients. However, localized allograft

infection in SOT may have a direct impact on immediate and long-term allograft function, particularly among lung transplant recipients [26].

Adenovirus disease is defined by the presence of attributable signs and symptoms combined with one or more positive adenovirus diagnostic assays. Diagnostic tests include the detection of adenovirus in tissue biopsy specimens by immunohistochemistry or culture, demonstration of viremia by blood PCR, and/or detection of adenovirus DNA or antigen from other body fluids such as cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL) fluid, urine, or stool [26]. It is challenging to differentiate true adenovirus disease from asymptomatic viral shedding, which may occur for prolonged periods before and after clinical infection. Serology is of limited utility in the post-transplant setting due the prevention of antibody production in the immunosuppressed state. The primary approach to treatment is supportive care and a reduction in immunosuppression. Cidofovir is an option for the most critically ill, and a number of experimental therapies are in various stages of clinical development. Currently, there are no randomized clinical trials to guide an approach to adjunctive antiviral or immunoglobulin therapy [20, 26].

For a more extensive discussion of adenovirus epidemiology and management, refer to ▶ Chap. 32, "Transplant Adenovirus Infection: Clinical Syndromes, Diagnosis, and Management."

Human Herpesviruses 6 and 7

Human herpesviruses (HHV) 6 and 7 are closely related Betaherpesviruses that are typically acquired early in life, with the establishment of lifelong latency following primary infection. By adulthood, nearly 90% of individuals are seropositive for both viruses [31]. Latent HHV-6 is found in monocytes and macrophages, as well as bronchial, oropharyngeal, and salivary epithelial cells [27]. HHV-7 primarily infects CD4+ T cells [32]. Viral reactivation with associated lytic infection may occur in the setting of immunosuppression, usually within the first 1–2 months post-transplant [31, 32].

The majority of reactivation events in transplant recipients are asymptomatic. However, clinical syndromes, particularly for HHV-6, have been well described [31, 32]. In both HSCT and SOT recipients, HHV-6 reactivation manifests most commonly as a febrile illness associated with bone marrow suppression [33]. HHV-6 in HSCT recipients has also been implicated in delayed engraftment, graft loss, and the development of acute GVHD [34, 35]. The most concerning syndrome, typically only seen in heavily immunosuppressed HSCT recipients, is HHV-6-associated limbic encephalitis [36–38]. Clinical manifestations often include seizures, acute delirium, and memory deficits. MRI imaging characteristically shows medial temporal lobe enhancement with hippocampal area encephalitis. CSF can reveal a lymphocytic pleocytosis, and typically by this point the recipient is either viremic or has HHV6 detected in the CSF, or both. Encephalitis is a well-described entity in the HSCT population, but less common in the SOT setting. Hepatitis, enteritis, and pneumonitis have also been reported, although causality is hard to prove in these cases [31, 39, 40]. Clinically relevant HHV-7 disease is infrequently encountered in HSCT and SOT recipients, but has been reported to cause a nonspecific febrile illness

associated with thrombocytopenia and bone marrow suppression, particularly in children [41, 42].

The preferred diagnostic assay for both HHV-6 and HHV-7 is quantitative PCR of serum or plasma [43, 44]. PCR may also be used to quantify viral DNA from tissue, CSF, and BAL fluid specimens. While available through reference laboratories in North America and Europe, their availability elsewhere is limited. Interpretation is limited by the lack of assay standardization and validation, which prohibits the establishment of a threshold value for clinical significance [45]. Likewise, finding HHV-6/HHV-7 DNA in any specimen does not confirm causality, particularly since the majority of reactivation events in transplant recipients are not associated with symptoms [46, 47]. An infrequent but well-described phenomenon of chromosomally integrated HHV-6 should also be considered when interpreting HHV-6 viral loads. Suspicion for chromosomally integrated HHV-6 should be raised in the setting of extremely and persistently elevated HHV-6 viral loads that show minimal fluctuation over time, even with the administration of antiviral therapy [48, 49]. Recognition of this phenomenon is important in order to avoid unnecessary antiviral therapy in individuals who have persistently elevated HHV-6 viral loads but no clearly attributable symptoms. Hence, HHV-6 and HHV-7 PCR assays must be always be interpreted in the clinical context.

Antiviral treatment with ganciclovir, foscarnet, or cidofovir should be initiated in the setting of HHV-6 encephalitis. The evidence for administering antiviral treatment for other HHV-6 and HHV-7 syndromes such as bone marrow suppression is weak, but may be considered in the setting of viremia where symptomatic infection is plausible or failure of allo-HSCT engraftment is feared. The majority of reactivation events do not require treatment. For all cases of HHV-6 and HHV-7 viral reactivation, an adjunctive reduction in immunosuppression should be considered [31, 32].

Human Herpesvirus 8

HHV-8 is a DNA gammaherpesvirus that exhibits both latent and lytic phases. The virus primarily infects B cells and establishes lifelong latency. There is varied geographic prevalence of the virus with the highest seroprevalence in individuals from sub-Saharan Africa (>30–40%), followed by the Mediterranean (10–20%) and Asia/United States (<10%) [32, 50, 51]. Clinically apparent post-transplant HHV-8 infections are rare. For SOT recipients, incidence estimates range from <1% in nonendemic regions to 3–5% in endemic regions [31]. For HSCT recipients, data are more limited, but incidence estimates are <1%, with higher risk for allogeneic versus autologous transplants [52]. The majority of clinical HHV-8 infections in the post-transplant setting are presumed due to viral reactivation, though donor-transmitted infection through the solid organ has been observed [53–55]. A clear understanding of the epidemiology of HHV-8 infections in the transplant setting is limited due to the lack of routine pre-transplant serologic screening among donors and recipients, both in the SOT and HSCT populations. Individuals with HHV-8 infection may present with both infectious and neoplastic manifestations. Relevant to cytopenias, both HSCT and SOT recipients may present with cytopenias

concomitant with a spectrum of illness characterized by fever, maculopapular rash, splenomegaly, and hepatitis [31, 52]. In more severe cases, HHV-8 may progress to lymphoproliferative B-cell disorders, bone marrow failure, and hemophagocytic syndrome. Another HHV-8-associated syndrome, multicentric Castleman disease, is characterized by B-cell transformation to plasmablasts and associated with fevers, hepatosplenomegaly, and cytopenias [56, 57]. Kaposi's sarcoma is the most common HHV-8-associated neoplasm, and cytopenias are a less common clinical feature with this disease than with the aforementioned syndromes.

Transplant recipients and their donors who are living with HIV infection may be a unique population at risk for HHV-8 reactivation and disease. Higher rates of HHV-8 seropositivity occur in men with male sexual partners, individuals who are also at higher risk for HIV infection. Consequently as organ donation and transplantation among individuals living with HIV infection become increasingly accepted, clinicians should be mindful of the risks of HHV-8 syndromes, especially early in the post-transplant period when immunosuppression is greatest.

The diagnosis of HHV-8 syndromes is most definitively made with tissue biopsy of the involved site, with corresponding immunohistochemistry staining, in situ hybridization, and viral PCR [58]. Quantitative PCR of plasma and peripheral blood mononuclear cells can also be performed. Test interpretation is challenging due to lack of interlaboratory standardization and varying testing modalities, but may be helpful when interpreted within the clinical context.

As there are no controlled studies assessing the utility of antiviral therapy for HHV-8, these agents are not recommended in treatment. Management recommendations call for judicious reduction or cessation of immunosuppression [31, 52]. In the setting of Kaposi's sarcoma, early data indicate that the use of the mammalian target of rapamycin inhibitors (MTORi), sirolimus and everolimus, may also provide therapeutic benefit. In addition to the immunosuppressive properties of these agents, MTORi inhibit the growth of certain vascularized tumors. The mechanism is likely multifaceted, but presumed related in part to the drug's action of disrupting vascular endothelial growth factor production and activity, as well as impacting HHV-8-associated oncogenesis [59]. This approach may be particularly beneficial in the setting of transplant-associated Kaposi's sarcoma where preservation of allograft function is desired. In general, Kaposi's sarcoma treatment requires oncology consultation.

Epstein-Barr Virus (EBV) and Post-Transplant Lymphoproliferative Disorder

Post-transplant lymphoproliferative disorder (PTLD), a heterogeneous group of polyclonal and monoclonal lymphoid proliferations, is a well-recognized EBV-associated complication of both SOT and allogeneic HSCT [32, 60, 61]. Cytopenias of one or multiple cell lines may be a manifestation of PTLD as a consequence of marrow infiltration with monoclonal B cells, hypersplenism, and/or immune destruction. In addition, cytopenias result as a consequence of PTLD treatment.

Although the most feared EBV-associated disease after transplantation is PTLD, non-PTLD manifestations can also occur. Clinical features include an infectious mononucleosis type syndrome, organ-specific diseases such as hepatitis,

pneumonitis, and gastrointestinal symptoms and hematological abnormalities [60]. Specific hematologic findings include leukopenia, thrombocytopenia, hemolytic anemia, as well as hemophagocytosis. In severe cases, hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome may occur (discussed below). Clinical EBV-associated syndromes most commonly manifest in the early post-transplant period, often in the setting of enhanced immunosuppression. However, the timing of PTLD is more variable, spanning the entire post-transplant period [62].

Diagnosis of post-transplant EBV-related syndromes, including PTLD, includes interpreting EBV-specific diagnostic tests in the clinical context [60, 63]. Obtaining an EBV viral load in the blood is often the initial screening assay. This assay is most predictive of the risk of developing or having PTLD in individuals at highest risk, specifically EBV sero-mismatched recipients and possibly all intestinal transplant recipients. The diagnostic utility of EBV viral load testing is less established in adult SOT recipients who were EBV-seropositive pre-transplant, as elevated and often sustained viral load elevations have been observed and appear to be a poor marker of future PTLD risk [60]. Furthermore, serial EBV viral load measurements are of unclear value in tracking treatment success. The optimal assay type, interpretive diagnostic criteria, and monitoring algorithms remain uncertain. Interpretation of EBV viral load measurements in BAL fluid and CSF are even less well established. Serology is of limited utility except as a means to risk stratify and inform prevention strategies. Ultimately, diagnosis of EBV-associated PTLD requires biopsy of involved tissues and histopathologic evaluation, typically including RNA in situ hybridization targeting Epstein-Barr-encoded small nuclear RNA (EBER) [63]. Positron emission tomography-computerized tomography (PET-CT) is a useful adjunct for disease staging and assessment of treatment response [64].

Management of post-transplant EBV-related syndromes is multifaceted and depends on the specific clinical manifestations [60]. For non-PTLD EBV syndromes, the recommended initial intervention is a reduction in immunosuppression [65]. Many cases will resolve with this intervention alone. While this approach is also recommended in PTLD, it is combined with rituximab or cytotoxic chemotherapy and/or surgical resection [60, 66]. Adoptive immunotherapy using donor-derived or third-party EBV-specific cytotoxic T cells has been used successfully for the treatment of PTLD in allogeneic HSCT recipients, but experience is limited in SOT [67]. As there are no antiviral agents effective against EBV, their use is not recommended [68].

For a more extensive discussion of EBV epidemiology and management, refer to ► Chap. 27, "Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorders."

Histoplasma capsulatum

Histoplasmosis is caused by the dimorphic fungus, *Histoplasma capsulatum*, which occurs in unique geographic niches in several areas worldwide. In the United States, the organism is endemic to the Ohio and Mississippi River valleys. The clinical spectrum of illness ranges from a self-limited febrile illness to severe multi-organ dysfunction, depending on the size of the host inoculum and immune status of the

host. Estimated incidence of histoplasmosis after SOT is <1%, with post-HSCT histoplasmosis limited to case reports and small case series [69–71].

The infection commonly presents in an occult, subacute manner among transplant recipients and is often disseminated at the time of diagnosis [69, 71–74]. Pancytopenia is a frequent manifestation due to decreased hematopoiesis and/or marrow infiltration and may be an important early clue to diagnosis (Fig. 1). Other associated findings include fever/constitutional symptoms, pulmonary infection, hepatosplenomegaly, liver function abnormalities, and gastrointestinal involvement. Confirmatory diagnosis can be made with direct visualization of *H. capsulatum* yeast forms in involved tissues and/or culture growth. Bone marrow aspirate cultures are often positive. However, cultures may take up to 4 weeks to demonstrate growth. A more rapid diagnosis is often made by antigen detection enzyme immunoassays. In the setting of disseminated disease, the newer-generation assays have a sensitivity of 97% and 89% in urine and blood specimens, respectively [73–75]. Lower sensitivity is observed in isolated pulmonary infection. Test specificity is limited by its cross-reactivity with other endemic fungi, notably 90% with *Blastomyces*. Cross-reactivity has also been reported with *Coccidioides* spp., *Paracoccidioides brasiliensis*, *Talaromyces marneffei*, and *Sporothrix schenckii* [75]. Serology has limited value in the post-transplant setting due to blunting of the serologic response in the setting of immunosuppression.

Despite the severity of illness upon presentation, treatment efficacy with systemic antifungal therapy is 80–100% among infected transplant recipients [72]. A concomitant reduction in immunosuppression is a typically employed adjunctive treatment approach.

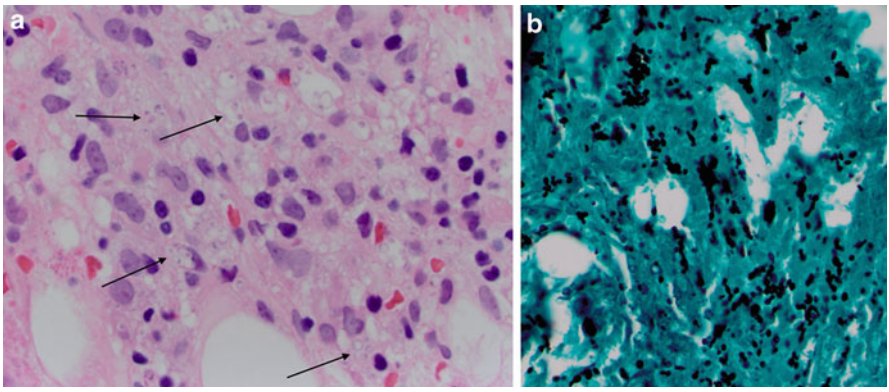


Fig. 1 Bone marrow biopsy specimen demonstrating multiple 1–4 μm, round- to oval-shaped *Histoplasma capsulatum* yeast forms. (**a**) Hemotoxylin and eosin stain (100X). Arrows highlighting multiple yeast forms demonstrating a central basophilic body, refractile capsule, and perinuclear clear zone (halo). (**b**) Grocott methenamine silver stain (50X) showing multiple densely staining yeast forms with rare budding and the characteristic "clusters of grapes" morphology

Mycobacteria

Mycobacterial infections are uncommon in SOT and HSCT recipients, but are associated with significant morbidity and mortality [76–80]. The true incidence of infection varies by geographic region and transplant type. For both *M. tuberculosis* and nontuberculous mycobacteria, disseminated infection occurs with higher frequency in transplant recipients than in their non-immunosuppressed counterparts. The timing of post-transplant mycobacterial infections is variable and reflects the intensity of the environmental exposure/inoculum and the underlying immunosuppression. Infections typically present subacutely, frequently with slowly progressive pulmonary and/or cutaneous manifestations. When the bone marrow is involved in disseminated infection, pancytopenia can result due to marrow infiltration with granulomas and decreased hematopoiesis. Bone marrow biopsy typically shows granulomas with acid-fast bacilli. Bone marrow and/or peripheral blood culture may be positive in disseminated disease. Cultures may take as long as 6–8 weeks before turning positive, and identification of the specific agent may take additional days [76]. There are limited data spanning over two decades on the performance of PCR and other molecular assays for the diagnosis of mycobacterial infections on bone marrow specimens [81–83]. However, among the multiple assays studied, the assays demonstrate variable sensitivity and specificity depending on the patient population, incidence of the disease, and specimen type (bone marrow aspirate versus core biopsy) tested. These factors, in addition to the cost and logistics of test validation, have limited their more widespread development to date. This is a promising area of investigation, however, as newer-generation molecular assays to diagnose mycobacterial infections become more mainstream. Treatment courses require multiple antimicrobial agents for a prolonged period, which are often poorly tolerated.

For a more extensive discussion of mycobacteria and their management, refer to ▶ Chaps. 18, "*Mycobacterium tuberculosis* in Transplantation"; ▶ 19, "Rapidly Growing Mycobacterial Infections in Transplant: Evolving Epidemiology and Treatment Options" and ▶ 20, "Slow-Growing Nontuberculous Mycobacteria in Transplant."

Drugs

Drugs are an important cause of cytopenias that can influence one or more cell lineages, with drugs accounting for 20–40% of all instances of cytopenias [84]. The proportion may be even higher in transplant recipients, particularly in the early post-transplant period, due to induction immunosuppressive therapy and intense maintenance immunosuppression. There are multiple mechanisms by which drugs may cause cytopenias, some of which are discussed in the sections of this chapter on thrombotic microangiopathy (TMA) and autoimmune disorders. However, the most common mechanisms in the transplant setting are cytopenias due to marrow suppression (decreased production) and/or increased destruction directly from the targeted cytotoxic agents. Identifying the culprit drug for a drug-induced hematological cytopenia lacks an evidence-based approach. Often, individuals are receiving multiple cytopenia-inducing agents that may potentiate the toxicity of any one drug. Most management decisions are based on clinical experience and the relative thresholds/risks of dose modification and/or drug discontinuation. In most instances,

cytopenias resolve with drug discontinuation. A summary of drugs commonly associated with cytopenias in the post-transplant setting is outlined in Table 3, with the more clinically significant drugs discussed below.

Immunosuppressive agents used in transplantation are commonly associated with hematologic derangements. Though antibody therapies have defined targets for their use as induction agents or for treatment of rejection, they are well known to be associated with collateral cytopenias. For example, rituximab, a chimeric anti-CD20 monoclonal antibody leading to B-cell depletion, has been associated with grade 3 or 4 cytopenias in 48% of patients (lymphopenia> neutropenia> anemia> thrombocytopenia) [85]. Likewise, alemtuzumab, a humanized monoclonal IgG1 antibody directed against CD52, is associated with a combined incidence of leukopenia and neutropenia of 47% and thrombocytopenia of 14% [85]. Antithymocyte globulin is less specific in its target, as despite its name the antibodies are directed against and cross-react with multiple blood cell types, resulting in a broad range of cytopenias of varying degrees based on the dose and duration of therapy. For HSCT recipients, the chemotherapeutic agents, methotrexate and/or cyclophosphamide, may be used in the early post-transplant setting for prevention of graft-versus-host disease (GVHD). Both drugs have known marrow suppressive effects. However, cyclophosphamide has a unique mechanism of action such that it can prevent GVHD without impairing engraftment [86]. Regarding methotrexate, doses administered in this setting are variable, but are generally lower than those standardly used for treatment of the underlying malignancy with less associated toxicity and minimal effect on engraftment [87, 88].

Agents typically used for longer term immunosuppression in both SOT and HSCT also are commonly associated with cytopenias and are frequently dose related [85]. Though the antimetabolites mycophenolate mofetil and azathioprine can lead to marrow suppression of all three cell lineages, leukopenia is the most common and pronounced. Dose reduction is a frequent management approach, particularly when use of other marrow suppression agents are more pressing from a clinical standpoint. The calcineurin inhibitors, tacrolimus and cyclosporine, have also been associated with cytopenias of all lineages. Tacrolimus, however, has been more frequently implicated for its hematologic (and other) adverse effects. Bone marrow toxicity associated with the two most commonly used MTORi, sirolimus and everolimus, is a well-known phenomenon. Anemia is the most common cytopenia and typically the most pronounced, but leukopenia and thrombocytopenia occur in 10–17%. Generally these occur within the first month of initiating therapy and are potentiated with concomitant use of other marrow suppressive drugs.

Several antimicrobial agents used routinely in transplantation for prophylaxis and treatment of infection are associated with cytopenias. The most pronounced toxicity among these agents is leukopenia, which can occur with ganciclovir/valganciclovir and trimethoprim-sulfamethoxazole (TMP-SMX), though multi-lineage suppression can occur with higher doses [10, 85, 89]. Linezolid is associated with bone marrow suppression, particularly thrombocytopenia, with longer durations of therapy (>10 days). The risk with tedizolid, the newer agent in the oxazolidinone class, appears to be less [90]. This is frequently a therapy-limiting issue in transplant recipients and a

Table 3 Drugs associated with cytopenias that are frequently used in transplantation

	Lineage affected			
	WBCs	Hemoglobin	Platelets	Comments
Anti-infectives				
Penicillins	√	√	√√	Can cause severe DIHA and DIITP, most common with piperacillin, PCN
Cephalosporins		√	√	Can cause severe DIHA, most common with cefotetan, CTX
Oxazolidinones	√	√	√√	↑ severity with longer treatment durations
Trimethoprim-sulfamethoxazole (TMP-SMX)	√√	√	√	Dose-dependent effect, can cause DIITP
Vancomycin	√		√	Associated with DIITP
Nitrofurantoin		√		
Chloramphenicol	√	√	√	Can cause serious and fatal blood dyscrasias
Rifampicin	√	√	√	TCP especially at high doses, DIITP
Isoniazid	√	√	√	
Ethambutol	√		√	
Amphotericin		√√		
Flucytosine	√	√	√	
Dapsone	√	√√		Can also cause methemoglobinemia
Ganciclovir/ valganciclovir	√√√	√√	√√	Dose dependent
Ribavirin	√√	√√	√	Associated with severe hemolytic anemia
Quinine	√	√	√	Can cause DIITP
Immunosuppressive agents				
Calcineurin inhibitors	√	√	√	
mTOR inhibitors	√	√√	√√	
Antimetabolites: MMF, AZA	√√	√√	√√	Dose dependent
Alemtuzumab	√√√		√	Profound lymphocytopenia
Thymoglobulin	√√√	√	√√	
Rituximab	√√	√√	√√	
Chemotherapeutic agents[a]	√√√	√√√	√√√	
Miscellaneous				
Colchicine	√	√	√	Blood dyscrasias reported
ACE inhibitors		√		
Amiodarone			√	

(continued)

Table 3 (continued)

	Lineage affected			
	WBCs	Hemoglobin	Platelets	Comments
Haloperidol	√			Fatal reports of WBC dyscrasias
Heparin			√	HITT due to DIITP
Thiazide diuretics	√	√	√	

Estimated frequencies: √ <10%, √√ 10–20%, √√√ >20%.
DIHA drug-induced hemolytic anemia, *DIITP* drug-induced immune thrombocytopenic purpura, *HITT* heparin-induced thrombocytopenia with thrombosis, *WBC* white blood cell, *PCN* penicillin, *TCP* thrombocytopenia, *CTX* ceftriaxone, *MMF* mycophenolate mofetil, *AZA* azathioprine
[a]Includes alkylating agents, antitumor antibiotics, topoisomerase inhibitors, antimetabolites, and mitotic inhibitors

reason to avoid use of these agents early after HSCT. Dapsone-induced neutropenia has been reported, occasionally severe enough to result in agranulocytosis. Dapsone can also result in acquired methemoglobinemia. Beta-lactam antibiotics and TMP-SMX can cause hemolytic anemia [91]. Although vancomycin triggers drug-induced immune thrombocytopenic purpura or granulocytopenia infrequently per dose or per course, the cumulative and widespread use of vancomycin leads to many cases. It rarely occurs after the first dose, rather later in a prolonged treatment course.

While a comprehensive list of all drugs associated with cytopenias that are used post-transplant is beyond the scope of this review, and idiosyncratic reactions are possible with many drugs, other drugs to consider in the differential diagnosis of cytopenias include antineoplastic agents (multi-lineage cytopenias), colchicine (thrombocytopenia), and ACE inhibitors (anemia) [92–94].

Hemophagocytic Lymphohistiocytosis

Acquired hemophagocytic syndrome, also referred to as hemophagocytic lymphohistiocytosis (HLH), is an uncommon but life-threatening condition in the post-transplant setting typically associated with progressive and profound cytopenias. Mechanistically, HLH is characterized by an aberrant immune response of abnormal T-cell activation in response to a precipitating cause. As a result, the release of macrophage-activating, proinflammatory cytokines leads to uncontrolled proliferation of hemophagocytic monocytes/macrophages/histiocytes that ingest and destroy cellular blood elements within the bone marrow and reticuloendothelial system (Fig. 2) [95]. In the post-transplant setting, HLH is typically triggered in association with viral infections, such as EBV, CMV, adenovirus, HHV-8, and parvovirus B19. However, HLH associated with several bacterial, fungal, and protozoan infections has also been reported [95, 96].

The true incidence of post-transplant HLH remains unknown due to variable diagnostic criteria reported through the literature, overlap with other clinical syndromes, and underreporting of the disease. This is particularly true in the post-HSCT setting where conditions such as capillary leak syndrome, engraftment syndrome, cytokine release syndrome, and TMA may occur concomitantly [97]. Among the SOT population, the incidence is estimated to be <1%. For both the HSCT and SOT

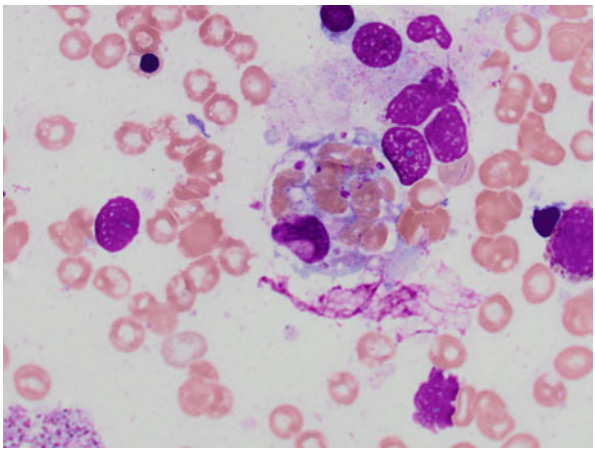


Fig. 2 Bone marrow aspirate (Wright stain, 100X) depicting an activated macrophage engulfing cellular blood components characteristically observed with hemophagocytic lymphohistiocytosis

populations, most cases of HLH occur within 2 months post-transplant, though cases occurring many years later have been reported [96–98]. The clinical features include fever, sweats, weight loss, hepatosplenomegaly, rash, jaundice with characteristic laboratory aberrancies including cytopenias (of two or more cell lineages), hyperferritinemia (>500 ug/L), hypertriglyceridemia, elevated lactate dehydrogenase (LDH), abnormal liver function tests, and hypofibrinogenemia [99]. Characteristically, soluble CD25 receptor (soluble IL-2 receptor alpha [sIL-2R]) is markedly elevated and now forms the backbone of diagnostic criteria, although this test is not widely available. Bone marrow aspiration and biopsy reveal large macrophages with intracytoplasmic erythrocytes, platelets, and leukocytes.

The primary approach to HLH management is the identification and treatment of the underlying driver of the uncontrolled hemophagocytic response, with targeted antimicrobial therapy and/or reduction of immunosuppression [96]. An array of adjunctive treatment approaches has been reported with varying success, but none have been subjected to randomized trials. Despite treatment, the mortality rate is approximately 50% in case reports and series among SOT recipients, with even higher mortality in HSCT recipients [97, 100].

Thrombotic Microangiopathy

Thrombotic microangiopathy (TMA) following SOT and HSCT is a well-recognized complication characterized by intravascular platelet aggregation, leading to thrombosis in the microcirculation with resultant thrombocytopenia and microangiopathic hemolytic anemia. Several risk factors have been identified for developing TMA. In HSCT recipients, risk factors include endothelial damaging conditioning regimens (including irradiation), GVHD, HLA mismatch, and angioinvasive viral and fungal infections [96, 101–103]. In SOT recipients, risk factors include antibody-mediated allograft rejection, severe renal ischemia (renal transplantation), and viral infections [96, 104]. For both populations, calcineurin inhibitors and/or sirolimus may exert a

direct toxic effect on the vasculature and lead to TMA independently, or potentiate established TMA precipitated by other causes. TMA typically manifests in the early post-transplant period, usually within the first 3 months. The incidence is variable, estimated in up to 35% of post-HSCT recipients, with a higher incidence among allogeneic versus autologous HSCT recipients [101].

There are no specific clinical features or laboratory abnormalities that define post-transplant TMA. The identified risk factors, proposed pathophysiologic processes, and clinical presentation are heterogeneous, which lead to a common end result of anemia, thrombocytopenia, and peripheral blood schistocytes (Fig. 3) [102]. Early in the clinical course, individuals often demonstrate hypertension, with neurologic abnormalities in more severe cases. Additional laboratory abnormalities may include elevated serum creatinine, LDH, bilirubin, and serum/urine free hemoglobin. Haptoglobin is usually decreased. These laboratory findings are typically used for diagnosis, as the history and exam manifestations are often nonspecific. Renal biopsy may help to confirm the diagnosis histologically, but renal involvement is not always present. Given the potential bleeding risk of the procedure and the lack of correlation between the laboratory and pathological findings, renal biopsy to diagnose TMA is rarely performed in clinical practice [101].

Management of post-transplant TMA primarily includes the withdrawal of suspected immunosuppressive agents if possible and assessment for a contributing infection. In severe cases, plasma exchange, rituximab, and eculizumab have been deployed with varying success [96].

Autoimmune Cytopenias

Autoimmune disorders, of which autoimmune cytopenias are the most common, are a challenging complication in the post-transplant setting. All cell lineages may be affected, either individually or in combination, with the most common being autoimmune hemolytic anemia, followed by immune thrombocytopenia. In HSCT

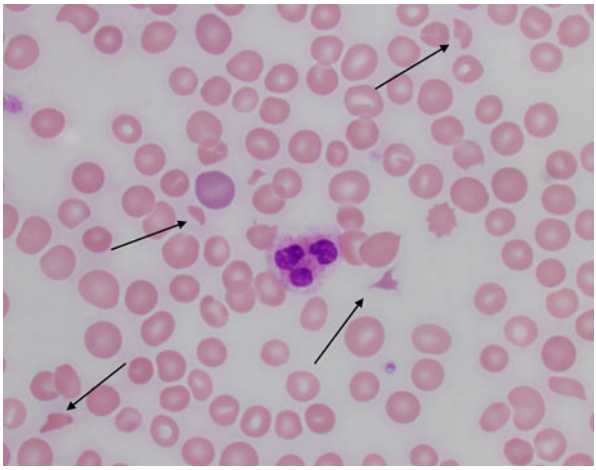


Fig. 3 Peripheral blood smear (Wright stain, 100X). Arrows highlight schistocytes as typically found in thrombotic microangiopathy

recipients, the incidence is estimated to be ~1–6% [101, 105, 106]. The strongest risk factors for the development of autoimmune hemolytic anemia (AIHA) include unrelated donor status, nonmalignant underlying disease, and chronic GVHD [107]. Among SOT recipients, immune cytopenias have been reported following liver, intestinal, heart, pancreas, and kidney transplants. Based on a single center review of 764 SOT recipients, immune cytopenias were found in 19 patients (2.4%), with the highest incidence identified in multivisceral transplant recipients [93].

In both HSCT and SOT populations, the etiology of cytopenias is often multifactorial, such that establishing an autoimmune component is a diagnostic challenge. Adding to the complexity, immune cytopenias may also be triggered by a preceding viral infection or manifest in conjunction with underlying PTLD [106]. Clinical findings range from asymptomatic laboratory abnormalities, to mild symptoms such as fatigue and/or easy bruisability related to the affected cell line, severe bleeding, neutropenic sepsis, and/or anemia. Hemolytic anemia is suspected if anemia is accompanied by an elevated reticulocyte count, LDH, and bilirubin, and unmeasurably low haptoglobin (particularly in the absence of recent RBC transfusion). An autoimmune etiology for the hemolytic anemia can often be confirmed by a positive direct antiglobulin test (DAT) with a broadly reactive RBC eluate. Less commonly, spherocytes are also seen on peripheral blood smear. Diagnosis of autoimmune thrombocytopenia and neutropenia is largely by exclusion of other etiologies in the appropriate clinical context [108]. The disease course is variable, ranging from spontaneous remissions to life-threatening complications. Furthermore, as the pathogenesis of these disorders is poorly understood, the treatment response to traditional first-line therapies and modifications of immunosuppression regimens is variable. In the post-HSCT setting, AIHA is associated with decreased survival [101].

Passenger Lymphocyte Syndrome

Though anemia following organ transplantation is a common finding, passenger lymphocyte syndrome (PLS) is a unique cause of hemolysis in the transplant population [108]. PLS is triggered when viable lymphocytes of donor origin are adoptively transferred as passengers with the allograft. These lymphocytes are then stimulated in the transplant recipient by exposure to recipient or transfused red cell antigens, either by primary or secondary (anamnestic) response, leading to red cell hemolysis.

This syndrome is typically observed in SOT in the setting of minor ABO or other red cell antigen incompatibilities between donor and recipient [95]. The risk and severity of this syndrome is proportional to the volume of lymphoid tissue transplanted, being more frequent in heart-lung transplant (around 70%), followed by liver transplant (~40%) and kidney transplant (~17%) [109]. Though PLS in intestinal transplant is limited to case reports, the association of this syndrome with the volume of transplanted lymphoid tissue suggests these recipients may be at even higher risk [110]. Additional risk factors include (1) blood group O to A transfer, (2) sensitizing events such as pregnancy and/or blood transfusions, (3) cyclosporine use, and (4) infection in the immediate post-transplant period [111]. The onset is typically

abrupt, occurring between 1 and 3 weeks post-transplant. Advances in the understanding of transplant immunology have allowed more successful ABO-mismatched organ transplants over time, thus the necessity of awareness of PLS as a potential complication of this practice. The degree of hemolysis is often mild and resolves spontaneously within a few weeks of SOT. However some cases require transfusion support during this period, and fulminant cases resulting in death have been reported. Diagnosis is made by the development of a positive direct antiglobulin test 7–14 days after transplantation and the presence of anti-A or anti-B in the RBC eluate (as well as in the plasma) [109].

While this syndrome can also be observed in the setting of HSCT, it is less common despite estimates that approximately 40–50% of all HSCTs are performed across the ABO blood group barrier. The introduction of reduced intensity conditioning regimens, however, has led to a higher incidence of PLS, up to 30% [112]. Assessing the independent incidence of PLS as separate from other etiologies of hemolysis and anemia in the post-HSCT setting is challenging, particularly with varying conditioning regimens and GVHD prophylaxis strategies. Immune hemolysis typically begins near the end of the first week or during the second week post HSCT. Manifestations may range from a gradual, mild drop in hemoglobin to abrupt, severe hemolysis with associated renal failure [108]. Graft manipulation to minimize isohemagglutinins prior to infusion can significantly lessen the clinical impact [113].

Laboratory findings are those typical of hemolysis, as described above. Leukocyte and platelet counts are unaffected. The process is usually transient and self-limiting, resolving without major complication in ~3 months due to the finite lifespan of passenger lymphocytes. Treatment is largely supportive with donor compatible transfusion, volume resuscitation, and/or steroids. Exchange transfusion can be considered in severe cases [95, 108–111].

Etiologies Unique to SOT: Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) after SOT results from the engraftment and proliferation of lymphocytes from the organ donor, which in turn attack HLA-disparate tissues in the transplant recipient. As compared to GVHD in the HSCT population, GVHD following SOT is rare. The risk correlates with the quantity of lymphoid tissues transplanted, with small bowel (5.6–19.2%) [114] and liver transplantation (1–2%) [115] posing the highest risk. The risk is additive in the setting of multivisceral transplantation [114, 116]. The lowest risk occurs in lung and kidney transplantation [117]. Other recognized risk factors include recipient age >65 years and a higher degree of HLA matching between donor and recipient. GVHD after SOT is regarded as an early complication with a median onset of 33 d post-transplant in a series of 30 cases [117]. Most cases manifest within the first 90 days after transplant.

The main clinical manifestations are similar to those seen in the more classic form of GVHD in HSCT, with involvement of the skin and GI tract. Unlike HSCT-associated GVHD, bone marrow involvement manifesting as pancytopenia is a

frequent finding [115, 116]. Cutaneous findings are the most common and occur in ~90–100% of reported cases. Skin eruptions appear early in the disease course, with a maculopapular exanthem being most commonly reported [118]. The triad of fever, diarrhea, and pancytopenia, especially when accompanied by a rash, warrants consideration of the diagnosis. The transplant allograft is generally spared given it is perceived as "self" by the offending donor lymphocytes. Diagnosis is often delayed as the findings are often attributed to other diagnoses such as viral infection or drug toxicity. Early skin and/or GI biopsy demonstrating lymphocytic infiltrates of the epithelium, apoptotic bodies, lymphocytic exocytosis, and cellular vacuolization are highly supportive of the diagnosis and can support interventions to mitigate the process. The diagnosis should also be investigated with an analysis for lymphocyte macrochimerism in the peripheral blood, bone marrow, and other affected tissues [115, 116]. Left unchecked, GVHD after SOT is frequently lethal, with mortality most commonly due to sepsis/infection and multi-organ failure in the setting of prolonged marrow aplasia [117]. A trial of high dose steroids has historically been the initial approach to treatment [114, 116]. In recent years, more novel approaches with biologic agents and extracorporeal photopheresis have also been used with varying success [116, 119].

Etiologies Unique to HSCT: Graft Failure

Graft failure as an etiology of cytopenias is an infrequent but often fatal complication of HSCT. Diagnostic criteria are based on established cell count parameters. It can be primary, often defined as the failure to surpass an absolute neutrophil count of 500/mm^3 by day 28 post-graft infusion [120, 121]. Secondary or late graft failure occurs in those individuals who satisfy criteria for initial engraftment but subsequently develop severe cytopenias of at least two cell lineages. Cytopenias of one cell lineage or milder cytopenias which are clinically significant following engraftment may be a sign of poor graft function. These may ultimately resolve with time or progress to late graft failure. Late graft failure is more commonly associated with allogeneic HSCT than with autologous transplantation [122]. The reported incidence of graft failure varies and is clearly dependent on the quality and quantity of transplanted cells. Among autologous HSCT recipients, graft failure approximates 1–3% [123]. Conversely, depending on the specifics of the transplant, graft failure among allogeneic HSCT recipients occurs in 2–20% [124–126]. Risk factors include host- and donor-related immune factors, increased donor and/or recipient age, recipient disease-related factors including previous treatment, pre-transplant conditioning regimen, post-transplant infections, post-transplant immune suppression regimens, nature of any HLA mismatch, and graft source/cell numbers infused [120, 121]. Additionally, graft failure may result from relapse of the primary disease for which HSCT was indicated, with acute leukemias and myelodysplastic syndromes portending the highest risk [120]. Mortality ultimately arises secondary to bleeding and/or infection.

Even when an allograft HSCT does engraft, and the overall leukocyte count normalizes, it is important to recognize that individual cell lines may normalize at

vastly different rates. For example, typically neutrophil recovery is comparatively early, whereas individual lymphocyte subsets can take many months to recover. Consequently, there are implications for the infection risk over time, with the recovery of granulocytes corresponding to a reduction in bacteremia and candida infections, whereas the ongoing functional lymphopenia results in persistent complex fungal and viral infection risk.

Overall, establishing the etiology of graft failure is complex as it is very frequently multifactorial [120]. Chimerism studies can be performed for monitoring in individuals at risk for allogeneic graft failure. The kinetic change of the proportion of donor cells over time is central to projecting subsequent graft function. Successful management of graft failure centers around preventive measures and early identification/treatment of potential reversible causes [121, 127]. In select individuals, lymphocyte infusion from the original stem cell donor, use of a CD34+ cell selected graft from the same donor without additional conditioning chemotherapy, or a second allogeneic HSCT can be offered [121, 128].

Approach to Diagnosis of Transplant-Associated Cytopenias

The differential diagnosis of cytopenias involving one or multiple lineages is broad. Transplantation introduces additional considerations for determining an etiology, particularly since the etiology of pancytopenia is often multifactorial in the post-transplant setting. As launching a workup for all possible causes in the transplant population is not feasible, it is important to consider key variables that will serve to guide the diagnostic workup.

Characterization of the abnormality: When cytopenias are discovered, it is important to confirm a true result to rule out laboratory error, hemodilution, or other exogenous event as an explanation for the finding. Once confirmed, review of the patient's complete blood count is needed to determine which cell lineage(s) are involved. In addition to reviewing the absolute leukocyte, hemoglobin, and platelet values, it is important to clinically assess the severity of the abnormality and the urgency with which intervention is required. Active and/or significant bleeding may be manifested with clinical findings such as tachycardia, pallor, petechiae, ecchymoses, and mucosal bleeding involving the GI, respiratory, and reproductive tracts. Leukopenia, especially with associated severe neutropenia, may be accompanied by fever, mouth ulcers, gingivitis, and sepsis.

Timing: An important aspect in determining the etiology of cytopenias is identifying the time of onset and the trajectory over time. Exposures, symptoms, and medication changes that occurred around the time of onset of the lab abnormalities can assist with prioritizing the differential diagnosis. The development of cytopenias in relation to the date of transplantation is similarly helpful (Table 1).

Associated clinical findings: A thorough assessment for additional history, physical examination, and laboratory findings can further refine the differential diagnosis. Important points from the patient history include medication use (including prescribed medications, obtained over-the-counter medications, and herbal

preparations), weight loss, seasonal association, travel, environmental exposures, and ill contacts. Adjunctive exam findings to note include skin rashes or lesions, hepatosplenomegaly, lymphadenopathy, and jaundice. The WBC differential and peripheral blood smear may provide useful clues with findings of atypical lymphocytosis, visualization of intracellular pathogens, malignant-appearing cells, and/or spherocytes or schistocytes associated with hemolysis. In addition, abnormalities of liver function tests, creatinine, ferritin, LDH, and/or triglycerides may aid with diagnosis.

Consideration for adverse drug effects: As drug-associated cytopenias are very common in the post-transplant setting, it is important to consider whether a drug effect may be causing potentiation of a nondrug etiology of cytopenia rather than the drug solely causing the abnormalities. For example, CMV can cause cytopenia directly, as can its first-line treatment, ganciclovir. As such, this provides an opportunity to manipulate the medication regimen through dose reduction or discontinuation to help clarify the clinical findings.

If, based on an assessment of the above factors, an infectious etiology of the cytopenia remains a consideration, the most important starting point is a detailed history and physical examination. This should include gathering detailed information about the patient's zoonotic, geographic, and other environmental exposures that may have been overlooked previously. Based on this information, opportunities for additional targeted diagnostic testing may become apparent. For example, obtaining a history of recent international travel in the setting of cytopenias and fever raises the possibilities of dengue fever, typhoid fever, malaria, and viral hepatitis. Seasonality is an important factor when considering the likelihood of tickborne infections such as Ehrlichia/Anaplasma and viral infections such as adenovirus, parainfluenza virus, and enteroviruses. Environmental exposure to excavated soil or construction sites located within the Ohio River Valley increases the possibility of histoplasmosis.

In cases where the preliminary workup is negative, the cytopenia(s) persist despite reducing or discontinuing marrow suppressive drugs, and/or a more expedient/aggressive workup is indicated, a hematology evaluation is recommended. Often a bone marrow examination will follow as the next diagnostic step to assess for marrow infiltrating lesions including neoplastic processes, hyper- or hypo-proliferative cytopenic states, and infection [129]. For HSCT recipients with pancytopenia, there is a low threshold to perform a bone marrow examination to assess for relapsed primary disease as an etiology for graft failure. There are limited data to assess the utility of bone marrow examination in SOT recipients. Two studies of 85 sequential kidney and 44 sequential liver transplant recipients undergoing bone marrow biopsy primarily for workup of cytopenias at a single center demonstrated nonspecific findings of normo/hypocellular marrow in 67% and 82%, respectively [130, 131]. Malignancy was diagnosed in 6/85 (7.1%) of renal and 1/44 (2.3%) of liver transplant recipients, and no infections were diagnosed. Aside from diagnosing relapsed primary disease in the setting of HSCT, unfortunately, the bone marrow examination is often inconclusive for determining the underlying cause of cytopenias in transplant recipients. However, it remains an important diagnostic step in the evaluation of challenging cases.

General Approach to Management of Transplant-Associated Cytopenias

The most important initial step in management of transplant-associated cytopenias is recognizing those conditions that are life-threatening and initiating prompt, aggressive interventions. The most important of these include life-threatening bleeding or hemolysis, HLH, and neutropenia-associated bacterial or fungal sepsis. Additionally, any of the aforementioned infections and/or autoimmune cytopenias may lead to acute complications requiring urgent interventions.

Life-threatening bleeding requires prompt intervention to control the bleeding source and provide supportive transfusion with red blood cells, platelets, and coagulation factors as the clinical picture dictates. In the absence of life-threatening bleeding, red blood cell transfusions are generally recommended when the patient is symptomatic from lack of adequate tissue oxygenation, which often but not always occurs when hemoglobin concentrations decrease to <7.0 g/dl [132]. In the setting of myocardial ischemia, severe hypoxemia or acute hemorrhage the actionable hemoglobin level for RBC transfusion is higher in order to minimize the risk of end-organ injury. Prophylactic platelet transfusion is indicated for platelet counts $\leq 10 \times 10^9$/L for stable patients, in the absence of acute bleeding. For the patient awaiting high-risk interventions such as tissue biopsy or surgery, higher targets are typically sought, for example, $>50 \times 10^9$/L [132]. In the setting of drug-induced or autoimmune hemolysis, life-threatening hemolysis in the acute setting can be managed with transfusion of incompatible blood, provided that the presence of underlying alloantibodies has been excluded. Whole blood exchange transfusion can also be considered in centers providing this service.

In the setting of leukopenia/neutropenia and suspected sepsis, expedient antimicrobial therapy and supportive care are paramount. Though there is no evidence that sepsis and septic shock require different treatment in neutropenic vs. non-neutropenic individuals, count recovery has historically been considered to be an important component of infection cure.

Granulocyte transfusions have a long history of usage in neutropenic patients with severe infections [133, 134]. In the 1960 and 1970s, granulocyte transfusions were limited by low cell collection yields and challenging cell storage issues. With the availability of granulocyte colony-stimulating factor (G-CSF) in the 1990s, larger granulocyte quantities were able to be mobilized from stimulated donors with increased ex vivo cell longevity [133]. Further, G-CSF-stimulated granulocytes demonstrate enhanced functional activity [135]. The utility of granulocyte transfusions has been studied in a variety of patient populations, predominantly those with neutropenia related to chemotherapy and/or stem cell transplantation. Despite demonstrated benefit in uncontrolled studies, the logistical challenges in procuring cells has meant this approach is infrequently used in clinical practice in adults [133]. Adverse recipient and donor reactions may contribute to the limited use, as do the logistics of the procedure and the relatively low granulocyte dose administered. Conversely, granulocyte transfusions present a better therapeutic option for children due their smaller size and the relative

higher granulocyte dose achieved with transfusion. Current guidelines for granulocyte transfusion include those individuals with temporary neutropenia ($<0.2–0.5 \times 10^9$/L) who have severe infections unresponsive to aggressive antibiotic treatment for >48 h [133]. In our experience in adult patients, granulocyte transfusions should be restricted to neutropenic patients with proven or suspected invasive infections, such as *Mucorales*, where standard therapies are poor and mortality is high. Overall, the quality of evidence to support routine granulocyte transfusions to reduce mortality and other adverse outcomes related to infection in individuals with neutropenia is low [136].

The use of the hematopoietic growth factors, granulocyte colony-stimulating factor (G-CSF), and granulocyte monocyte colony-stimulating factor (GM-CSF) for treatment of leukopenia in transplantation provides a more feasible option [137]. Among individuals with chemotherapy-induced neutropenia, numerous randomized controlled trials demonstrate that administration of these agents effectively reduces the time to neutrophil recovery and length of hospitalization [138]. G-CSF may also be administered in HSCT recipients in the setting of marginal graft function related to marrow suppression from a necessary medication, such as an antimicrobial or immunosuppressive agent. Although the routine use of these factors in the absence of infection is generally well tolerated in this population, respiratory deterioration with associated ARDS has been described with G-CSF−/GM-CSF-induced neutrophil recovery [132]. As leukopenia with associated neutropenia among SOT recipients is also a common occurrence, G-CSF treatment is often considered as a management option in this setting; however, data to support this approach are limited and conflicting. The exact immunomodulatory effects of G-CSF on the adaptive immune response and T-cell activity are not known. Specifically, there is concern its use will trigger allograft acute cellular rejection (ACR) [139–141]. Collective data indicate there is minimal risk of ACR in the liver and kidney transplant populations, but there are conflicting findings in heart and lung recipients [4, 5, 8, 139–142]. The majority of studies are retrospective, with differing patient populations, definitions of leukopenia/neutropenia, and G-CSF dosage regimens which limit comparisons across studies. In addition, concurrent medication adjustments, particularly with immunosuppressive regimens, are frequent and confound the findings. Despite data limitations, G-CSF is widely used in SOT recipients to allow for continuation and full dosing of myelosuppressive agents as deemed necessary for individual drug regimens.

There are varying thresholds for interventions to manage cytopenias based on the underlying transplant type and timing post-transplant. For cytopenia cases requiring less urgent intervention, a more methodical management approach is prudent. Particularly among transplant recipients, the underlying cause is often multifactorial and potentiated by concomitant medications which can often be modified as an initial approach. Quantitative or qualitative immune suppression modification, where the net state of immune suppression is enhanced, reduced, or the agent changed, is a common intervention. This step may be critical for determining an individual patient's subsequent course and requires careful consideration in a multidisciplinary context.

Conclusion

Cytopenias are a common occurrence in both HSCT and SOT recipients, particularly in the early post-transplant time period. The etiology is frequently multifactorial and potentiated by medications. Thorough evaluation of patient history, physical exam, and preliminary laboratory values can assist with prioritizing the differential diagnosis and focusing the diagnostic workup. Treatment approaches are commonly multifaceted, often requiring manipulation of immune suppression.

Key Points

- Transplant-associated cytopenias of variable spectrum and severity occur commonly among HSCT and SOT recipients. The etiology is frequently multifactorial.
- Both primary and reactivation infections, the most common of which are viral, may cause transplant-associated cytopenias due to bone marrow suppression and/or infiltration.
- Immune-mediated processes causing cytopenias, including those precipitated directly by host-graft interactions, present a diagnostic and therapeutic challenge in the transplant population.
- Medications often cause cytopenias alone or potentiate cytopenias due to other etiologies in transplant recipients. A patient's drug regimen provides a modifiable variable for intervention.
- Important factors which assist with prioritizing the differential diagnosis of an individual's cytopenia include the characterization of the abnormality, evaluation of associated clinical findings, post-transplant timing, and identification of potential environmental/drug exposures.
- The management of transplant-associated cytopenias foremost requires recognition of conditions that are life-threatening in order to facilitate prompt intervention. All cases are best managed in a multidisciplinary context.

Cross-References

- ► Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients: Prevention, Diagnosis, and Treatment
- ► Cytomegalovirus in Solid Organ Transplant Recipients: Prevention, Diagnosis, and Treatment
- ► Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorders
- ► *Mycobacterium tuberculosis* in Transplantation
- ► Rapidly Growing Mycobacterial Infections in Transplant: Evolving Epidemiology and Treatment Options
- ► Slow-Growing Nontuberculous Mycobacteria in Transplant
- ► Transplant Adenovirus Infection: Clinical Syndromes, Diagnosis, and Management

References

1. Valent P. Low blood counts: immune mediated, idiopathic, or myelodysplasia. Hematology Am Soc Hematol Educ Program. 2012;2012:485–91.
2. Dale DC, Cottle TE, Fier CJ, Bolyard AA, Bonilla MA, Boxer LA, et al. Severe chronic neutropenia: treatment and follow-up of patients in the severe chronic neutropenia international registry. Am J Hematol. 2003;72(2):82–93.
3. Common Terminology Criteria for Adverse Events (CTCAE): National Cancer Institute. 2018. Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/aeguidelines.pdf.
4. Zafrani L, Truffaut L, Kreis H, Etienne D, Rafat C, Lechaton S, et al. Incidence, risk factors and clinical consequences of neutropenia following kidney transplantation: a retrospective study. Am J Transplant. 2009;9(8):1816–25.
5. Poon T, Guerra CM. Evaluation of Filgrastim therapy in kidney transplant recipients. Prog Transplant. 2017;27(4):360–4.
6. Alraddadi B, Nierenberg NE, Price LL, Chow JK, Poutsiaka DD, Rohrer RJ, et al. Characteristics and outcomes of neutropenia after orthotopic liver transplantation. Liver Transpl. 2016;22(2):217–25.
7. Mavrakanas TA, Fournier MA, Clairoux S, Amiel JA, Tremblay ME, Vinh DC, et al. Neutropenia in kidney and liver transplant recipients: risk factors and outcomes. Clin Transpl. 2017;31(10):e13058
8. Tague LK, Scozzi D, Wallendorf M, Gage BF, Krupnick AS, Kreisel D, et al. Lung transplant outcomes are influenced by severity of neutropenia and granulocyte colony-stimulating factor treatment. Am J Transplant. 2019. 20(1):250–261
9. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol. 2010;20(4):202–13.
10. Paya C, Humar A, Dominguez E, Washburn K, Blumberg E, Alexander B, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant. 2004;4(4):611–20.
11. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-guidelines of the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13512.
12. Nakamae H, Storer B, Sandmaier BM, Maloney DG, Davis C, Corey L, et al. Cytopenias after day 28 in allogeneic hematopoietic cell transplantation: impact of recipient/donor factors, transplant conditions and myelotoxic drugs. Haematologica. 2011;96(12):1838–45.
13. Reddehase MJ. Mutual interference between cytomegalovirus and reconstitution of protective immunity after hematopoietic cell transplantation. Front Immunol. 2016;7:294.
14. Razonable RR, Hayden RT. Clinical utility of viral load in management of cytomegalovirus infection after solid organ transplantation. Clin Microbiol Rev. 2013;26(4):703–27.
15. Eid AJ, Arthurs SK, Deziel PJ, Wilhelm MP, Razonable RR. Clinical predictors of relapse after treatment of primary gastrointestinal cytomegalovirus disease in solid organ transplant recipients. Am J Transplant. 2010;10(1):157–61.
16. Ljungman P, de la Camara R, Robin C, Crocchiolo R, Einsele H, Hill JA, et al. Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European conference on infections in leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(8):e260–e72.
17. Anderson LJ, Tsou C, Parker RA, Chorba TL, Wulff H, Tattersall P, et al. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. J Clin Microbiol. 1986;24(4):522–6.
18. Centers for Disease C. Risks associated with human parvovirus B19 infection. MMWR Morb Mortal Wkly Rep. 1989;38(6):81–8, 93–7.
19. Eid AJ, Ardura MI, Practice ASTIDCo. Human parvovirus B19 in solid organ transplantation: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33:e13535.

20. Obeid KM. Infections with DNA viruses, adenovirus, polyomaviruses, and parvovirus B19 in hematopoietic stem cell transplant recipients and patients with hematologic malignancies. Infect Dis Clin N Am. 2019;33(2):501–21.
21. Potter CG, Potter AC, Hatton CS, Chapel HM, Anderson MJ, Pattison JR, et al. Variation of erythroid and myeloid precursors in the marrow and peripheral blood of volunteer subjects infected with human parvovirus (B19). J Clin Invest. 1987;79(5):1486–92.
22. Eid AJ, Brown RA, Patel R, Razonable RR. Parvovirus B19 infection after transplantation: a review of 98 cases. Clin Infect Dis. 2006;43(1):40–8.
23. Katoh D, Ochi Y, Hiramoto N, Morita M, Yabushita T, Shimomura Y, et al. Parvovirus B19 infection in adult patients after allogeneic stem cell transplantation: our experience of five cases and literature review. Bone Marrow Transplant. 2019. 55:636–656
24. Karrasch M, Schmidt V, Hammer A, Hochhaus A, Rosee P, Petersen I, et al. Chronic persistent parvovirus B19 bone marrow infection resulting in transfusion-dependent pure red cell aplasia in multiple myeloma after allogeneic haematopoietic stem cell transplantation and severe graft versus host disease. Hematology. 2017;22(2):93–8.
25. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. 2014;27(3):441–62.
26. Florescu DF, Stohs EJ. Approach to infection and disease due to adenoviruses in solid organ transplantation. Curr Opin Infect Dis. 2019;32(4):300–6.
27. Fischer SA. Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV. Transplantation. 2008;86(10):1327–39.
28. Rustia E, Violago L, Jin Z, Foca MD, Kahn JM, Arnold S, et al. Risk factors and utility of a risk-based algorithm for monitoring cytomegalovirus, Epstein-Barr virus, and adenovirus infections in pediatric recipients after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22(9):1646–53.
29. Symeonidis N, Jakubowski A, Pierre-Louis S, Jaffe D, Pamer E, Sepkowitz K, et al. Invasive adenoviral infections in T-cell-depleted allogeneic hematopoietic stem cell transplantation: high mortality in the era of cidofovir. Transpl Infect Dis. 2007;9(2):108–13.
30. Srinivasan A, Klepper C, Sunkara A, Kang G, Carr J, Gu Z, et al. Impact of adenoviral stool load on adenoviremia in pediatric hematopoietic stem cell transplant recipients. Pediatr Infect Dis J. 2015;34(6):562–5.
31. Pellett Madan R, Hand J, Practice ASTIDCo. Human herpesvirus 6, 7, and 8 in solid organ transplantation: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13518.
32. Dadwal SS. Herpes virus infections other than Cytomegalovirus in the recipients of hematopoietic stem cell transplantation. Infect Dis Clin N Am. 2019;33(2):467–84.
33. Razonable RR, Rivero A, Brown RA, Hart GD, Espy MJ, van Cruijsen H, et al. Detection of simultaneous beta-herpesvirus infections in clinical syndromes due to defined cytomegalovirus infection. Clin Transpl. 2003;17(2):114–20.
34. Le Bourgeois A, Labopin M, Guillaume T, Delaunay J, Foucher Y, Tessoulin B, et al. Human herpesvirus 6 reactivation before engraftment is strongly predictive of graft failure after double umbilical cord blood allogeneic stem cell transplantation in adults. Exp Hematol. 2014;42(11):945–54.
35. Ljungman P, Wang FZ, Clark DA, Emery VC, Remberger M, Ringden O, et al. High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. Br J Haematol. 2000;111(3):774–81.
36. Zerr DM, Gupta D, Huang ML, Carter R, Corey L. Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2002;34(3):309–17.
37. Zerr DM, Fann JR, Breiger D, Boeckh M, Adler AL, Xie H, et al. HHV-6 reactivation and its effect on delirium and cognitive functioning in hematopoietic cell transplantation recipients. Blood. 2011;117(19):5243–9.

38. Hill JA, Koo S, Guzman Suarez BB, Ho VT, Cutler C, Koreth J, et al. Cord-blood hematopoietic stem cell transplant confers an increased risk for human herpesvirus-6-associated acute limbic encephalitis: a cohort analysis. Biol Blood Marrow Transplant. 2012;18(11): 1638–48.
39. Dulery R, Salleron J, Dewilde A, Rossignol J, Boyle EM, Gay J, et al. Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study. Biol Blood Marrow Transplant. 2012;18(7):1080–9.
40. Seo S, Renaud C, Kuypers JM, Chiu CY, Huang ML, Samayoa E, et al. Idiopathic pneumonia syndrome after hematopoietic cell transplantation: evidence of occult infectious etiologies. Blood. 2015;125(24):3789–97.
41. Anton A, Cervera C, Pumarola T, Moreno A, Benito N, Linares L, et al. Human herpesvirus 7 primary infection in kidney transplant recipients. Transplantation. 2008;85(2):298–302.
42. Razonable RR, Brown RA, Humar A, Covington E, Alecock E, Paya CV, et al. Herpesvirus infections in solid organ transplant patients at high risk of primary cytomegalovirus disease. J Infect Dis. 2005;192(8):1331–9.
43. Zerr DM. Human herpesvirus 6 (HHV-6) disease in the setting of transplantation. Curr Opin Infect Dis. 2012;25(4):438–44.
44. Caserta MT, Hall CB, Schnabel K, Lofthus G, Marino A, Shelley L, et al. Diagnostic assays for active infection with human herpesvirus 6 (HHV-6). J Clin Virol. 2010;48(1):55–7.
45. Flamand L, Gravel A, Boutolleau D, Alvarez-Lafuente R, Jacobson S, Malnati MS, et al. Multicenter comparison of PCR assays for detection of human herpesvirus 6 DNA in serum. J Clin Microbiol. 2008;46(8):2700–6.
46. Betts BC, Young JA, Ustun C, Cao Q, Weisdorf DJ. Human herpesvirus 6 infection after hematopoietic cell transplantation: is routine surveillance necessary? Biol Blood Marrow Transplant. 2011;17(10):1562–8.
47. Epstein DJ, Tan SK, Deresinski S. HHV-6 and septic shock: tenuous proof of causation. Am J Transplant. 2019;19(1):303.
48. Hall CB, Caserta MT, Schnabel K, Shelley LM, Marino AS, Carnahan JA, et al. Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection. Pediatrics. 2008;122(3):513–20.
49. Pellett PE, Ablashi DV, Ambros PF, Agut H, Caserta MT, Descamps V, et al. Chromosomally integrated human herpesvirus 6: questions and answers. Rev Med Virol. 2012;22 (3):144–55.
50. Qu L, Jenkins F, Triulzi DJ. Human herpesvirus 8 genomes and seroprevalence in United States blood donors. Transfusion. 2010;50(5):1050–6.
51. Chatlynne LG, Ablashi DV. Seroepidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV). Semin Cancer Biol. 1999;9(3):175–85.
52. Cesaro S, Tridello G, van der Werf S, Bader P, Socie G, Ljungman P, et al. Incidence and outcome of Kaposi sarcoma after hematopoietic stem cell transplantation: a retrospective analysis and a review of the literature, on behalf of infectious diseases working party of EBMT. Bone Marrow Transplant. 2019. 55:110–116
53. Dollard SC, Douglas D, Basavaraju SV, Schmid DS, Kuehnert M, Aqel B. Donor-derived Kaposi's sarcoma in a liver-kidney transplant recipient. Am J Transplant. 2018;18(2):510–3.
54. Lebbe C, Porcher R, Marcelin AG, Agbalika F, Dussaix E, Samuel D, et al. Human herpesvirus 8 (HHV8) transmission and related morbidity in organ recipients. Am J Transplant. 2013;13(1):207–13.
55. Pietrosi G, Vizzini G, Pipitone L, Di Martino G, Minervini MI, Lo Iacono G, et al. Primary and reactivated HHV8 infection and disease after liver transplantation: a prospective study. Am J Transplant. 2011;11(12):2715–23.
56. Speicher DJ, Sehu MM, Mollee P, Shen L, Johnson NW, Faoagali JL. Successful treatment of iatrogenic multicentric Castleman's disease arising due to recrudescence of HHV-8 in a liver transplant patient. Am J Transplant. 2014;14(5):1207–13.

57. Patel A, Bishburg E, Zucker M, Tsang P, Nagarakanti S, Sabnani I. Concomitant Kaposi sarcoma and multicentric Castleman's disease in a heart transplant recipient. Heart Lung. 2014;43(6):506–9.
58. Gantt S, Casper C. Human herpesvirus 8-associated neoplasms: the roles of viral replication and antiviral treatment. Curr Opin Infect Dis. 2011;24(4):295–301.
59. Detroyer D, Deraedt K, Schoffski P, Hauben E, Lagrou K, Naesens M, et al. Resolution of diffuse skin and systemic Kaposi's sarcoma in a renal transplant recipient after introduction of everolimus: a case report. Transpl Infect Dis. 2015;17(2):303–7.
60. Allen UD, Preiksaitis JK, Practice ASTIDCo. Post-transplant lymphoproliferative disorders, Epstein-Barr virus infection, and disease in solid organ transplantation: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13652.
61. Dierickx D, Habermann TM. Post-transplantation lymphoproliferative disorders in adults. N Engl J Med. 2018;378(6):549–62.
62. Opelz G, Dohler B. Lymphomas after solid organ transplantation: a collaborative transplant study report. Am J Transplant. 2004;4(2):222–30.
63. Parker A, Bowles K, Bradley JA, Emery V, Featherstone C, Gupte G, et al. Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients – BCSH and BTS guidelines. Br J Haematol. 2010;149(5):675–92.
64. Bianchi E, Pascual M, Nicod M, Delaloye AB, Duchosal MA. Clinical usefulness of FDG-PET/CT scan imaging in the management of posttransplant lymphoproliferative disease. Transplantation. 2008;85(5):707–12.
65. Reshef R, Vardhanabhuti S, Luskin MR, Heitjan DF, Hadjiliadis D, Goral S, et al. Reduction of immunosuppression as initial therapy for posttransplantation lymphoproliferative disorder (bigstar). Am J Transplant. 2011;11(2):336–47.
66. Trappe RU, Dierickx D, Zimmermann H, Morschhauser F, Mollee P, Zaucha JM, et al. Response to Rituximab induction is a predictive marker in B-cell post-transplant lymphoproliferative disorder and allows successful stratification into rituximab or R-CHOP consolidation in an international, prospective, multicenter phase II trial. J Clin Oncol. 2017;35(5):536–43.
67. Gottschalk S, Rooney CM. Adoptive T-cell immunotherapy. Curr Top Microbiol Immunol. 2015;391:427–54.
68. AlDabbagh MA, Gitman MR, Kumar D, Humar A, Rotstein C, Husain S. The role of antiviral prophylaxis for the prevention of Epstein-Barr virus-associated posttransplant lymphoproliferative disease in solid organ transplant recipients: a systematic review. Am J Transplant. 2017;17(3):770–81.
69. Natarajan M, Swierzbinski MJ, Maxwell S, Zelazny AM, Fahle GA, Quezado M, et al. Pulmonary Histoplasma infection after allogeneic hematopoietic stem cell transplantation: case report and review of the literature. Open Forum Infect Dis. 2017;4(2):ofx041.
70. Haydoura S, Wallentine J, Lopansri B, Ford CD, Saad D, Burke JP. Disseminated histoplasmosis in allogeneic bone marrow transplant: a diagnosis not to be missed. Transpl Infect Dis. 2014;16(5):822–6.
71. Kauffman CA, Freifeld AG, Andes DR, Baddley JW, Herwaldt L, Walker RC, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the Transplant-Associated Infection Surveillance Network (TRANSNET). Transpl Infect Dis. 2014;16(2):213–24.
72. Miller R, Assi M, Practice ASTIDCo. Endemic fungal infections in solid organ transplant recipients-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13553.
73. Grim SA, Proia L, Miller R, Alhyraba M, Costas-Chavarri A, Oberholzer J, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. Transpl Infect Dis. 2012;14(1):17–23.
74. Assi M, Martin S, Wheat LJ, Hage C, Freifeld A, Avery R, et al. Histoplasmosis after solid organ transplant. Clin Infect Dis. 2013;57(11):1542–9.
75. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, McKinsey DS, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin Infect Dis. 2011;53(5):448–54.

76. Longworth SA, Daly JS, Practice ASTIDCo. Management of infections due to nontuberculous mycobacteria in solid organ transplant recipients-guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019; 33(9):e13588.
77. Pena T, Klesney-Tait J. Mycobacterial infections in solid organ and hematopoietic stem cell transplantation. Clin Chest Med. 2017;38(4):761–70.
78. Al-Anazi KA, Al-Jasser AM, Al-Anazi WK. Infections caused by non-tuberculous mycobacteria in recipients of hematopoietic stem cell transplantation. Front Oncol. 2014;4:311.
79. Abad CLR, Razonable RR. Mycobacterium tuberculosis after solid organ transplantation: a review of more than 2000 cases. Clin Transpl. 2018;32(6):e13259.
80. Doucette K, Fishman JA. Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. Clin Infect Dis. 2004;38(10):1428–39.
81. Gamboa F, Manterola JM, Lonca J, Matas L, Vinado B, Gimenez M, et al. Detection and identification of mycobacteria by amplification of RNA and DNA in pretreated blood and bone marrow aspirates by a simple lysis method. J Clin Microbiol. 1997;35(8):2124–8.
82. Singh UB, Bhanu NV, Suresh VN, Arora J, Rana T, Seth P. Utility of polymerase chain reaction in diagnosis of tuberculosis from samples of bone marrow aspirate. Am J Trop Med Hyg. 2006;75(5):960–3.
83. Gous N, Scott LE, Wong E, Omar T, Venter WD, Stevens W. Performance of the Roche LightCycler real-time PCR assay for diagnosing extrapulmonary tuberculosis. J Clin Microbiol. 2012;50(6):2100–3.
84. Andres E, Dali-Youcef N, Serraj K, Zimmer J. Recognition and management of drug-induced cytopenias: the example of idiosyncratic drug-induced thrombocytopenia. Expert Opin Drug Saf. 2009;8(2):183–90.
85. Khalil MAM, Khalil MAU, Khan TFT, Tan J. Drug-induced Hematological Cytopenia in kidney transplantation and the challenges it poses for kidney transplant physicians. J Transp Secur. 2018;2018:9429265.
86. Al-Homsi AS, Roy TS, Cole K, Feng Y, Duffner U. Post-transplant high-dose cyclophosphamide for the prevention of graft-versus-host disease. Biol Blood Marrow Transplant. 2015;21(4):604–11.
87. Ramsay NK, Kersey JH, Robison LL, McGlave PB, Woods WG, Krivit W, et al. A randomized study of the prevention of acute graft-versus-host disease. N Engl J Med. 1982;306(7):392–7.
88. Yoshida S, Ohno Y, Nagafuji K, Yoshimoto G, Sugio T, Kamimura T, et al. Comparison of calcineurin inhibitors in combination with conventional methotrexate, reduced methotrexate, or mycophenolate mofetil for prophylaxis of graft-versus-host disease after umbilical cord blood transplantation. Ann Hematol. 2019;98(11):2579–91.
89. Fox BC, Sollinger HW, Belzer FO, Maki DG. A prospective, randomized, double-blind study of trimethoprim-sulfamethoxazole for prophylaxis of infection in renal transplantation: clinical efficacy, absorption of trimethoprim-sulfamethoxazole, effects on the microflora, and the cost-benefit of prophylaxis. Am J Med. 1990;89(3):255–74.
90. Lee EY, Caffrey AR. Thrombocytopenia with tedizolid and linezolid. Antimicrob Agents Chemother. 2018;62(1):e01453–17
91. Garratty G, Arndt PA. Drugs that have been shown to cause drug-induced immune hemolytic anemia or positive direct antiglobulin tests: some interesting findings since 2007. Immunohematology. 2014;30(2):66–79.
92. Todd BA, Billups SJ, Delate T, Canty KE, Kauffman AB, Rawlings JE, et al. Assessment of the association between colchicine therapy and serious adverse events. Pharmacotherapy. 2012;32(11):974–80.
93. Schoettler M, Elisofon SA, Kim HB, Blume ED, Rodig N, Boyer D, et al. Treatment and outcomes of immune cytopenias following solid organ transplant in children. Pediatr Blood Cancer. 2015;62(2):214–8.
94. Cheungpasitporn W, Thongprayoon C, Chiasakul T, Korpaisarn S, Erickson SB. Renin-angiotensin system inhibitors linked to anemia: a systematic review and meta-analysis. QJM. 2015;108(11):879–84.

95. Smith EP. Hematologic disorders after solid organ transplantation. Hematology Am Soc Hematol Educ Program. 2010;2010:281–6.
96. Yang Y, Yu B, Chen Y. Blood disorders typically associated with renal transplantation. Front Cell Dev Biol. 2015;3:18.
97. Vatsayan A, Pateva I, Cabral L, Dalal J, Abu-Arja R. Post-hematopoietic stem cell transplant hemophagocytic lymphohistiocytosis or an impostor: case report and review of literature. Pediatr Transplant. 2018;22(4):e13174.
98. Marinella MA. Hematologic abnormalities following renal transplantation. Int Urol Nephrol. 2010;42(1):151–64.
99. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124–31.
100. Karras A, Thervet E, Legendre C. Groupe Cooperatif de transplantation d'Ile de F. Hemophagocytic syndrome in renal transplant recipients: report of 17 cases and review of literature. Transplantation. 2004;77(2):238–43.
101. Holbro A, Passweg JR. Management of hemolytic anemia following allogeneic stem cell transplantation. Hematology Am Soc Hematol Educ Program. 2015;2015:378–84.
102. Kojouri K, George JN. Thrombotic microangiopathy following allogeneic hematopoietic stem cell transplantation. Curr Opin Oncol. 2007;19(2):148–54.
103. Elsallabi O, Bhatt VR, Dhakal P, Foster KW, Tendulkar KK. Hematopoietic stem cell transplant-associated thrombotic Microangiopathy. Clin Appl Thromb Hemost. 2016;22(1):12–20.
104. Reindl-Schwaighofer R, Oberbauer R. Blood disorders after kidney transplantation. Transplant Rev (Orlando). 2014;28(2):63–75.
105. Li Z, Rubinstein SM, Thota R, Savani M, Brissot E, Shaw BE, et al. Immune-mediated complications after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2016;22(8):1368–75.
106. Miloh T, Arnon R, Roman E, Hurlet A, Kerkar N, Wistinghausen B. Autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura in pediatric solid organ transplant recipients, report of five cases and review of the literature. Pediatr Transplant. 2011;15(8):870–8.
107. Neunert CE, Despotovic JM. Autoimmune hemolytic anemia and immune thrombocytopenia following hematopoietic stem cell transplant: a critical review of the literature. Pediatr Blood Cancer. 2019;66(4):e27569.
108. Petz LD. Immune hemolysis associated with transplantation. Semin Hematol. 2005;42(3):145–55.
109. Yazer MH, Triulzi DJ. Immune hemolysis following ABO-mismatched stem cell or solid organ transplantation. Curr Opin Hematol. 2007;14(6):664–70.
110. Foell D, Glasmeyer S, Senninger N, Wolters H, Palmes D, Bahde R. Successful management of passenger lymphocyte syndrome in an ABO-compatible, nonidentical isolated bowel transplant: a case report and review of the literature. Transfusion. 2017;57(6):1396–400.
111. Nadarajah L, Ashman N, Thuraisingham R, Barber C, Allard S, Green L. Literature review of passenger lymphocyte syndrome following renal transplantation and two case reports. Am J Transplant. 2013;13(6):1594–600.
112. Worel N. ABO-mismatched allogeneic hematopoietic stem cell transplantation. Transfus Med Hemother. 2016;43(1):3–12.
113. Larghero J, Rea D, Esperou H, Biscay N, Maurer MN, Lacassagne MN, et al. ABO-mismatched marrow processing for transplantation: results of 114 procedures and analysis of immediate adverse events and hematopoietic recovery. Transfusion. 2006;46(3):398–402.
114. Ganoza A, Mazariegos GV, Khanna A. Current status of graft-versus-host disease after intestinal transplantation. Curr Opin Organ Transplant. 2019;24(2):199–206.
115. Zhang Y, Ruiz P. Solid organ transplant-associated acute graft-versus-host disease. Arch Pathol Lab Med. 2010;134(8):1220–4.

116. Green T, Hind J. Graft-versus-host disease in paediatric solid organ transplantation: a review of the literature. Pediatr Transplant. 2016;20(5):607–18.
117. Assi MA, Pulido JS, Peters SG, McCannel CA, Razonable RR. Graft-vs.-host disease in lung and other solid organ transplant recipients. Clin Transpl. 2007;21(1):1–6.
118. Kim GY, Schmelkin LA, Davis MDP, El-Azhary RA, Farrell AM, Meves A, et al. Dermatologic manifestations of solid organ transplantation-associated graft-versus-host disease: a systematic review. J Am Acad Dermatol. 2018;78(6):1097–101 e1.
119. Houston BL, Yan M, Tinckam K, Kamel-Reid S, Chang H, Kuo KH, et al. Extracorporeal photopheresis in solid organ transplant-associated acute graft-versus-host disease. Transfusion. 2016;56(4):962–9.
120. Lowsky RMH. Mechanisms and treatment of graft failure. In: Foreman SJ, Antin JH, Appelbaum FR, editors. Thomas' hematopoietic cell transplantation. 5th ed. Chichester: Wiley; 2016. p. 944–54.
121. Ozdemir ZN, Civriz Bozdag S. Graft failure after allogeneic hematopoietic stem cell transplantation. Transfus Apher Sci. 2018;57(2):163–7.
122. Wolff SN. Second hematopoietic stem cell transplantation for the treatment of graft failure, graft rejection or relapse after allogeneic transplantation. Bone Marrow Transplant. 2002;29(7):545–52.
123. Pottinger B, Walker M, Campbell M, Holyoake TL, Franklin IM, Cook G. The storage and reinfusion of autologous blood and BM as back-up following failed primary hematopoietic stem-cell transplantation: a survey of European practice. Cytotherapy. 2002;4(2):127–35.
124. Stotler C, Bolwell B, Sobecks R, Dean R, Serafino S, Rybicki L, et al. Are backup BM harvests worthwhile in unrelated donor allogeneic transplants? Bone Marrow Transplant. 2010;45(1):49–52.
125. Ponce DM, Lubin M, Gonzales AM, Byam C, Wells D, Ferrante R, et al. The use of back-up units to enhance the safety of unrelated donor cord blood transplantation. Biol Blood Marrow Transplant. 2012;18(4):648–51.
126. Stelljes M, van Biezen A, Slavin S, Olavarria E, Clark RE, Nagler A, et al. The harvest and use of autologous back-up grafts for graft failure or severe GVHD after allogeneic hematopoietic stem cell transplantation: a survey of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 2008;42(11):739–42.
127. Rondon G, Saliba RM, Khouri I, Giralt S, Chan K, Jabbour E, et al. Long-term follow-up of patients who experienced graft failure postallogeneic progenitor cell transplantation. Results of a single institution analysis. Biol Blood Marrow Transplant. 2008;14(8):859–66.
128. Krishnamurthy P, Potter VT, Barber LD, Kulasekararaj AG, Lim ZY, Pearce RM, et al. Outcome of donor lymphocyte infusion after T cell-depleted allogeneic hematopoietic stem cell transplantation for acute myelogenous leukemia and myelodysplastic syndromes. Biol Blood Marrow Transplant. 2013;19(4):562–8.
129. Weinzierl EP, Arber DA. The differential diagnosis and bone marrow evaluation of new-onset pancytopenia. Am J Clin Pathol. 2013;139(1):9–29.
130. Borcek P, Ozdemir BH, Akar Ozkan E, Taslica FZ, Haberal M. Bone marrow biopsy in patients with renal transplant: spectrum of findings and diagnostic use. Exp Clin Transplant. 2015;13(Suppl 1):263–5.
131. Borcek P, Ozdemir BH, Sercan C, Yilmaz Akcay E, Karakus S, Haberal M. Histologic changes in bone marrow biopsies from liver transplant patients. Exp Clin Transplant. 2016;14(Suppl 3):109–11.
132. Kochanek M, Schalk E, von Bergwelt-Baildon M, Beutel G, Buchheidt D, Hentrich M, et al. Management of sepsis in neutropenic cancer patients: 2018 guidelines from the infectious diseases working party (AGIHO) and intensive care working party (iCHOP) of the German Society of Hematology and Medical Oncology (DGHO). Ann Hematol. 2019;98(5):1051–69.
133. Klein K, Castillo B. Historical perspectives, current status, and ethical issues in granulocyte transfusion. Ann Clin Lab Sci. 2017;47(4):501–7.

134. West KA, Gea-Banacloche J, Stroncek D, Kadri SS. Granulocyte transfusions in the management of invasive fungal infections. Br J Haematol. 2017;177(3):357–74.
135. Hubel K, Dale DC, Engert A, Liles WC. Current status of granulocyte (neutrophil) transfusion therapy for infectious diseases. J Infect Dis. 2001;183(2):321–8.
136. Estcourt LJ, Stanworth SJ, Hopewell S, Doree C, Trivella M, Massey E. Granulocyte transfusions for treating infections in people with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2016;4:CD005339.
137. Dale DC, Crawford J, Klippel Z, Reiner M, Osslund T, Fan E, et al. A systematic literature review of the efficacy, effectiveness, and safety of filgrastim. Support Care Cancer. 2018;26(1):7–20.
138. Mhaskar R, Clark OA, Lyman G, Engel Ayer Botrel T, Morganti Paladini L, Djulbegovic B. Colony-stimulating factors for chemotherapy-induced febrile neutropenia. Cochrane Database Syst Rev. 2014;2014(10):CD003039.
139. Hamel S, Kuo V, Sawinski D, Johnson D, Bloom RD, Bleicher M, et al. Single-center, real-world experience with granulocyte colony-stimulating factor for management of leukopenia following kidney transplantation. Clin Transpl. 2019;33(6):e13541.
140. Casciello N, Hulbert A, Snyder L, Byrns J. Incidence of acute cellular rejection following granulocyte colony-stimulating factor administration in lung transplantation: a retrospective case-cohort analysis. Clin Transpl. 2017;31(5)e12965
141. Vrtovec B, Haddad F, Pham M, Deuse T, Fearon WF, Schrepfer S, et al. Granulocyte colony-stimulating factor therapy is associated with a reduced incidence of acute rejection episodes or allograft vasculopathy in heart transplant recipients. Transplant Proc. 2013;45(6):2406–9.
142. Nguyen AB, Lourenco L, Chung BB, Imamura T, Rodgers D, Besser SA, et al. Increase in short-term risk of rejection in heart transplant patients receiving granulocyte colony-stimulating factor. J Heart Lung Transplant. 2018;37(11):1322–8.

Common and Emerging Infections in Pediatric Transplant Recipients

69

Jennifer E. Schuster, Hilary Miller-Handley, and Lara Danziger-Isakov

Contents

Introduction 1710
Vaccine-Preventable Diseases 1710
Special Considerations in Pediatric Vaccination 1710
Rotavirus 1710
Measles 1711
Varicella 1711
Human Papillomavirus 1711
Influenza 1712
Additional Age-Specific Considerations 1712
Common, Non-Vaccine-Preventable Infections 1713
Respiratory Viral Infections 1713
Cytomegalovirus 1716
Epstein-Barr Virus 1717
Emerging Infections 1718
Enterovirus D68 1718
Acute Flaccid Myelitis 1719
Arboviruses 1719
Diagnostic Testing 1719
Determination of Serostatus 1719
Clostridioides Difficile 1720
Unique Aspects of Antimicrobial Therapy for Pediatric Transplant Patients 1721
Lack of Antimicrobial Dosing Data and Indication in Pediatrics 1721
Variations in Pediatric Pharmacokinetics 1721

J. E. Schuster
Division of Infectious Diseases, Children's Mercy Hospital, Kansas City, MO, USA
e-mail: jeschuster@cmh.edu

H. Miller-Handley · L. Danziger-Isakov (✉)
Division of Pediatric Infectious Diseases, Department of Infectious Disease, Cincinnati Children's Hospital and University of Cincinnati, Cincinnati, OH, USA
e-mail: Hilary.Miller-Handley@cchmc.org; Lara.Danziger-Isakov@cchmc.org

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6_68

Limitations in Route of Drug Administration 1722
Limitations in Adverse Event Assessment in Pediatrics 1722
Summary 1723
References 1723

Introduction

Pediatric transplant recipients have unique infectious risks compared to adult transplant recipients. Distinct challenges to pediatric providers include incomplete or missed primary immunizations, lack of primary immunity to common illnesses, inability to definitively determine infectious serostatus in infants, and recognition of pediatric-specific disease states. Further, the lack of pediatric indications and dosing data for antimicrobials as well as variations in pediatric pharmacokinetics and drug exposure target levels complicate treatment of infectious diseases in pediatric patients.

Vaccine-Preventable Diseases

As part of the pretransplant evaluation, vaccination history should always be reviewed. In the pediatric population, this is particularly important. Studies have shown that pediatric transplant recipients, particularly those less than 2 years of age, have a high rate of incomplete vaccination [1]. Pediatric recipients may be incompletely vaccinated pretransplant for a variety of reasons, including immunosuppressive therapy for primary illness, young age, and prolonged hospitalizations. Ideally, vaccines should be given at least 4 weeks, and no less than 2 weeks, prior to transplantation [2]. Vaccination schedules in the United States are updated yearly and can be found on the Advisory Committee on Immunization Practice (ACIP) website. Pediatric transplant recipients, especially those who have not completed their primary series of vaccinations, should be vaccinated using an expedited schedule to ensure they are fully vaccinated prior to transplantation. Because many transplant recipients may not be able to be vaccinated fully pretransplant, all healthcare workers and close contacts of transplant recipients should be fully vaccinated. All live-attenuated vaccines are safe for close contacts except for smallpox and oral polio vaccination [3].

Special Considerations in Pediatric Vaccination

Rotavirus

Rotavirus vaccine, a live attenuated vaccine, is typically started at 2 months of age; the first dose of vaccine cannot be given after 15 weeks of age and the final dose must be given by 8 months of age. Some infant transplant candidates may

miss the eligible window due to chronic illness. Those who require prolonged hospitalization after birth also typically miss out on rotavirus vaccination with concerns for active viral shedding 10–14 days after vaccination and theoretical risk for nosocomial transmission [4]; administration within a healthcare facility is typically avoided. However, newer data suggest that although viral shedding occurs, transmission in the hospital setting is unlikely, and this could be considered for hospitalized infants [5]. As children born with primary immunodeficiency who require hematopoietic cell transplant (HSCT) are not candidates for any live-attenuated vaccines, the rotavirus vaccination would be deferred for this patient population.

Measles

Measles, mumps, and rubella vaccine (MMR), another live-attenuated viral vaccine, is also currently not routinely recommended after solid-organ transplantation [2], although newer studies have shown that live-attenuated vaccines may be safe and effective in specific posttransplant populations [6]. The ability to safely pursue posttransplant immunization with live-attenuated vaccines is important because young children undergoing transplantation may not have received primary immunization with MMR prior to transplantation despite efforts to ensure accelerated vaccination schedules pretransplantation. Due to the increasing proportion of unvaccinated individuals that has resulted in decreased herd immunity, measles is reemerging globally in areas where it was previously determined eradicated. During outbreaks, it is best for transplant recipients to avoid high volume areas, particularly those who did not receive the MMR vaccination.

Varicella

Varicella remains a common cause of infection in pediatric patients posttransplant [7]. Live-attenuated varicella vaccination is approved as early as 9 months of age, but many infants are still unable to receive this vaccination pretransplant [3]. A recombinant, adjuvated zoster vaccine (Shingrix, GSK, Philadelphia, PA) has been approved for people over 50 year of age, but no studies have been performed in pediatrics.

Human Papillomavirus

Many pediatric transplant recipients are too young to receive adolescent vaccinations such as the human papillomavirus (HPV) vaccination pretransplantation. One in four Americans will be infected with HPV within their lifetime, causing an increase risk cervical, anal, penile, and oropharyngeal malignancies. Transplant recipients have a significantly increased incidence of HPV-related malignancy making vaccination a

priority in these patients [8]. Although a two-dose series is acceptable in many children ages 9–14 years, a three-dose series is recommended for all children with immunocompromising conditions, regardless of the age. A study in 50 adult recipients vaccinated with four-valent HPV vaccine posttransplantation showed a lower vaccination response compared to healthy controls [9]. However, another study in 20 adolescent liver and kidney recipients vaccinated similarly showed no difference in vaccine response compared to controls [10]. More studies are needed to understand variations in immunogenicity in transplant patients. Given concern of possible decrease immunogenicity of vaccine and increasing risk of malignant transformation, the American Society of Transplantation recommends regular screening, including annual cervical and anal Papanicolaou (pap) smear, starting at 21 years of age [11].

Influenza

Influenza is another important infection in patients posttransplantation. In a study of pediatric liver transplant recipients, influenza was the most common vaccine preventable disease that causes hospitalization [12]. Data suggest that influenza vaccine has decreased immunogenicity in posttransplant recipients compared to healthy controls [13, 14]. The optimal influenza vaccination dosing strategy in pediatric HSCT recipients posttransplant is uncertain. All children under 9 years of age who have not received prior influenza vaccination are recommended to receive a two-dose series. A repeat of the two-dose series in the first season after HSCT in pediatric recipients under 9 years of age may be considered with potential loss of imprinting during the HSCT process. Some experts also consider giving a second dose of influenza vaccine outside this age range [15]. If pediatric SOT recipients might benefit from multiple doses is unknown.

Additional Age-Specific Considerations

Age-specific vaccine requirements may prevent administration of vaccines pretransplant when children are likely to have the best immune response. Posttransplantation, children may not be able to receive vaccines that protect against diseases for which they are at increased risk. For example, children undergoing solid organ transplant are at increased risk for *S. pneumoniae* infections [12]. Currently two vaccinations are on the market for *S. pneumoniae*, including 23-valent polysaccharide vaccine (PPSV23), which elicits a T-cell independent response and 13-valent protein-conjugated vaccine (PCV13), which induces a T-cell dependent response. In general, PCV13 tends to have a higher response; PPSV23 covers 10 additional strains of *S. pneumoniae* and therefore both are recommended for protection. However, currently the 23-valent polysaccharide pneumococcal vaccination (PPSV23) is only approved for children >2 years of age.

Hepatitis A vaccine is recommended for children with chronic liver disease but only licensed for children >12 months of age, so infants with chronic liver disease

(e.g., biliary atresia) awaiting transplant remain unvaccinated. Increasing use of eculizumab, a monoclonal antibody directed against C5 in the complement cascade, as an immune modulator in transplant recipients increases the risk of encapsulated organisms including meningococcal disease. Meningococcal vaccines are recommended for patients receiving eculizumab; meningococcal ACWY vaccine should be given down to 2 months of age in all high-risk individuals, however, the current meningococcal B vaccines are not licensed in the United States in children <10 years of age. Canadian and European labeling demonstrate safety and immunogenicity data down to 2 months of age for Bexsero (GSK).

Common, Non-Vaccine-Preventable Infections

Many children undergoing transplantation lack primary immunity to common, non-vaccine-preventable pathogens. By adulthood, immunity to such pathogens, including non-influenza respiratory viruses, cytomegalovirus (CMV), and Epstein-Barr virus (EBV), is common. Thus, compared with adults, children have increased susceptibility to these pathogens since primary infection typically occurs after, rather than before, transplantation. When primary infection occurs, children are on immunosuppressive therapies that not only limit their ability to respond to the current infection but also inhibit development of memory responses that protect against future infections. Although primary infection with respiratory viruses typically occurs in the first 2 years of life, many infants undergoing transplantation, e.g., those with cardiomyopathy or liver disease, have been frequently or chronically hospitalized since birth, thus limiting their exposure to pathogens. Young children and adolescents who are CMV and EBV negative are at high risk of primary infection both from donor positive grafts and community exposures in daycare and school. The unique aspects of these infections, including aspects of preventative measures in pediatrics, are discussed in further detail.

Respiratory Viral Infections

Solid Organ Transplants

The burden of respiratory viral infections (RVI) in the pediatric solid organ transplant (SOT) population is significant. A retrospective study, which including 1,096 children at 9 pediatric hospitals, reported that 149 (14.5%) children had ≥ 1 RVI in the first year posttransplant. Rhinovirus (RV) (51.6%) was the most commonly detected virus, followed by respiratory syncytial virus (RSV) (25.2%), parainfluenza virus (PIV) (18.2%), human metapneumovirus (HMPV) (12.6%), and influenza (11.9%). RVI rates varied based on the graft: multivisceral (45.5%), intestine (30%), heart (18.3%), lung (16.7%), liver (15.6%), and kidney (5.5%). Although children were followed for 1 year post-SOT, the majority of initial RVI occurred during the first 100 days. Children with RVI were significantly younger than those without an RVI (median age 1.7 years vs. 7.9 years, $p < 0.001$), highlighting the

increased susceptibility in infants and toddlers. Children with RSV and RV required respiratory support in 33% and 30% of cases, respectively, and children with these viruses also had the highest rates of ICU admission (RV: 33% and RSV: 28%). Lower respiratory tract infection (LRTI) at initial presentation occurred in 56 (35%) children. Mortality within 3 months post-RVI was uncommon, 4.4%. Of the seven deaths, one was likely attributed to RSV and one was possibly attributed to PIV. Rates of subsequent pulmonary complications occurred in nine (5.7%) patients [16].

In a cohort of pediatric lung transplant recipients, 28 (51%) developed an RVI, with 41% of infections occurring in the first 90 days. RV was the most commonly detected virus, and young age was identified as a risk factor for any RVI. Three (5.5%) children died secondary to RVI (two with adenovirus and one with RSV). Interestingly, RVI was not associated with chronic rejection or death, unlike adult literature [17].

Although rates of influenza infection were among the lowest hospitalized RVI, solid organ transplant patients remain at high-risk for complications. In a cohort of pediatric liver transplant patients, influenza-related hospitalizations occurred in 5.6% of patients, with 16.1% occurring during the transplant hospitalization and 63.6% occurring in the first year posttransplant. One-quarter of influenza-infected patients required intensive care unit management, and three deaths occurred (case-fatality rate 2%). In the same cohort, RSV was more common than influenza with 6.2% of all patients. Over 50% required intensive care unit management, and the case fatality rate was 4.4% [7].

Hematopoietic Cell Transplants

Pediatric HSCT patients have higher rates of RVI than pediatric SOT patients. In a retrospective cohort of 9 centers, 259/1560 (16.6%) children who underwent HSCT developed 307 RVI within the first year post-HSCT. Similar to children undergoing SOT, children with an RVI were younger than those without an RVI (4.8 vs. 7.1 years, $p < 0.001$). Interestingly, the overall incidence of RVI was not significantly different between autologous (14.2%) and allogeneic (17.4%) HSCT. Initial RVI occurred a median of 56 days post-HSCT, and RV was the most common pathogen identified. Pathogen epidemiology in this population was similar to pediatric SOT: RV ($n = 162$, 62.5%), PIV ($n = 53$, 20.5%), RSV ($n = 40$, 15.4%), influenza ($n = 29$, 11.2%), and HMPV ($n = 17$, 6.6%). Although most patients (72%) had upper respiratory tract infection, some pathogens, particularly HMPV, were more associated with LRTI, which was identified in 59% of HMPV-infected children. Although need for respiratory support (14%) and ICU management (5%) were less common than in pediatric SOT patients, HMPV was associated with significant morbidity with 35% and 12% of HMPV cases required respiratory support and ICU admission, respectively. Twenty-eight (11%) of children died within the first year post-HSCT, and 50% of those cases were considered to be likely or possibly attributable to the RVI event. The majority (82%) of deaths occurred within the first 60 days after the RVI event. The highest all-cause fatality rate (24%) occurred in children with HMPV, highlighting the significant morbidity and mortality associated with this pathogen

in pediatric HSCT patients [18]. Other studies have highlighted the morbidity associated with RSV in pediatric HSCT patients.

Detection of respiratory viruses pre-HSCT is problematic. In a large cohort of pediatric and adult HSCT patients, children were more likely to have a respiratory virus detected prior to HSCT (46% vs. 23%, $p < 0.001$). HSCT delay is typically pursued when RVI pre-HSCT occurs. Children have higher rates of asymptomatic respiratory virus detection pre-HSCT compared with adults (31% vs. 6%). Although symptomatic RVI pre-HSCT has been associated with worse outcomes, with deaths described in two children, one each with pre-HSCT adenovirus and HMPV, the role of asymptomatic shedding remains unclear. Asymptomatic shedding of respiratory viruses pre-HSCT may be associated with decreased time out of the hospital but not increased mortality or need for bronchoscopy although this is extrapolated from primarily adult data [19].

Preventative Strategies

Despite the burden of RVI in both pediatric HSCT and SOT patients, limited prophylactic and therapeutic agents are available. The use of palivizumab, an RSV monoclonal antibody targeting the fusion protein, is licensed for children at high-risk of hospitalization due to RSV infection. Currently, the American Academy of Pediatrics recommends consideration for the use of palivizumab for children <2 years of age undergoing cardiac transplantation during the RSV season. Consideration for use is also recommended for others of the same age who are "profoundly immunocompromised during the RSV season." [20] Further specifications regarding the degree of immune suppression are not available, and therefore, palivizumab use if highly variable in the transplant setting. A survey of pediatric SOT centers from 2005 to 2006 highlighted this variation, and 10% of respondents noted that they extended prophylaxis in children >24 months. Additionally, some centers reported administering prophylaxis to transplant candidates. Importantly, only about 65% of transplant centers reported receiving third-party reimbursement or medical assistance for palivizumab, which highlights the significant cost burden associated with prophylaxis. Centers reported a decreased rate of RSV infection in children who received prophylaxis compared with those who did not receive prophylaxis (4% vs. 11%), $p = 0.03$ [21]. Similarly, wide variation was noted in a survey of pediatric lung transplant centers: 10/17 offered prophylaxis to transplant candidates while 9/17 offered it to recipients. While all centers used palivizumab for prophylaxis, the specific criteria varied widely based on age with some centers following national guidelines and others using local and institution guidelines [22].

Recent data suggest that the morbidity associated with RSV in both the SOT and HSCT population may not be as high as previously suspected. In a retrospective single-center study of 10 SOT and 7 HSCT pediatric patients with RSV detected as an outpatient, 20% and 40%, respectively, required hospitalization. However, overall disease was not severe with no child requiring ICU admission [23]. A survey of Canadian pediatric transplant centers reported that 25% of RSV cases were managed in the outpatient setting although 29% of children did require ICU management. Interestingly, 43% of cases were nosocomial, suggesting that improvement in

infection control practices, as opposed to palivizumab, may be the most favorable, and cost-beneficial, to prevent RSV disease in transplant patients [24]. Identification of select risk factors may be beneficial to targeting prophylaxis. In a cohort of pediatric liver transplant patients, both age < 2 years and multivisceral transplants were significantly associated with RSV-related hospitalizations [7]. One hospital which instituted restrictive criteria for palivizumab use in pediatric HSCT patients (i.e., children <12 months with need for chronic oxygen children with severe combined immunodeficiency pre-HSCT or within the first 100 days post-HSCT), did not note an increased risk of RSV disease [25]. Thus the target population for palivizumab and the cost-efficacy remain unclear.

Cytomegalovirus

Primary CMV infection typically occurs in childhood, so children undergoing transplant are often CMV-negative recipients. Thus, they are at risk for development of primary disease posttransplant, either from the graft or typical exposures, including daycare. CMV-negative children undergoing HSCT are at risk of primary CMV infection after transplant. CMV-positive children undergoing HSCT are at risk for CMV reactivation, particularly those who receive CMV-negative grafts or grafts with T-cell depletion. Cord blood transplants are more commonly used in pediatric transplants compared with adults, and these grafts lack CMV-specific T-cells.

For CMV-negative children undergoing SOT, those that receive CMV positive grafts are at highest risk for the development of subsequent DNAemia and potentially disease. CMV negative children who receive CMV negative grafts remain at risk for primary infection. In one study, 6.6% of pediatric SOT recipients (D−/R-) developed primary CMV infection within the first year post-SOT [26]. Primary infection often occurs during childhood, so CMV negative children are at high risk for primary acquisition of the virus, even if a negative donor is used. Lastly, the correct designation of recipient status is difficult in children <12–18 months of age as antibody positivity may be reflective of maternal status. In these cases, recipient status is typically not known and should be assigned to the highest risk class based on the donor, as discussed further in the diagnostics section.

CMV can be a devastating disease in children. In a cohort of pediatric small bowel transplant recipients, 7/98 (7%) developed CMV disease despite prophylaxis. Those with disease had an 11-fold increased risk of death although only 1/7 deaths was attributed to CMV [27]. Interestingly, CMV negative children undergoing HSCT from a CMV negative donor had an increased risk of relapse (3.15, 95%CI 1.46–6.76) [28].

Although CMV has been associated with graft function decline in pediatric renal transplant recipients, the association of CMV with other infections, rejection, and graft dysfunction in the pediatric population is lacking compared with adults. Pediatric dosing of valganciclovir is complex. Traditional weight-based dosing is no longer recommended outside of congenitally infected infants. The more complex body surface area dosing is suggested including for prevention of CMV down to

4 months of age and may more accurately represent adult pharmacokinetics [29]. Compared with adults, the duration of recommended CMV prophylaxis, either with ganciclovir or valganciclovir, in children is slightly shorter with recommendations for close monitoring of DNAemia and disease [30].

New pharmacologic agents are being studied for the prevention of CMV and treatment of resistant disease. However, pediatric data for these agents is limited. In particular, letermovir, which is increasingly being studied and used prophylactically in adults undergoing HSCT and SOT, has a lack of pediatric dosing data. Similarly, the use of maribavir, for CMV-resistant infections, has not been not well studied in the pediatric population. Thus, pediatric practitioners rely on preemptive surveillance or prophylaxis with older, and often more toxic, agents to prevent CMV infection. Identification of CMV resistant disease often results in the use of multiple agents with significant toxicities.

Epstein-Barr Virus

Many children undergoing transplantation have not had exposure to primary EBV, and rates of seroconversion during childhood are higher than CMV. In a large cohort of children from Minnesota, only 31% of children ages 1–5 years were EBV positive, but by age 15–19 years, 71% of children were seropositive. Importantly, significant variations in EBV antibody seropositivity by race and ethnicity occurred. White, non-Hispanic children were less commonly (26%) antibody positive compared with Black children (74%) [31]. The differing rates of antibody positivity suggest that the risk of primary infection is significantly higher in some groups, which is important to acknowledge in children undergoing transplant. Similar to CMV, antibody status in children <12–18 months may reflect maternal serostatus, rather than pediatric immunity, so expert opinion recommends considering seropositive children <18 months to be seronegative for risk stratification purposes.

EBV seronegative children are at high risk for primary infection posttransplant. In a cohort of EBV seronegative children undergoing renal transplant, 50/103 (48.5%) developed primary EBV compared with 40/201 (19.9%) of seropositive children. Both EBV seropositivity and age > 14 years at the time of transplant were associated with lower risk of developing EBV infection, highlighting primary disease risk in young, seronegative children [32]. Seroconversion in EBV negative kidney transplant recipients occurred a median of 7.3 months posttransplant, suggesting that primary infection occurs in the early posttransplant period [33]. Primary EBV infection may be asymptomatic, and in a cohort of pediatric renal transplant patients, 42% of children <5 years with primary EBV infection were asymptomatic compared with 26% of children ≥12 years [34]. Therefore, intermittent screening for EBV DNAemia may be important in seronegative children.

Posttransplant lymphoproliferative disorder (PTLD) is one of the major causes of EBV-associated morbidity and mortality. Children have higher rates PTLD than adults, likely related to higher rates of EBV seronegativity and risk of primary infection posttransplant. The highest rates of pediatric PTLD occur in children

undergoing lung/heart-lung transplant (4% in the first year and 16% by 5 years) and small bowel transplant (14%) in all children, regardless of EBV serostatus [35]. The relationship between EBV serostatus and development of PTLD has been well described, and EBV seronegativity is a well-described risk factor for development of PTLD [35]. In a cohort of pediatric renal transplant patients, 7/10 (70%) patients who developed PTLD were seronegative at the time of transplant [32]. Among pediatric heart transplant recipients, EBV seronegative patients were also at high risk, with up to 25% developing PTLD [36].

Age at the time of transplant, likely related to the development of primary EBV infection, is another important factor when evaluating PTLD risk. In a multivariate analysis of a cohort of pediatric renal transplant patients, those $\leq$5 years and EBV D+/R- were more likely to develop PTLD (relative hazard: 3.2, $p = 0.03$ and 7.7, $p = 0.01$, respectively) [37]. However, another study of pediatric renal transplant patients with primary EBV infection demonstrated that the highest rate, 63.6%, of PTLD occurred in adolescents $\geq$12 compared with an overall rate of 24% in all children [34]. Among children undergoing liver transplant, age $<$ 1 year and EBV seronegative status at the time of transplant were risk factors for PTLD (relative risk 13.3, $p = 0.002$) [38]. In pediatric heart transplant recipients, the highest rates of PTLD occurred in children transplanted at 1–10 years of age [36]. Interestingly, data from pediatric renal and liver transplant patients identified that EBV seronegative children were more likely to have chronically high viral loads than their seropositive counterparts, but the presence of high viral loads did not correlate with subsequent development of PTLD [39, 40].

Emerging Infections

Transplant patients may be the "canary in a coal mine" for emerging infections due to their immunosuppressed status. Similarly, pediatric patients are a unique cohort in whom many emerging diseases are initially identified. Providers who care for pediatric transplant patients need to recognize diseases that are unique or have unusual manifestations in pediatric patients, which may have an exacerbated presentation in pediatric transplant patients. Herein, we provide a few select examples of such diseases.

Enterovirus D68

Enterovirus D68 (EV-D68) is an emerging virus that can cause significant disease in the pediatric population. The majority of patients are previously healthy, but children can present with significant respiratory distress. It is not known whether the virus may be exacerbated in transplant patients. In a large cohort of 614 patients, of whom $>$90% were children, only 2% of patients were noted to be immunocompromised [41]. In a cohort of adult HSCT patients, symptoms associated with EV-D68 ranged from mild disease managed in the outpatient setting to death associated with

respiratory failure [42]. Given the significant respiratory distress described during EV-D68 epidemics, this may be a severe pathogen in pediatric lung transplant patients, which given the predilection for school-aged children, may be more commonly seen in pediatric patients than adults. No specific treatment is available, but aggressive management of bronchospasm may be needed.

Acute Flaccid Myelitis

Acute flaccid myelitis (AFM) is an emerging disease with increasing rates biennially [43]. The majority of cases are reported in pediatric patients. In a large study of 120 cases in patients $\leq$21 years, only one patient had a transplant history (cardiac) [44]. At least two cases adult transplant patients with AFM have been reported, including one HSCT and one renal transplant patient. EV-D68 was identified from both patients, one from a respiratory specimen and one from CSF [45, 46]. Although the relationship between AFM and EV-D68 remains unclear, it is possible that persons who are immune suppressed may be more likely to have unusual manifestations of EV-D68. Both AFM and EV-D68 represent examples of diseases in which pediatric transplant practitioners may identify new diseases prior to the emergence in adults.

Arboviruses

Emerging diseases, such as arboviruses, have been described in the general pediatrics population although pediatric transplant data are limited and often extrapolated from adult transplant data. However, these infections may have distinctive presentations in pediatrics that have unique implications for transplant patients. For example, Zika virus was initially identified due to congenital abnormalities in newborns because only a mild illness occurred in adults. Although cohorts of adult transplant patients with Zika infection have been described, the implications of transplanting infants and children with Zika virus are not known. Similarly, less data are available for emerging arboviruses in pediatric transplant patients. Existing literature suggests that dengue can be severe in the pediatric transplant patients although there is a paucity of literature for other viruses, e.g., chikungunya and West Nile Virus [47].

Diagnostic Testing

Determination of Serostatus

Diagnostic testing in pediatric patients presents additional issues compared to adults. Infants present a unique problem when determining presence of prior infection and risk for future infection. During the third trimester of pregnancy, infants receive

passive transfer of maternal antibodies that provide protection for the infant in the early antenatal phase. These antibodies can be detectable for up to 15–18 months after birth, although a recent study suggests that maternal cytomegalovirus (CMV) antibodies clear by 12 months of age [48]. Assigning pretransplant serostatus to predict risk of posttransplant events, specifically for EBV and CMV, is therefore complicated by confounding by maternal antibody [30, 49]. This ambiguity can complicate the interpretation of serology from both the transplant candidate and the potential donor if they are under 12 months of age. For CMV, serostatus positivity can be confirmed by detection of viral shedding in the urine, blood, or oral secretions. Recently, Burton et al. showed that 30–50% of infants with positive serology also had positive viral culture or PCR with increasing positivity correlating to increasing age; thus confirming CMV infection in a significant proportion of transplant candidates [48]. However, a negative test does not exclude prior infection as shedding can be intermittent in candidates, and CMV viral PCR testing is not routinely performed in potential donors. Additionally, attempts have been made to assess CMV status through detection of CMV-specific T-cells but testing under 12 months of age has not been reliable [50]. Due to this specific difficulty in ascertainment of CMV serostatus in infant donors and recipients, it is currently recommended that risk assessment in this age group should assume the highest risk level for purposes of CMV prevention [30]. Infant serology for Epstein-Barr virus (EBV) also suffers from similar issues; however, limited information of EBV PCR testing and EBV-specific cellular immunity in this infant transplant candidates exists.

Clostridioides Difficile

A second issue with diagnostic testing in pediatric patients concerns assessment of *Clostridioides difficile* (previously *Clostridium difficile*) infection (CDI). The incidence of hospitalizations in children secondary to CDI infection has increased over the past two decades [51], and pediatric SOT recipients are increased risk for CDI infection compared to immunocompetent children [52]. Additionally, up to 24% of pediatric SOT with diarrhea will have positive testing for CDI [53]. With the increased utilization of nucleic acid amplification testing (NAAT) that detects genes encoding *C difficile* toxins, the sensitivity of testing has increased [54]. The primary issue is that *C difficile* including toxigenic strains are normal commensal organisms excreted by infants. The proportion of infants excreting toxigenic organisms decreases from 35–40% in the first month of life to approximately 10% by 12 months of age. Further, resolution of diarrhea in a cohort of children under 2 years of age occurred independent of whether the children received therapy or did not underscoring the likelihood that positive testing in this age group does not accurately predict active invasive infection [55]. The increased incidence of loose stools in infants coupled with the impact of certain immunosuppressive medications such as mycophenolate mofetil further complicates the interpretation of testing. In fact, the 2017 Infectious Diseases Society of America Guidelines on CDI recommend against testing for CDI for infants under 2 years of age unless other infectious and noninfectious etiologies have been excluded [54]. Therefore, pediatric-

specific understanding to interpret infectious-disease related testing is essential in the transplant population.

Unique Aspects of Antimicrobial Therapy for Pediatric Transplant Patients

Lack of Antimicrobial Dosing Data and Indication in Pediatrics

Although new antimicrobial agents are on the market for multidrug-resistant organisms (MDRO), a paucity of pediatric literature exists despite the fact that children are susceptible to multidrug-resistant pathogens. Since these drugs are generally not studied in pediatrics, limited pharmacokinetic (PK) and adverse effect data are available in the pediatric population. Pediatric exclusivity (PE), which provides financial incentives through market protection to drug companies for performing pediatric specific studies, encourages clinical trials to obtain some additional PK and safety data that may not otherwise be obtained. Of the 25 infectious diseases drugs that underwent pediatric trials for PE, 7 had dosing changes and 6 had new or enhanced safety concerns identified, highlighting the importance of studying antimicrobial agents in pediatrics [56]. Despite these incentives, pediatric trials are generally performed well after the completion of adult trials.

Although children remain at risk for MDRO, newer drugs that target these pathogens have limited pediatric data. Population PK analyses have provided suggested dosing data for ceftolozane/tazobactam [57] and ceftazidime/avibactam [58], the latter of which has a labeled indication for intra-abdominal infections and complicated urinary tract infections in children $\geq$3 months. Fidaxomicin has a similar PK profile in children and adults and may be efficacious but does not have a pediatric indication at this time [59]. Pediatric data for letermovir is not yet available.

Additionally, the lack of medications approved may affect the ability to accept organs for some pediatric patients while awaiting information about dosing and side effects specific to pediatric patients. One example relates to the use of HCV positive organs which is expanding rapidly in adult solid organ transplantation [60, 61]. With the approval of direct acting antiviral therapy for HCV (DAA) including pan-genotypic agents, cure of HCV in solid organ transplant recipients is possible allowing for the use of HCV+ organs to expand the donor pool and decrease wait-list mortality. DAA are currently only approved as young as 12 years of age with approval received in May 2019 for pan-genotypic agents. This prohibits many pediatric transplant recipients from potential access to these organs as appropriate DAA dosing and documentation of HCV cure is uncertain.

Variations in Pediatric Pharmacokinetics

The importance of obtaining pediatric PK data is highlighted by both variation in PK from children to adults but also within the pediatric population. Children require

higher doses of voriconazole in order to meet recommended troughs, and significant interindividual variability occurs, particularly in younger children [62]. Similarly, daptomycin is cleared rapidly in young children [63], and higher doses are recommended in children <12 years. Glomerular filtration maturation throughout childhood influences vancomycin dosing, and attainment of recommended goal troughs can be difficult in young children. Higher vancomycin dosing (15 mg/kg/dose every 6 h) is recommended in children with invasive methicillin-resistant *Staphylococcus aureus* infections in order to achieve a target trough concentration of 15–20 μg/mL. In adults, this target trough, which correlated with an area under the curve (AUC)/minimum inhibitory concentration (MIC) of ≥400 in adults, has been associated with better outcomes [64]. However, the association of improved outcomes in children with achievement of goal trough doses is not as robust as in the adult population. Modeling data in children suggest that a target trough of 7–10 μg/mL may actually achieve an AUC/MIC >400 in children receiving vancomycin doses of 15 mg/kg/dose q6 [65]. Thus, dosing strategies of many commonly used drugs in the pediatric transplant population remain complicated. The use of adult troughs and outcomes may not be fully applicable, and a better understanding of drug-specific pediatric PK is important in order to limit associated toxicities.

Limitations in Route of Drug Administration

The route of drug administration in pediatric transplant patients is important to consider, particularly in young children who cannot swallow tablets or capsules. For example, oral posaconazole suspension must be given multiple times per day, is best absorbed with fatty food, and may not be well absorbed. The delayed-release tablet is an excellent alternative, with once daily administration. However, the delayed-released tablet cannot be crushed or chewed for children who are unable to swallow tablets. Early pediatric data from a small number of patients are available to guide dosing recommendations for intravenous posaconazole [66]. Therefore, in children requiring posaconazole therapy, options are limited.

Limitations in Adverse Event Assessment in Pediatrics

Although many drugs have pediatric dosing available, these drugs are often used off-label in the pediatric population. In particular, the fluoroquinolones provide an oral, broad-spectrum antimicrobial option for treatment of pediatric infections, despite limited pediatric indications. Adverse events observed in the adult population may not occur in pediatrics. For example, Achilles tendon rupture, which has been well described in adults taking fluoroquinolones, has not been reported in pediatrics. Children do develop arthropathy at rates of <2%, which is typically reversible with cessation of the drug. Similarly, fluoroquinolone-associated risks of cardiac death and glucose abnormalities are largely uncommon in children compared with adults. Differences in the rates of adverse effects may be related to overall less

comorbidities in the pediatric populations; however, prescribers should be aware that other adverse effects, related to the use of these drugs in children with developing organs, may occur. Due to decreased frequency of use in the pediatric population, uncommon events may not be detected.

Summary

In conclusion, practitioners caring for pediatric transplant patients face unique issues compared with adult transplant providers. As the field of pediatric transplantation continues to expand, additional pediatric data will guide best practices in the field, and pediatric-specific recommendations will increase. Future studies to better define antimicrobial dosing and adverse effects will be crucial for children undergoing pediatric transplantation.

References

1. Urschel S, Cremer S, Birnbaum J, et al. Lack of serologic immunity against vaccine-preventable diseases in children after thoracic transplantation. Transpl Int. 2010;23(6):619–27.
2. Rubin LG, Levin MJ, Ljungman P, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58(3):309–18.
3. Danziger-Isakov L, Kumar D, AICo P. Vaccination of solid organ transplant candidates and recipients: guidelines from the American society of transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13563.
4. Smith CK, McNeal MM, Meyer NR, Haase S, Dekker CL. Rotavirus shedding in premature infants following first immunization. Vaccine. 2011;29(45):8141–6.
5. Hiramatsu H, Suzuki R, Nagatani A, et al. Rotavirus vaccination can be performed without viral dissemination in the neonatal intensive care unit. J Infect Dis. 2018;217(4):589–96.
6. Pittet LF, Verolet CM, McLin VA, et al. Multimodal safety assessment of measles-mumps-rubella vaccination after pediatric liver transplantation. Am J Transplant. 2019;19(3):844–54.
7. Feldman AG, Sundaram SS, Beaty BL, Kempe A. Hospitalizations for respiratory syncytial virus and vaccine-preventable infections in the first 2 years after pediatric liver transplant. J Pediatr. 2017;182:232–238 e231.
8. Madeleine MM, Finch JL, Lynch CF, Goodman MT, Engels EA. HPV-related cancers after solid organ transplantation in the United States. Am J Transplant. 2013;13(12):3202–9.
9. Kumar D, Unger ER, Panicker G, Medvedev P, Wilson L, Humar A. Immunogenicity of quadrivalent human papillomavirus vaccine in organ transplant recipients. Am J Transplant. 2013;13(9):2411–7.
10. Gomez-Lobo V, Whyte T, Kaufman S, Torres C, Moudgil A. Immunogenicity of a prophylactic quadrivalent human papillomavirus L1 virus-like particle vaccine in male and female adolescent transplant recipients. Pediatr Transplant. 2014;18(3):310–5.
11. Chin-Hong PV, Reid GE, Practice ASTIDCo. Human papillomavirus infection in solid organ transplant recipients: guidelines from the American Society of Transplantation infectious diseases community of practice. Clin Transpl. 2019;33(9):e13590.
12. Feldman AG, Beaty BL, Curtis D, Juarez-Colunga E, Kempe A. Incidence of hospitalization for vaccine-preventable infections in children following solid organ transplant and associated morbidity, mortality, and costs. JAMA Pediatr. 2019;
13. Kumar D, Blumberg EA, Danziger-Isakov L, et al. Influenza vaccination in the organ transplant recipient: review and summary recommendations. Am J Transplant. 2011;11(10):2020–30.

14. Kumar D, Ferreira VH, Blumberg E, et al. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis. 2018;67(9):1322–9.
15. Engelhard D, Mohty B, de la Camara R, Cordonnier C, Ljungman P. European guidelines for prevention and management of influenza in hematopoietic stem cell transplantation and leukemia patients: summary of ECIL-4 (2011), on behalf of ECIL, a joint venture of EBMT, EORTC, ICHS, and ELN. Transpl Infect Dis. 2013;15(3):219–32.
16. Danziger-Isakov L, Steinbach WJ, Paulsen G, et al. A multicenter consortium to define the epidemiology and outcomes of pediatric solid organ transplant recipients with inpatient respiratory virus infection. J Pediatr Infect Dis Soc. 2019;8(3):197–204.
17. Liu M, Mallory GB, Schecter MG, et al. Long-term impact of respiratory viral infection after pediatric lung transplantation. Pediatr Transplant. 2010;14(3):431–6.
18. Fisher BT, Danziger-Isakov L, Sweet LR, et al. A multicenter consortium to define the epidemiology and outcomes of inpatient respiratory viral infections in pediatric hematopoietic stem cell transplant recipients. J PediatrInfec Dis Soc. 2018;7(4):275–82.
19. Campbell AP, Guthrie KA, Englund JA, et al. Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. Clin Infect Dis. 2015; 61(2):192–202.
20. American Academy of. Pediatrics committee on infectious D, American Academy of Pediatrics bronchiolitis guidelines C. updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. Pediatrics. 2014;134(2):415–20.
21. Michaels MG, Fonseca-Aten M, Green M, et al. Respiratory syncytial virus prophylaxis: a survey of pediatric solid organ transplant centers. Pediatr Transplant. 2009;13(4):451–6.
22. Danziger-Isakov LA, Arslan D, Sweet S, Benden C, Goldfarb S, Wong J. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the international pediatric lung transplant collaborative. Pediatr Transplant. 2012;16(6):638–44.
23. Chu HY, Chin J, Pollard J, Zerr DM, Englund JA. Clinical outcomes in outpatient respiratory syncytial virus infection in immunocompromised children. Influenza Other Respir Viruses. 2016;10(3):205–10.
24. Robinson JL, Grenier D, MacLusky I, Allen UD. Respiratory syncytial virus infections in pediatric transplant recipients: a Canadian Paediatric surveillance program study. Pediatr Transplant. 2015;19(6):659–62.
25. Teusink-Cross A, Davies SM, Danziger-Isakov L, El-Bietar J, Grimley MS. Restrictive palivizumab use does not Lead to increased morbidity and mortality in pediatric hematopoietic stem cell transplantation patients. Biol Blood Marrow Transplant. 2016;22(10):1904–6.
26. Danziger-Isakov LA, Worley S, Michaels MG, et al. The risk, prevention, and outcome of cytomegalovirus after pediatric lung transplantation. Transplantation 2009;87(10): 1541–1548.
27. Florescu DF, Langnas AN, Grant W, et al. Incidence, risk factors, and outcomes associated with cytomegalovirus disease in small bowel transplant recipients. Pediatr Transplant. 2012;16(3): 294–301.
28. Behrendt CE, Rosenthal J, Bolotin E, Nakamura R, Zaia J, Forman SJ. Donor and recipient CMV serostatus and outcome of pediatric allogeneic HSCT for acute leukemia in the era of CMV-preemptive therapy. Biol Blood Marrow Transplant. 2009;15(1):54–60.
29. Bradley D, Moreira S, Subramoney V, et al. Pharmacokinetics and safety of valganciclovir in pediatric heart transplant recipients 4 months of age and younger. Pediatr Infect Dis J. 2016; 35(12):1324–8.
30. Kotton CN, Kumar D, Caliendo AM, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102(6): 900–31.
31. Condon LM, Cederberg LE, Rabinovitch MD, et al. Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. Clin Infect Dis. 2014;59(4):501–8.

32. Colombini E, Guzzo I, Morolli F, et al. Viral load of EBV DNAemia is a predictor of EBV-related post-transplant lymphoproliferative disorders in pediatric renal transplant recipients. Pediatr Nephrol. 2017;32(8):1433–42.
33. Hocker B, Fickenscher H, Delecluse HJ, et al. Epidemiology and morbidity of Epstein-Barr virus infection in pediatric renal transplant recipients: a multicenter, prospective study. Clin Infect Dis. 2013;56(1):84–92.
34. Smith JM, Corey L, Healey PJ, Davis CL, McDonald RA. Adolescents are more likely to develop posttransplant lymphoproliferative disorder after primary Epstein-Barr virus infection than younger renal transplant recipients. Transplantation. 2007;83(11):1423–8.
35. Green M, Michaels MG. Epstein-Barr virus infection and posttransplant lymphoproliferative disorder. Am J Transplant. 2013;13(Suppl 3):41–54; quiz 54.
36. Chinnock R, Webber SA, Dipchand AI, Brown RN, George JF. Pediatric heart transplant S. a 16-year multi-institutional study of the role of age and EBV status on PTLD incidence among pediatric heart transplant recipients. Am J Transplant. 2012;12(11):3061–8.
37. McDonald RA, Smith JM, Ho M, et al. Incidence of PTLD in pediatric renal transplant recipients receiving basiliximab, calcineurin inhibitor, sirolimus and steroids. Am J Transplant. 2008;8(5):984–9.
38. Wu JF, Ho MC, Ni YH, et al. Timing of Epstein-Barr virus acquisition and the course of posttransplantation lymphoproliferative disorder in children. Transplantation. 2009;87(5):758–62.
39. Yamada M, Nguyen C, Fadakar P, et al. Epidemiology and outcome of chronic high Epstein-Barr viral load carriage in pediatric kidney transplant recipients. Pediatr Transplant. 2018;22(3):e13147.
40. Green M, Soltys K, Rowe DT, Webber SA, Mazareigos G. Chronic high Epstein-Barr viral load carriage in pediatric liver transplant recipients. Pediatr Transplant. 2009;13(3):319–23.
41. Midgley CM, Watson JT, Nix WA, et al. Severe respiratory illness associated with a nationwide outbreak of enterovirus D68 in the USA (2014): a descriptive epidemiological investigation. Lancet Respir Med. 2015;3(11):879–87.
42. Waghmare A, Pergam SA, Jerome KR, Englund JA, Boeckh M, Kuypers J. Clinical disease due to enterovirus D68 in adult hematologic malignancy patients and hematopoietic cell transplant recipients. Blood. 2015;125(11):1724–9.
43. National Center for Immunization and Respiratory Diseases, Division of Viral Diseases. AFM Confirmed U.S. Cases. https://www.cdc.gov/acute-flaccid-myelitis/afm-cases.html. Published 2019. Accessed 9 Apr 2019.
44. Sejvar JJ, Lopez AS, Cortese MM, et al. Acute flaccid myelitis in the United States, august-December 2014: results of Nationwide surveillance. Clin Infect Dis. 2016;63(6):737–45.
45. Wali RK, Lee AH, Kam JC, et al. Acute neurological illness in a kidney transplant recipient following infection with Enterovirus-D68: an emerging infection? Am J Transplant. 2015;15(12):3224–8.
46. Giombini E, Rueca M, Barberi W, et al. Enterovirus D68-associated acute flaccid myelitis in immunocompromised woman, Italy. Emerg Infect Dis. 2017;23(10):1690–3.
47. Freeman MC, Coyne CB, Green M, Williams JV, Silva LA. Emerging arboviruses and implications for pediatric transplantation: a review. Pediatr Transplant. 2019;23(1):e13303.
48. Burton CE, Dragan T, Mabilangan CA, et al. Assignment of cytomegalovirus infection status in infants awaiting solid organ transplant: viral detection methods as adjuncts to serology. Pediatr Transplant. 2018;22(5):e13229.
49. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-guidelines of the American Society of transplantation infectious diseases community of practice. Clin Transpl. 2019:e13512.
50. Burton CE, Sester M, Robinson JL, Eurich DT, Preiksaitis JK, Urschel S. Assigning cytomegalovirus status in children awaiting organ transplant: viral shedding, CMV-specific T cells, and CD27-CD28-CD4+ T cells. J Infect Dis. 2018;218(8):1205–9.
51. Khanna S, Baddour LM, Huskins WC, et al. The epidemiology of *Clostridium difficile* infection in children: a population-based study. Clin Infect Dis. 2013;56(10):1401–6.

52. Crews JD, Koo HL, Jiang ZD, Starke JR, DuPont HL. A hospital-based study of the clinical characteristics of *Clostridium difficile* infection in children. Pediatr Infect Dis J. 2014; 33(9):924–8.
53. Ciricillo J, Haslam D, Blum S, et al. Frequency and risks associated with *Clostridium difficile*-associated diarrhea after pediatric solid organ transplantation: a single-center retrospective review. Transpl Infect Dis. 2016;18(5):706–13.
54. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):E1–E48.
55. Gonzalez-Del Vecchio M, Alvarez-Uria A, Marin M, et al. Clinical significance of *Clostridium difficile* in children less than 2 years old a case-control study. Pediatr Infect Dis J. 2016; 35(3):281–5.
56. Wharton GT, Murphy MD, Avant D, et al. Impact of pediatric exclusivity on drug labeling and demonstrations of efficacy. Pediatrics. 2014;134(2):e512–8.
57. Larson KB, Patel YT, Willavize S, et al. Ceftolozane/tazobactam population pharmacokinetics and dose selection for further clinical evaluation in pediatric patients with complicated urinary tract or complicated intraabdominal infections. Antimicrob Agents Chemother. 2019;
58. Bradley JS, Armstrong J, Arrieta A, et al. Phase I study assessing the pharmacokinetic profile, safety, and tolerability of a single dose of ceftazidime-avibactam in hospitalized pediatric patients. Antimicrob Agents Chemother. 2016;60(10):6252–9.
59. O'Gorman MA, Michaels MG, Kaplan SL, et al. Safety and pharmacokinetic study of Fidaxomicin in children with *Clostridium difficile*-associated diarrhea: a phase 2a multicenter clinical trial. J Pediatric Infect Dis Soc. 2018;7(3):210–8.
60. Goldberg DS, Abt PL, Blumberg EA, et al. Trial of transplantation of HCV-infected kidneys into uninfected recipients. N Engl J Med. 2017;376(24):2394–5.
61. Woolley AE, Singh SK, Goldberg HJ, et al. Heart and lung transplants from HCV-infected donors to uninfected recipients. N Engl J Med. 2019;380(17):1606–17.
62. Friberg LE, Ravva P, Karlsson MO, Liu P. Integrated population pharmacokinetic analysis of voriconazole in children, adolescents, and adults. Antimicrob Agents Chemother. 2012; 56(6):3032–42.
63. Abdel-Rahman SM, Benziger DP, Jacobs RF, Jafri HS, Hong EF, Kearns GL. Single-dose pharmacokinetics of daptomycin in children with suspected or proved gram-positive infections. Pediatr Infect Dis J. 2008;27(4):330–4.
64. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. Clin Infect Dis. 2011;52(3):285–92.
65. Frymoyer A, Guglielmo BJ, Hersh AL. Desired vancomycin trough serum concentration for treating invasive methicillin-resistant staphylococcal infections. Pediatr Infect Dis J. 2013; 32(10):1077–9.
66. Nickless JR, Bridger KE, Vora SB, Brothers AW. Evaluation of intravenous Posaconazole dosing and pharmacokinetic target attainment in pediatric patients. J Pediatric Infect Dis Soc. 2018;

Index

A
Abdominal wall graft, 1518, 1523
Abscess, 509, 511
Absolute lymphocyte count (ALC), 577
Acanthamoeba, 1608
  clinical presentation, 1455
  disseminated cutaneous infection, 1464
  drugs and doses, 1464
  ecology and pathobiology, 1453
  granulomatous amebic encephalitis (GAE), 1455
  miltefosine, 1463
  renal transplant recipient, 1456
  risk factors and incidence, 1453
  susceptibility testing, 1465
  tissue diagnosis, 1457
  total artificial heart device, 1456
  transplantation, 1460
Acanthamoeba keratitis (AK), 1452, 1453, 1465
Accelerate PhenoTest BC kit, 1546, 1548
Acinetobacter infections
  carbapenems, 377–379
  cefiderocol, 382
  clinical impact in transplant, 374–376
  combination therapy, 381–382
  diagnosis, 376–377
  epidemiology, 372–374
  polymyxins, 379–380
  prevention, 382–383
  sulbactam, 380
  tetracyclines and glycylcyclines, 380–381
Acinetobacter species, 299–306
Acquired hemophagocytic syndrome, 1690
Acquired immunodeficiency syndrome (AIDS), 1232
Active immunoprophylaxis, 673
Active tuberculosis, 414–416
Acute Chaga's disease (ChD)
  hematopoietic stem cell transplantation (HSCT), 1313
  solid organ transplant (SOT), 1312, 1313
Acute flaccid myelitis (AFM), 1719
Acute graft-versus-host-disease (aGVHD), 47, 1640
Acute lymphoblastic leukemia (ALL), 1464
Acute myeloid leukemia (AML), 6, 7
Acute respiratory distress syndrome (ARDS), 1235
Acute retinal necrosis (ARN), 1596, 1599, 1600
Acyclovir, 163, 596, 632
  resistance, 633
Adaptive immune system, 11
Adenovirus (AdV), 67, 68, 116, 761–763, 1647, 1655, 1681–1682
  pneumonia, 761
Adjunctive therapy, 716
Adoptive CMV-specific T cell therapy, 603–604
Advisory Committee on Immunization Practices (ACIP), 879, 888, 896
Aerosolized pentamidine, 1191
Aerosolized ribavirin, 144
Airway complications, 1619
Alanine transaminase (ALT), 928
Albendazole, 1146, 1430
Alcohol based hand sanitizers (ABHS), 242
Aldolase, 1384
Alemtuzumab, 21, 29, 46
Allogeneic, 727, 728, 734
Allogeneic hematopoietic stem cell transplant (allo-HSCT), 1254, 1596
  recipient, 686
Allograft rejection, 4, 690
Alloimmunity, 13
Allorecognition, 14

© Springer Nature Switzerland AG 2021

M. I. Morris et al. (eds.), *Emerging Transplant Infections*,
https://doi.org/10.1007/978-3-030-25869-6

Altered mental status, 1663, 1667–1669
early posttransplant period, 1664–1665
heart transplantation, 1667
intestinal and multivisceral transplantation, 1667
kidney transplantation, 1666
late posttransplant period, 1665–1666
liver transplantation, 1666
lung transplantation, 1667
management and prevention, 1669–1670
postoperative period, 1662–1664
Alternaria alternata, 1519, 1524
Amastigotes, 1299
American Academy of Pediatrics, 1715
American Society of Hematology, 1321
American Society of Transplantation (AST), 890, 1144, 1269, 1270, 1452, 1460, 1461
American Society of Tropical Medicine and Hygiene (ASTMH), 1354
American trypanosomiasis, *see* Chagas disease (ChD)
Aminoglycoside therapy, 474
Amphotericin, 1232, 1466
Amphotericin B (AmB), 1121, 1122, 1125, 1126, 1146, 1147, 1156, 1165, 1166, 1169, 1170, 1172, 1218, 1244, 1357
deoxycholate, 1465
Anal intraepithelial neoplasia, 836
Anemia, 1674, 1688
Anesthetics, 1662
Anidulafungin, 1095, 1098
Animal and insect precautions, 247
Anterior uveitis, 1597
Antibacterial prophylaxis, 156–158
Antibiotic(s), 1662
allergy assessment, 137–139
prophylaxis, 158–159
Antibody-dependent enhancement (ADE), 989
Antibody-mediated rejection (AMR), 1620
Anti-CD25 monoclonal antibodies, 20
Antiemetic drugs, 1651
Antifungal agents, 1120, 1127, 1128, 1219
Antifungal monotherapy, 1125
Antifungal prophylaxis, 159–162, 1123
Antifungal therapy, 59
first line therapy, 1122, 1125, 1126
polyene-azole, 1127
polyene-echinocandin, 1126
prophylaxis, 1121, 1122
salvage treatment, 1127
step down therapy, 1126
therapeutic drug monitoring (TDM), 1128, 1128
treatment duration, 1127
Antigenemia, 582
Anti-HBc-positive donors, 939, 940
liver transplantation, 938–939
non-liver transplantation, 940
Anti-HEV IgM assays, 915
Antileishmanial therapy, 1361
Anti-malarial drugs, 1386
Antimicrobial prophylaxis, 1187
Antimicrobial resistance, 344, 349, 354, 1536–1537
Antimicrobial stewardship, 7, 52, 132
Antimicrobial stewardship programs (ASP)
administrative support, 135
antibiotic allergy assessment, 137–139
antibiotic stewardship, 141–144
antifungal stewardship, 146–147
antimicrobial agent selection, 137
antiviral stewardship, 144–146
clinical pathways and guideline development, 140
diagnostic and therapeutic uncertainty, 133
diagnostic test stewardship, 140–141
dose optimization and drug interactions, 139
formulary management, 135–136
intravenous-to-oral conversion, 139
mid-level providers, 134
multidisciplinary approach, 134
nursing staffs, 134
outcomes measures, 147
pharmacists, 134
physicians, 133
post-prescriptive review, 136
pre-prescription authorization, 136
Antimicrobial susceptibility testing (AST), 1537
Antimicrobial therapy, 5, 6
Antimotility agent, 1651
Antiparasitic therapy, 1323
Antiproliferative agents, 19
Antirejection therapy, 1209, 1212
Antiretroviral therapy (ART), 866–868, 1401
Anti-secretory agent, 1651
Anti-thymocyte globulin (ATG), 46, 868
Anti-*Toxoplasma* chemoprophylaxis
CD4+ T cell count, 1271
D+/R-*vs*. D-/R-patients, 1256, 1270
gastrointestinal intolerance, 1271
hematopoietic stem cell transplantation (HSCT), 1271
kidney and liver transplant, 1256, 1270
myelotoxicity, 1271
protocols, 1271, 1272
solid organ transplant (SOT), 1256, 1270

trimethoprim-sulfamethoxazole (TMP-SMX), 1258, 1270, 1270
Antitrypanosomal treatment, 1321
Antiviral(s), 685, 688
prophylaxis, 938
therapy, 916
Aqueous humor, 1259–1260
Arbovirus(es), 976, 988, 995, 997, 999, 1001, 1002, 1719
Area under the curve (AUC), 1463
Artemether/lumefantrine, 1390
Artemisinin-based combination therapy (ACT), 1385
Arthemeter/lumefantrine, 1389
Arthroconidia, 1209
Ascaris lumbricoides, 1426–1428
AsperGenius assay, 1554
Aspergillosis
antifungal therapies, 1092
diagnosis, 1081–1082
prevention, 1100–1103
risk factors, 1079
treatment, 1090–1098
Aspergillus, 183–184, 1499, 1507
A. fumigatus, 160, 1078–1081, 1087–1089, 1097–1100
galactomannan (GM) test, 1118
infections, 146
polymerase chain reaction, respiratory infection, 1552–1559
Aspergillus spp.
azole resistant strains, 1080–1081
bronchoscopy, 1082
clinical manifestations, 1082
combination therapy, 1098–1099
(1-3)-β-D-glucan, 1085
diagnostic algorithm, 1090
distribution, 1081
duration of therapy, 1099
echinocandins, 1095–1097
galactomannan antigen detection, 1084–1085
ibrexafungerp, 1098
immunohistochemistry (IHC), 1084
isavuconazole, 1094
itraconazole, 1094, 1095
microRNA, 1089
monoclonal antibodies, 1087–1088
polymerase chain reaction (PCR), 1085–1087
polyenes, 1097
posaconazole, 1095
positron emission tomography (PET), 1089–1090
prevention, 1100–1103
rezafungin, 1097
susceptibility testing, 1099
urinary antigen detection, 1088
volatile organic compounds, 1088
voriconazole, 1090–1094
Astrovirus, 1642, 1648, 1656
Atovaquone (AVQ), 1191, 1198, 1265
proguanil, 1389, 1390
Atypical bacteria
fastidious bacteria, 1607
mycobacterial infections, 1607
Autoimmune cytopenias, 1692–1693
Autologous HSCT recipients, 686
Azathioprine, 19
Azithromycin (AZT), 1265, 1464, 1466
Azoles, 63, 1466
Aztreonam-avibactam, 332, 362

B

Bacterial infections, 172–177
atypical bacteria, 1607
bloodstream, 50–54
C. difficile, 54–56
typical bacteria, 1606
Bacteriuria, asymptomatic, 143–145
Balamuthia mandrillaris
amebae, 1459
antigens, 1459
clinical presentation, 1458
cerebrospinal fluid (CSF) studies, 1458
drugs and doses, 1467
ecology and pathobiology, 1455
in vitro activity, 1466
risk factors and incidence, 1455
transplantation, 1462
Balantidium coli infection
clinical presentation, 1424
diagnosis, 1424
epidemiology, 1424
treatment, 1425
Baloxavir marboxil, 751
Basiliximab, 20, 27
B cells, 14, 47
Beef tapeworm, 1435
Behavior, 179, 189, 191
Belatacept, 20, 26
Benznidazole, 1325, 1327
Beta-1,3-D-glucan (BDG) assays, 1118
Beta-D-glucan (BDG), 1164
1,3-Beta-D-glucan (BG), 58
Beta-glucan testing, 1192, 1195

Beta-lactamases, 355
Ambler classification, 355
vs. beta-lactamase inhibitor activity, 355
Beta-lactam-beta-lactamase inhibitors (BL-BLIs), 53
Bezlotoxumab, 56, 533
BioFire FolmArray gastrointestinal panel (BioFire GIP), 1643
BK polyomavirus, 808
infection, 809
prophylaxis, 165
See also Hemorrhagic cystitis; Polyomavirus-associated nephropathy
Blastocystis spp., 1416–1418
Blastomycosis, 180, 1226, 1231, 1236
clinical impact in transplant patients, 1234
diagnosis, 1235–1236
disease epidemiology, 1233–1234
pathogenesis and clinical manifestations, 1234–1235
prophylaxis, 1237
treatment, 1236–1237
Blood/bone marrow, 1259, 1260, 1273
Blood donor screening, 979
Bloodstream infections
antibiotic prophylaxis, 51
bacterial pneumonia, 53
central venous catheters (CVCs), 50
diagnosis, 51, 52
management, 52–53
pre-engraftment phase, 50
Bloodstream infections, emerging diagnostics
magnetic resonance-based methods, 1550–1551
metagenomic sequencing, 1552
molecular methods, 1537–1550
Blood-sucking bugs, 1295
Blood transfusion, 1313, 1314
Blood vessels, 1522
Bocaviruses, 763
Bone infection, 1521
Bone marrow transplant
clinical impact, 291
epidemiology, 290
Borna disease virus-1, 1036
Bortezomib, 34
Brain abscess(es), 492, 1144, 1145
Brazilian Ministry of Health, 1015, 1019
Brentuximab, 34
Brincidofovir, 68, 565, 597, 763
for human adenovirus (HAdV) infection, 795, 796
Bronchiolitis obliterans syndrome, 1626
Bronchoalveolar lavage (BAL), 1082, 1088, 1230, 1259
fluid, 61
Burkholderia, 1497–1499
Burkholderia cepacia, 176, 396
antibiotic resistance mechanism, 399
antibiotics, 403
antibiotic therapy, 403
associated with contaminated medicines, medical fluids and devices, 396–404
associated with cystic fibrosis, 399
cysteamine, 403
immunosupressive therapies post-transplant, 402
infection control measures, 401
inter-operative therapies, 402
oligoG, 403
outcomes of infection, 400
phage therapy, 403
prophylaxis strategies, 402
supportive therapy, 402
and transplantation, 400–401
virulence features, 397–399
Burkholderia gladioli, 395–396
Burkholderia multivorans, 399–400
Burkholderia pseudomallei, 392–395
management of, 394
and transplantation, 394

C

Calcineurin inhibitors (CNIs), 17, 139, 1640, 1662
Candida, 1519, 1580
C. albicans, 57
C. auris, 57–60
C. glabrata, 57, 59
C. krusei, 57, 59
Candida infections, 146, 1046, 1518
Candida auris, 1064
culture-based diagnostic methods, 1057–1058
diagnostics, 1056
donor derived, 1055–1056
heart transplant, 1049–1051
kidney transplant, 1053–1054
liver transplant, 1051–1053
lung transplant, 1047–1049
mortality, 1047
pancreas transplantation, 1054–1055
polymerase chain reaction, 1059
small intestine, 1055
T2 magnetic resonance, 1059–1060
therapeutic drug monitoring, 1064–1065
treatment, 1060
Capillaria philippinensis, 1434–1435

Carbapenem, 140
Carbapenemase, 326–328
Carbapenem-resistant Enterobacterales (CRE) infection
  antibiotics, 331
  clinical impact in transplant, 323–324
  disease diagnostics and clinical evaluation, 326–328
  epidemiology, 317–323
  management, 330–333
  risk factors in solid organ and hematopoietic stem cell transplant recipients, 322
Carbapenem resistant *Enterobacteriaceae* (CRE), 176, 299–306
Carbapenems, 53, 377–379
Cardiomyocytes, 1317
Cardiomyopathy, 1301, 1302
Caspofungin, 1095, 1099
Castleman disease, 695
Catheter-associated rapid grower mycobacteria (RGM) infections, 443
Catheter-associated urinary tract infection (CAUTI), 102–103
Catheter-related infections, 468
Cavitary lesions, 491
CCR5 blockade, 868
CD134, 687
CD4+ cells, 687
CDC Free-Living and Intestinal Amebas (FLIA) Laboratory, 1457
Cefiderocol, 53, 332–333, 361, 382, 403
Ceftazidime-avibactam, 329, 360–361
Ceftolozane-tazobactam, 329, 360
Cell cytotoxicity neutralization assay (CCNA), 529
Cellular immunotherapy, 1601
Center for International Blood and Marrow Transplant Research (CIBMTR), 4
Center of Disease Control (CDC), 1298
Centers for Disease Control and Prevention (CDC), 132, 877, 1452
Central line-associated bloodstream infection (CLABSI), 101
Central nervous system infection, 1146
Central pontine myelinosis (CPM), 1664
Central venous catheters (CVCs), 50
Cerebral toxoplasmosis, 1254, 1265
Cerebrospinal fluid (CSF), 1230, 1259–1260, 1309, 1455, 1457
Cervarix®, 850
Cervical Papanicolaou (Pap) test, 844–845
CFTR modulators, 1505–1506
Chagas disease (ChD), 184–186
  acute diagnosis, 1308–1310
  burden of disease, 1297–1299
  chronic diagnosis, 1310
  epidemiology, 1294–1299
  laboratory diagnosis, 1306–1308
  modes of transmission, 1295–1297
  natural history and pathogenesis, 1299–1304
  post-transplant management, 1322–1324
  pre-transplant risk assessment and management, 1320–1321
  prevention, 1328
  public health problem, 1294
  reactivation diagnosis, 1310–1311
  successive phases, 1294
  transplant physicians, 1294
  transplant recipients, 1311–1320
  treatment, 1324–1328
Chagas disease (ChD) reactivation
  asymptomatic, 1311, 1315
  heart solid organ transplant (SOT), 1315, 1317
  hematopoietic stem cell transplantation (HSCT), 1317
  non-heart solid organ transplant (SOT), 1315
  parasitemia, 1310, 1311, 1314
  semi-quantitative xenodiagnosis, 1311
Chemoprophylaxis, 674–675, 716, 1262–1264, 1270, 1386
Chemotherapy, 669
Chest X-ray (CXR), tuberculosis, 413
Chikungunya virus (CHIKV) infection, 191
  clinical impact on transplantation, 994
  diagnosis, 996
  disease epidemiology, 990
  management, 998
  screening for, 1002
  vaccine, 1000
  vector control, 999
Chimerism, 688
Chlamydia trachomatis, 1524
Chloroquine (CQ), 1389
Chorioretinitis, 1596
Chromosomally integrated HHV-6, 691
Chronic Chagas cardiomyopathy (CCC), 1302, 1315
Chronic graft-*versus*-host disease (cGVHD), 47, 411
Chronic hepatitis B (CHB)
  hematopoietic stem cell transplant recipients, 934
  liver transplant recipients, 933
  managing transplant recipients, 942–943
  solid organ transplant recipients, 933

Chronic lung allograft dysfunction (CLAD), 728
Cidofovir, 564, 598, 685, 688, 697, 1655
  for human adenovirus (HAdV) infection, 794
Ciprofloxacin, 1410
Cladophialophora bantiana, 1144
Climate change
  and bacterial infections, 93
  and fungal infections, 92–93
  and infectious diseases, 89–93
  and parasitic infections, 90
  and vector-borne infections, 91–92
Clindamycin (CLD), 1198, 1265, 1390
Clinical Laboratory Standards Institute (CLSI), 291, 1559
Clofazimine, 474
Clostridioides difficile, 100, 103, 104, 159, 1720–1721
  clinical manifestation, 519
  diagnosis of infection, 528–531
  epidemiology, 520–524
  pathogenesis of, 524–528
  probiotics, 535
  prophylaxis, 534
  treatment modalities and options, 531–534
Clostridioides difficile infection (CDI), 132, 141, 1645, 1656
  diagnosis, 54
  intestinal microbiome diversity, 54
  management, 55–56
  recurrent CDI, 56
  risk factors, 54
  secondary prophylaxis, 56
CNS infections, emerging diagnostics
  metagenomic sequencing, 1564–1565
  multiplex polymerase chain reaction, 1564
Coccidioides spp., 180
  C. immitis, 1210
  C. posadasii, 1210
Coccidioides-infected donor, 1216
Coccidioidomycosis, 180–182
  antifungal treatment, 1217, 1218
  clinical manifestations, 1210, 1211
  diagnosis, 1211, 1212
  drug interactions, 1218
  epidemiology, 1208–1209
  follow up, 1218
  hematopoietic stem cell transplantation (HSCT), 1218, 1220
  mycology and immunity, 1209, 1210
  non-pharmacologic prevention strategies, 1216
  organ donors, 1215
  prophylaxis strategies, 1214
  recognition, 1217
  screening, 1212, 1213
  solid organ transplantation, 1213, 1214
  transplant recipients, 1212, 1216
  treatment, 1217
Cognitive specialties, 5
Colistin methanesulfonate, 379
Combination antiretroviral therapy (cART), 863, 865–867
Complement-mediated lysis, 1306
Computed tomography (CT), 1114
Computerized clinical decision support (CCDS), 141
Congestive heart failure (CHF), 1095, 1302
Cord blood, 686
  cells, 1256, 1269
Cord colitis, 1640
Coronal fluid-attenuated inversion recovery (FLAIR), 973
Coronavirus, 760–761
Corticosteroids, 19, 1198
Cotrimoxazole, 1258
Covalently closed circular DNA (cccDNA), 929
Cowdry bodies, 1650
Cryptococcal antigen, 1159–1161, 1172
Cryptococcal meningitis, 1156, 1157, 1159, 1161, 1166, 1169, 1170
Cryptococcemia, 1159
Cryptococcosis, 182–183
  clinical impact, 1158–1160
  diagnosis, 1160–1164
  epidemiology, 1156–1157
  management, 1165–1169
  prevention and control, 1170–1172
  solid organ transplant, 1157
  stem cell transplant, 1158
  treatment modalities, 1169–1170
Cryptococcus, 60, 1155–1158, 1160
  C. adeliensis, 1158
  C. albidus, 1156
  C. curvatus, 1164
  C. gattii, 183, 1155, 1156, 1161, 1164, 1167
  C. laurentii, 1156
  C. neoformans, 1155, 1156, 1160, 1161, 1164, 1167
  C. neoformans var. *grubii*, 1156
  C. neoformans var. *neoformans*, 1156

Cryptogenic organizing pneumonia, 1626
Cryptosporidiosis, 1407
Cryptosporidium, 1649, 1656
Cryptosporidium infection
  clinical presentation, 1400
  diagnosis, 1400–1401
  epidemiology, 1399
  extra-intestinal manifestations of, 1408
  as GVHD mimic, 1407
  in hyper-IgM syndrome, 1408
  and renal failure, 1407
  treatment, 1401
CTX-M, 314, 326, 329
Cutaneous leishmaniasis (CL)
  clinical manifestations, 1362
  diagnosis, 1362
  disease endemicity, 1355
  disease epidemiology, 1360, 1361
  geographical distribution, 1355, 1362
  immunohistochemical examination, 1365
  SOT and HSCT, 1361
  transplant recipients, 1363
  treatment, 1365, 1366
Cyclospora cayetanensis infection
  clinical presentation, 1409
  diagnosis, 1409
  epidemiology, 1408
  treatment, 1409
Cyclosporiasis, 1649
Cyclosporine, 1460
Cysteamine, 403
Cystic fibrosis, 174, 176, 178, 179, 183, 184, 191, 346, 1496
  bacteriophage therapy, 1504–1505
  Burkholderia cepacia complex, 1497–1499
  considerations in patients with, 1507
  cystic fibrosis transmembrane conductance regulator (CFTR) modulators, 1505–1506
  lung transplant recipients, 1497
  M. abscessus infections, 1502–1504
  Scedosporium, 1499–1502
Cystoisospora belli infection
  clinical presentation, 1411
  diagnosis, 1412
  epidemiology, 1411
  treatment, 1412
Cysts, 1185
Cytochrome P450 (CYP) 3A4 system, 1463
Cytomegalovirus (CMV), 145–146, 577–579, 690, 693, 1588, 1599, 1651, 1678–1679
  antigenemia, 582–583
  antiviral agents with efficacy, 553
  antivirals, 599
  clinical impact in solid organ transplant, 549–550
  clinical manifestations, 579–581
  culture and pathology, 553
  diagnosis, 65–67
  disease clinical evaluation and diagnosis, 550–555
  disease epidemiology, 547–549
  donor serology, 548
  drug resistant infection, 66
  epidemiology and risk factors, 574–576
  histopathology, 582
  immune assays, 554–555
  immune reconstitution, 576–577
  immunoglobulins, 559–560
  nucleic acid testing, 552–553
  preemptive therapy, 65, 585–595
  prevention, 555–559
  prophylaxis, 66, 163–165, 595–599
  prophylaxis *vs.* pre-emptive therapy, 558
  quantitative nucleic acid testing, 583–585
  reactivation, 65
  resistance, 599–604
  retinitis, 1596
  risk factors, 65
  serology, 551, 582
  treatment of active infection, 560–562
  treatment resistant and refractory disease, 562–565
  vaccines, 559, 604–605
  vascularized composite allograft (VCA) infection, 1525, 1716–1717
  viral culture, 582
  viral load testing, 551
Cytopathic effect, 1645
  of human adenovirus (HAdV), 790
Cytotoxic T lymphocytes (CTL), 576
  therapy, 670

D

Dacryocystitis, 1598
Dacryocystorhinostomy, 1606
D-Alanine-D-Alanine (D-Ala D-Ala), 291
Dapsone, 1191
Daptomycin, 52
Decompensated cirrhosis, 952–954
Deep local infection, 1145

Deferasirox, 1130
Definitive cure, 1328
Delirium, 1661, 1662, 1666, 1667
Dematiaceous fungi, 1140, 1143, 1145, 1148
Dengue virus (DENV), 191
Dengue virus (DENV) infection
  clinical impact on transplantation, 992–994
  diagnosis, 995–996
  disease epidemiology, 989–990
  management, 997–998
  screening for, 1002
  vaccine, 1000
  vector control, 999
De novo acquisition, 1209
De novo infection, 1317
Dermatophytes, 1579
1-3-β-D-glucan, 1058–1059
Diabetes mellitus, 1482
Diagnostics
  for bloodstream infections, 1537–1552
  for CNS infections, 1563–1565
  for gastrointestinal infections, 1565–1568
  for respiratory infections, 1552–1563
Diarrhea
  adenovirus, 1647
  astrovirus, 1648
  bacterial enteric pathogens, 1646
  C. difficile, 1645
  CMV PCR serum/blood, 1645
  colonoscopy, 1650, 1651
  Cryptosporidium, 1649
  Cyclospora, 1649
  definition, 1639
  emerging infections, 1642–1643
  Entamoeba, 1649
  Giardia, 1648
  Microsporidia, 1649
  multiplex polymerase chain reaction, 1643
  norovirus, 1647, 1648
  rotavirus, 1648
  sapovirus, 1648
  Strongyloides, 1650
  transplant recipients, 1651–1657
Dientamoeba fragilis infection
  clinical presentation, 1422
  diagnosis, 1422–1423
  epidemiology, 1422
  treatment, 1423
Diffuse alveolar hemorrhage, 1625
Digital anorectal examination (DARE), 848
Dihydroartemisinin/piperaquine, 1389, 1390
Dihydrofolate reductase, 1185
Dihydropteroate synthase, 1185
Diphyllobothrium latum, 1440
Dipylidium caninum, 1441
Direct-acting antiviral agents (DAAs), 952
  benefits in patients with HCC, 956
  functional and clinical evolution, 954
  impact in post-LT outcomes, 958
  impact on LT-waiting lists, 956
  regimes after liver transplantation, 957–958
  in use of HCV-positive donors, 960–961
Direct agglutination test (DAT), 1354
Direct allo-recognition, 14
Directly observed therapy (DOT) programs, 414
Discrete typing units (DTUs), 1299
Disseminated infection, 1146
Disseminated nocardiosis, 493
Dog tapeworm, 1441
Dolichomegacolon, 1304
Donor(s)
  hematopoietic stem cell transplantation (HSCT), 1321
  organ, 1209, 1215
  pre-transplant treatment, 1321
  solid organ transplant (SOT), 1320, 1321
Donor-derived infection, 506, 507, 974, 1283
Donor-derived organ transplant transmission, 1376
Donor-to-host transmission, 1375
Doxycycline, 1390, 1391
Drug-drug interaction (DDI), 147, 1218
Drug hypersensitivity syndrome, 1587
Drug reaction with systemic symptoms (DRESS), 1587
Dwarf tapeworm, 1438

E

Eastern Equine Encephalitis virus (EEEV), 1035
Echinocandins, 1052, 1060, 1095–1097, 1146, 1147
Eculizumab, 30–31
Electroencephalography, 1669
Electronic consultation, 6
Electronic ID consultation, 6
Electron microscopy (EM), 1648
Emerging diagnostics
  for bloodstream infections, 1537–1552
  for CNS infections, 1563–1565
  for gastrointestinal infections, 1565–1568
  for respiratory infections, 1552–1563
Empiric antimicrobial therapy, 1631–1632

Encephalitis, 578, 686, 690, 972, 974
Encephalitozoon cuniculi, 1141
Endemic, 1012, 1014, 1020, 1021
  fungal infections, 179–184
  human herpes viruses, 188–189
Endocarditis, 1145
Endophthalmitis, 1597, 1598
  abscesses, 1604
  clinical symptoms, 1604
  liposomal amphotericin B, 1605
  therapeutic failure, 1605
  transplant recipients, 1604
  vitrectomy, 1605
  yeasts and molds, 1604
Endosymbionts, 1460
Engraftment, 686, 687
  syndrome, 1625, 1640
Entamoeba, 1649
Entamoeba histolytica infection
  clinical presentation, 1419
  diagnosis, 1419–1421
  epidemiology, 1418
  treatment, 1421
Enteritis, 1642, 1656
Enteroaggregative *E. coli* (EAEC), 1647
Enterocytozoon bieneusi, 1141
Enteroinvasive *E. coli* (EIEC), 1647
Enteropathogenic *E. coli* (EPEC), 1647
Enterotoxigenic *E. coli* (ETEC), 1647
Enterovirus D68 (EV-D68), 1718–1719
Enzyme immunoassays (EIAs), 1236, 1647
Enzyme-linked fluorescent immunoassays (EIA), 529
Enzyme-linked immunosorbent assay (ELISA), 54, 1306, 1354, 1648, 1649
Enzyme linked immunospot (ELISPOT), 65
Epidemic, 1012, 1014, 1019–1021
Epstein-Barr virus (EBV), 67, 656
  infection, 1525, 1717–1718
Epstein-Barr virus driven post-transplant lymphoproliferative disorder
  after hematopoietic stem cell transplantation, 660–661
  after solid organ transplantation, 657–658
  algorithm for treatment of, 667
  antiviral agents and intravenous immune globulin, 670
  cell-mediated immune response, 664
  chemoprophylaxis, 674–675
  chemotherapy, 669
  clinical impacts on transplantation, 661–663
  diagnosis, 663–666
  disease epidemiology, 656–661
  immunoprophylaxis, 673–674
  management and prevention, 666–677
  nucleic acid detection, 663–664
  preventive strategies, 672
  radiation and surgery, 669
  radiographic imaging, 665
  rituximab, 668
  serologic testing, 663
  tissue histopathology, 665
  treatment in hematopoietic stem cell transplantation, 670
  treatment in solid organ transplantation, 668–670
  viral load monitoring and preemptive strategies of prevention, 675–677
Eravacycline, 332, 381
Escherichia coli, 159
ET-12 strain, 404
European Aspergillus PCR Initiative (EAPCRI), 1553
European Centre for Disease Prevention and Control, 714
European Committee on Antimicrobial Susceptibility Testing (EUCAST), 1121, 1559
European Conference of Infections in Leukemia (ECIL 7), 720
European group for Blood and Marrow Transplantation Infectious Diseases Working Party (EBMT-IDWP), 1262
European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) Consensus Group, 1081
European Society of Clinical Microbiology and Infectious Diseases (ESCMID), 1144
Everyday living, 240–243
Exhaled metabolites, 1559–1560
Exotic pets, 247
Extended spectrum beta-lactamase (ESBL), 53, 176
Extended-spectrum β-lactamase-producing Enterobacterales (ESBL-EB) infection
  antibiotics, 331
  clinical impact in transplant, 323
  disease diagnostics and clinical evaluation, 325–326
  disease epidemiology, 314–317
  management, 328–330
Extracorporeal membrane oxygenation (ECMO), 1478, 1480, 1486
Extrathoracic infections, 1208

F
Face transplant, 1516, 1523
Fasciolopsis buski, 1442
Fastidious bacteria, 1607
Favipiravir, 1034
Febrile neutropenia, 141–143
Fecal microbiota transplantation (FMT), 56, 1656
Fibrosing cholestatic hepatitis (FCH), 933, 958
Fidaxomicin, 55, 56
FilmArray, 1540, 1547, 1564
Filmarray ME test, 1161
Fishtank granuloma, 463, 466
Fish tapeworm, 1440
Flouroquinolones, 474
Fluconazole, 59, 60, 1060, 1064, 1156, 1164, 1166, 1167, 1169, 1170, 1172, 1217, 1242–1243, 1463, 1464
Flucytosine, 1146, 1156, 1165, 1166, 1169, 1172, 1464
Flumist®, 753
Fluorescence in-situ hybridization (FISH), 1538
Fluorescent techniques, 1649
Fluoroquinolone(s), 51, 68, 415, 1607, 1662
Focal segmental glomerular sclerosis (FSGS), 870
Folliculitis, 1584, 1587
Food and Drug Administration (FDA), 1296, 1306
Foodborne illness, 246
Food safety, 245–247
Foscarnet, 563, 633, 685, 688
  therapy, 602
Fosfomycin, 363
Free-living amoebae (FLAs), 1452, 1467
Fresh/formalin-fixed paraffin embedded tissue (FFPE), 1117, 1118
Fungal infections, 1155, 1157, 1172, 1184, 1185
  causative pathogens, 1603
  clinical syndromes, 1603
  endophthalmitis, 1604, 1605
  vs. herpesvirus, 1602
  molds, 60–64
  rhinocerebral mucormycosis, 1605
  treatment, 1603
  yeast, 57–60
Fungemia, 1144
Fusarium spp., 1140–1142, 1144–1146, 1148

G
Galactomannan (GM), 61, 1142
  antigen detection, 1084–1085
Gammaherpesviridae, 694
Ganciclovir, 595, 596, 685, 688
  for human adenovirus (HAdV) infection, 794
Gardasil®, 850
Gas-chromatography mass spectrometry, 1559–1560
Gastrointestinal disease, 578, 580
Gastrointestinal disorders, 1358
Gastrointestinal infections, 1565–1568
Gastrointestinal tract disease, 1303
Geometric mean concentrations (GMC), 883, 891, 893, 896
Giardia, 1648
Giardia spp. infection
  clinical presentation, 1414
  diagnosis, 1415
  epidemiology, 1414
  treatment, 1415
Glasgow Coma Scale, 1391
Glucocorticoids, 48, 1599
Glucose-6-phosphate dehydrogenase (G6PD), 1385
Glutamate dehydrogenase (GDH), 54, 529, 1645
Graft dysfunction, 788, 1619–1620
Graft failure, 1695–1696
Graft loss, 690
Graft rejection, 1515
Graft versus host disease (GVHD), 44, 47, 48, 141, 351–352, 686, 692, 698, 893–895, 1256, 1258, 1262, 1268, 1317, 1581, 1640, 1694
Gram-negative bacterial infections, 1606
Gram-negative infections, 349
Gram-positive bacterial infections, 52, 53, 1606
Granulocyte colony stimulating factor (G-CSF), 1130, 1699
Granulocyte-macrophage colony stimulating factor (GM-CSF), 1130
Granulocyte transfusions, 1698
Granulomas, 1210
Granulomatous amebic encephalitis (GAE), 1452, 1453, 1455
Grocott-Gomori's methenamine-silver (GMS), 1116

H
Hand hygiene, 100, 101, 103–105, 112, 113, 115, 116, 241, 242
Hand transplantation, 1517, 1523
Haploidentical transplantation, 49
HBsAg-positive donors
  liver transplantation, 942
  non-liver transplantation, 942

Heart transplant, 689, 1256, 1268, 1666–1667
human adenovirus (HAdV) infection in, 788–789
Helminths, 1425
Hematology, 1112, 1119, 1124, 1127
Hematopoietic stem cell transplant(s), 155, 158, 159, 161–164, 166, 167, 1714–1715
Hematopoietic stem cell transplant recipients
blastomycosis (*see* Blastomycosis)
histoplasmosis (*see* Histoplasmosis)
paracoccidioidomycosis (*see* Paracoccidioidomycosis)
Hematopoietic stem cell recipients, 4
Hematopoietic stem cell transplantation (HSCT), 133, 176, 179, 253, 819, 820, 822, 1014, 1021, 1110, 1218, 1220, 1256, 1268, 1271, 1282, 1283, 1343, 1378, 1379, 1639, 1640
acute Chagas disease, 1313
anti-Hepatitis B, 49
antiviral therapy, 642
aplastic period, 46
Aspergillus in (*see Aspergillus* spp.)
bacterial infections, 50–56
B-cells, 47
Chagas disease (ChD) reactivation, 1317
donors, 1321
EBV infection and PTLD after, 660
fungal infections, 57–64
GVHD, 47, 48
human adenovirus (HAdV) infection, 784–786
haploidentical donors, 49
immune reconstitution, 45
immunoglobulins, 47
infections, 44
NK and T-cells, 46, 47
outcome of PTLD after, 662
parasitic infections, 68, 69
post-transplant immunization, 638
pre-transplant immunization, 638
respiratory viral pathogens in (*see* Respiratory viral pathogens in transplant recipients)
treatment for EBV disease and PTLD in, 670
umbilical cord blood transplantation (UCBT), 48, 49
vaccinations, 69
viral infections, 64–68
varicella zoster virus (VZV) immunization in seronegative, 637
varicella zoster virus (VZV) immunization in seropositive, 644
yellow fever (YF) vaccination, 1017
Hematopoietic stem cell transplant (HSCT) recipients, influenza infection
adjuvanted vaccine, 721
donor vaccination and pre-HSCT vaccination, 721
fever, 714
high dose vaccination, 720
ICU admission, 712
incidence, 711
lower respiratory tract infection, 714
prophylaxis, 716
repeated vaccination, 720
timing of vaccination, 720
Hematoxylin-eosin (H&E), 1116
staining, 1194
Hematoxylin-eosin-safran (HES), 1261
Hemophagocytic lymphohistiocytosis, 696, 1690
Hemophagocytic syndrome (HPS), 1258
Hemorrhagic cystitis, 788
clinical evaluation and diagnostics, 820
diagnosis and evaluation of, 812
disease epidemiology and risk factors, 820–821
pathology and clinical impact, 817–820
treatment, 821–823
Hemovigilance methods, 1384
Hepatitis A vaccine, 1712
Hepatitis A virus (HAV) infection, 265
clinical presentation, 883–886
diagnosis, 882–883
epidemiology, 878–880
food precautions, 887–888
HAV-ALFSG, 886
immunocompromised hosts, vaccination in, 889–890
postexposure prevention, 896–897
post-SOT, 895
posttransplant vaccination, 892–895
pre-donation vaccination, 892
pre-existing, 885
pretransplant vaccination, 890–892
structure, 880
subclinical presentations, 887
transmission, 880–882
travel recommendations, 895–896
vaccination against, 266
vaccine series, 888
Hepatitis B immune globulin (HBIG), 937
Hepatitis B virus (HBV), 187, 188
vaccination against, 264
Hepatitis B virus (HBV) infection, in transplants
chronic hepatitis B, natural history, 933–934
diagnostic evaluation, 929–932

Hepatitis B virus (HBV) infection (*cont.*)
management, 934–943
reactivation and implications, 932–933
screening tests, 929
Hepatitis C virus (HCV), 187, 188
Hepatitis C virus (HCV) infection, 952
post-transplant management, 957–963
pre-transplant management, 952–957
with decompensated cirrhosis, 952–954
Hepatitis D virus (HDV) infection, 944
Hepatitis E virus (HEV), 188
Hepatitis E virus (HEV) infection, in transplants
antigen detection, 915
chronic infections, 913–914
clinical manifestations, 913
disease epidemiology, 911–913
extra-hepatic manifestations, 914–915
immunosuppressive regimen management, 916
molecular diagnosis, 915–916
prevention, 920
ribavirin monotherapy, 917–918
serological tests, 915
sofosbuvir, 918
Hepatocellular carcinoma (HCC), 928, 934
HEPLISAV-B vaccine, 936
Herpes simplex virus (HSV) 1 and 2, 1580–1582
acyclovir shortage, 647
antiviral prophylaxis after transplant, 642
clinical impact in transplant, 623–625
clinical issues and risk factors for infections in transplant patients, 624
disease diagnostics and clinical evaluation, 626–628
disease epidemiology, 618–621
laboratory methods for diagnosis of, 629–630
management, 631–633
in pregnant transplant recipients, 647
prevention of reactivation, 640
primary infection prevention, 636
reactivation, 623
recommendations for treatment in transplant patients, 632
Herpes simplex virus (HSV), 64, 66, 163, 188, 1521
β-Herpesviridae, 684
Herpesviruses
acute retinal necrosis (ARN), 1599, 1600
causative pathogens, 1602
clinical syndromes, 1598, 1602
keratitis, 1600
post-transplant lymphoproliferative disorder (PTLD), 1600
progressive outer retinal necrosis (PORN), 1600
retinitis, 1599
therapeutic failure, 1600, 1601
treatment, 1602
zoster ophthalmicus, 1600
Herpes zoster (shingles), 269
complications, 625
cutaneous, 625
in hematopoietic stem cell transplantation (HSCT) population, 273
incidence, 271, 621
Oka vaccine, 643
ophthalmicus, 625
vaccination, 645
vaccination against, 271
High-grade squamous intraepithelial lesions (HSIL)
development of, 837–838
progression to invasive cancer, 837–838
Histamine-2-receptor antagonists, 521
Histidine-rich protein 2 (HRP-2), 1384
Histocompatibility-allopeptide complex engagement, 13
Histoplasma, 180
Histoplasmosis, 179–180, 1226, 1230, 1231, 1685–1686
clinical impact on transplant patients, 1228–1229
clinical manifestations, 1229–1230
diagnosis, 1230–1231
disease epidemiology, 1227–1228
pathogenesis, 1229
prophylaxis, 1232–1233
treatment, 1231–1232
HIV, 182, 187–189
negative donors, 864–865
HIV positive donors, 865
donor screening, 867
history of HIV disease, 866
renal impairment, 867
Hookworm infection
clinical presentation, 1430
diagnosis, 1430
epidemiology, 1428
treatment, 1430
Household contacts, 241
Human adenoviruses (HAdV) infection
clinical presentation, 785–789
diagnosis, 789–794
epidemiology, 784–785

hematopoietic stem cell transplant, 785–786
management, 782
prevention, 796
solid organ transplant, 786–789
transmission mode, 782–784
treatment, 794–796
virology, 782
Human bocavirus (HboV), 1642
Human cosavirus (HCoSV), 1642
Human herpes virus, 1682–1684
prophylaxis, 557
Human herpesvirus-6 (HHV-6), 683
challenges, 691–692
chromosomally-integrated, 684–685
diagnosis and antivirals, 685
infection in hematopoietic stem cell transplant recipients, 685–688
infection in solid organ transplant recipients, 689–692
variants, 684
Human herpesvirus-7 (HHV-7), 692
challenges, 694
infection in hematopoietic stem cell transplant recipients, 692–693
infection in solid organ transplant population, 693
Human herpesvirus-8 (HHV-8), 189
challenges, 699
infection in hematopoietic stem cell transplant recipients, 698–699
infection in solid organ transplant recipients, 695–698
Human immunodeficiency virus (HIV), 937
Human immunodeficiency virus (HIV) positive patients, solid organ transplantation
antiretroviral therapy (ART), 867–868
contraindications, 863–864
donor options, 864–867
immunosuppression choices, 868
kidney, 861–862
liver, 861
pre-operative investigations, 863
prophylactic therapy, 872
recurrence of HIVAN, 869–870
rejection, 868–869
second viral strain, 870–871
vaccination, 872–873
Human macrophages, 1209
Human metapneumovirus, 757–758
Human microbiome, 16
Human papillomavirus (HPV), 1580, 1582
vaccination against, 267
vaccines, 1583, 1711–1712
Human papillomavirus (HPV) infection
anal cancer screening, 845–849
cervical cancer screening, 844–845
epidemiology in SOT and HSCT recipients, 838–844
pathogenesis, 834–838
prevention, 844–852
Human polyomavirus type I (BK virus), 68
Hymenolepis nana, 1438
Hyperammonemia syndrome, 507
Hyperbaric oxygen, 1130
Hyper-infection syndrome, 1283
Hyperkalemia, 1197
Hypnozoites, 1375
Hypogammaglobulinemia, 1483

I

Ibrexafungerp, 1098
Ibrutinib, 33
Idiopathic hyperammonemia syndrome, 1667
Idiopathic pneumonia syndrome, 686, 1625
IgM testing, 551
Imipenem/relebactam, 329, 330
Imipenemase, 317
Imipenem-relebactam, 362
Immune globulin (IG), 883, 887, 895
Immune reconstitution, 45
Immune reconstitution inflammatory syndrome (IRIS), 1168, 1169, 1258
Immunization
of household contacts, 277
with conjugated vaccine, 261
Immunochromatography, 1648
test, 1354
Immunocompromised, 1016, 1017
patients, 1186, 1187, 1190
Immunofluorescence assays, 1647
Immunoglobulin(s), 47, 732, 734–735, 737
Immunohistochemistry (IHC), 1084
Immunomodulatory and immunosuppressive properties of, 690–691
Immunosenescense, 12
Immunosuppression, 17, 549, 659, 1304, 1314, 1322, 1323, 1327, 1328, 1467, 1651
drugs, 1386
therapy(ies), 48, 1131, 1213, 1254, 1259
Impella, 1476
Improved protection against cytomegalovirus in transplantation (IMPACT) study, 556
Indirect fluorescent antibody test (IFAT), 1354
Indirect hemagglutination assay (IHA), 1306

Indirect immunofluorescence assay (IFA), 1306
Induction agents, 17
Infection control
  implementation of, 111
  interventions, 113
  risks, 103
Infection control risk assessment (ICRA), 107
Infectious disease (ID), specialists, 4–8
Infectious Diseases Society of America (IDSA), 1217, 1233, 1354
  guidelines, 1553, 1559
Inflammatory cytokines, 1209
Influenza, 114, 116, 119
  booster dosing, 753
  description, 750
  inactivated high-dose influenza, 753
  post-exposure prophylaxis, 754
  prevention, 752–754
  prophylaxis, 162–163
  treatment, 751–752
  vaccination, 1712
  vaccination against, 255
Influenza infection
  clinical presentation, 712–714
  diagnosis, 715
  hospital admission, 711–712
  ICU admission, 712
  incidence, 710–711
  prophylaxis, 716
  risk factors for mortality, 716
  treatment and outcomes, 715–716
  vaccination, 717–721
Innate immune system, 11, 1209
Interferon gamma (IFN-γ), 589
Interferon gamma release assay (IGRA), 177
Intergovernmental Panel on Climate Change (IPCC), 89
Internal transcribed spacer (ITS), 1118, 1142
International Society for Heart and Lung Transplantation (ISHLT)
  guidelines, 1082
International Society for Human and Animal Mycology (ISHAM) Fungal PCR consortium, 1120
International unit (IU), 911
Intestinal cestodes, 1435–1442
Intestinal parasitic infections in transplant recipients
  Ascaris lumbricoides, 1428
  Balantidium col, 1425
  Blastocystis, 1418
  Capillaria philippinensis, 1435
  cryptosporidiosis, 1407–1408
  Cyclospora, 1410
  Cystoisospora, 1413
  Dientamoeba fragilis, 1423
  Diphyllobothrium latum, 1440
  Dipylidium caninum i, 1442
  E. histolytica, 1421–1422
  Giardia, 1416
  hookworms, 1428–1431
  Hymenolepis nana, 1439
  intestinal helminths, 1425
  soil-transmitted helminths, 1426–1428
  Strongyloides stercoralis, 1433
  Taenia solium, 1438
  Trichuris trichiura, 1433
Intestinal transplant, human adenovirus (HAdV) infection in, 789
Intra-aortic balloon pump (IABP), 1476
Intracranial pressure (ICP), 1158, 1159, 1165, 1166
Intravenous immunoglobulin (IVIG), 734, 882, 894, 895, 897
Invasive aspergillosis (IA), 61, 1086
  antifungal therapies, 1092
  diagnosis of, 1081
  duration of therapy, 1099
  incidence of, 1078, 1079
  molecular and immunological diagnostic tests, 1083
  prevention, 1100–1103
  radiologic imaging, 1089
  risk factors, 1079
  treatment, 1090–1098
Invasive candidiasis, 1046
  diagnostic test performance, 1057
  treatment, 1061
  See also Candida infections
Invasive fungal disease (IFD), 57, 60, 1110
Invasive fungal infections (IFI), 1078, 1081, 1082, 1091, 1097, 1101
Invasive pulmonary aspergillosis (IPA), 62, 1553–1558
Invasive pulmonary mucormycosis, 62
In vitro susceptibility testing, 496
Iron chelators
  deferasirox, 1130
  host immunity and risk factors, 1130
  hyperbaric oxygen, 1130
Isavuconazole, 63, 1080, 1094, 1121, 1124, 1126, 1128, 1131, 1145, 1218, 1232
Ischemic stroke, 1667
Itraconazole, 1050, 1064, 1080, 1094, 1095, 1121, 1145–1147, 1217, 1236, 1242, 1464
Ivermectin, 1285–1286

J
Janus Kinase (JAK) inhibitors, 1190

K
Kaposi sarcoma-associated inflammatory cytokine syndrome, 696
Kaposi's sarcoma, 694
Keratitis, 1145, 1600
Kidney Disease: Improving Global Outcomes (KDIGO) guideline, 717
Kidney transplant, 689, 860, 863, 868, 871, 873, 1256, 1270, 1666
Kissing bugs, 1295

L
Lafoensia pacari, 1170
Laribacter hongkongensis, 1643
Laryngeal transplants, 1524
Latent tuberculosis infection (LTBI), 162, 416–418
Lateral flow assay (LFA), 1161
Lateral flow device (LFD), 1087, 1088
Lateral flow immunoassay (LFIA), 1118
Latex agglutination, 1306, 1648
  test, 1354
LCMV, *see* Lymphocytic choriomeningitis virus infection
Leclercia adecarboxylata, 1642
Legionella pneumophila, 108
Legionella species, 106, 108, 111
Leishmania
  atypical disease manifestations, 1342
  CL (*see* Cutaneous leishmaniasis (CL))
  clinical presentations, 1342
  endemic areas, 1342
  immunocompromised hosts, 1343
  life-threatening diseases, 1342
  parasites, 1608
  VL (*see* Visceral leishmaniasis (VL))
Leishmaniasis, 186
Leisure activities, 243–244
Letermovir, 66, 564, 597–598
Lever of unknown origin, 689
Limbic encephalitis, 687
Liposomal amphotericin B (L-AMB), 59, 60, 1099, 1124, 1131, 1357, 1365
Liposomal inhaled amikacin, 474
Liver transplantation, 689, 863, 1122, 1256, 1270, 1666
  direct-acting antiviral agent regimes after, 957–958
  human adenovirus (HAdV) infection in, 786–787
  yellow fever, 1019–1020
Lomentospora prolificans, 1144
Loop electroexcisional procedure, 844
Lower respiratory tract infection (LRTI), 732
Lumacaftor/ivacaftor, 1505
Lumbar puncture, 1160, 1166, 1168, 1172
Luminex xTAG gastrointestinal panel (Lumitex GPP), 1643
Lung rejection
  acute, 1620
  chronic, 1620–1621
Lung transplant, 400, 689, 726–728, 732–736, 1498, 1506, 1667
  human adenovirus (HAdV) infection in, 788
Lyme disease, 1607
Lymphatic system, 1301
Lymphocytic choriomeningitis virus infection, 1031
  clinical timelines for, 1033
  diagnosis, 1034
  signs and symptoms, 1032
  treatment, 1034
Lymphoid tissue, 1522

M
Macrolides, 1463
Magicplex™ Sepsis Real-time Test, 1549
Magnetic resonance-based methods, bloodstream infections, 1550–1551
Magnetic resonance imaging (MRI), 1114
Magnetic resonance technology, 58
Major histocompatibility complex (MHC), 47
Malaria, 186–187
  asexual and sexual erythrocytic stage, 1374
  diagnosis, 1384
  donor-derived infection, 1374, 1375
  hematopoietic stem cell transplantation (HSCT) guidelines, 1378, 1385
  hypnozoites, 1384
  incubation period, 1375
  infected donors, 1387
  infected recipient, 1387
  preemptive treatment, 1387
  presumptive treatment, 1387
  respiratory distress, 1375
  serology-based screening, 1384
  solid organ transplantation (SOT), 1375
  transfusion-transmitted disease agent, 1384
  transplant recipients, 1386
  treatment, 1385
Malassezia, 1579
Malignancy, 1621–1622

Mammalian target of rapamycin (mTOR) inhibitors, 19, 695
Maribavir, 564, 597
Marijuana, recreational, 243
Matrix assisted laser desorption ionization time of flight (MALDI-ToF), 1142, 1365
Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS), 59, 471, 1118
May-Grünwald-Giemsa (MGG), 1261
Measles, mumps, and rubella (MMR) vaccination, 274, 1711
Measles, 119
Medicare claims, 5
Megacolon, 1304
Megaesophagus, 1304
Melioidosis, 392, 394
Meningitis, 1156–1159, 1161, 1164, 1166, 1168–1170, 1172
Meningococcal disease, vaccination against, 262–263
Meningoencephalitis, 1300
Meropenem/vaborbactam, 329–332, 362
Metabolites, 1536, 1559–1560
Metagenomic next-generation sequencing (mNGS), 794
Metagenomic sequencing
  bloodstream infections, 1551–1552
  CNS infections, 1564–1565
  respiratory infection, 1560–1563
Metallo-β-lactamases, 317, 327, 332
Methicillin resistant *Staphylococcus aureus* (MRSA), 52, 111, 294, 295
MexAB-OprM, 357
Micafungin, 1097
MicroRNA, 1089
Microscopy, 1259
Microsporidia, 1140–1142, 1649
Migration and population shift, 84, 85
Miltefosine, 1147, 1358, 1366, 1463, 1464, 1466
Minimal inhibitory concentration (MIC), 1121, 1538, 1559
Minocycline, 380
Minor histocompatibility antigens, 47
Mixed inhibitory concentration (MIC), 1099
Mold(s)
  Aspergillus polymerase chain reaction, 61
  clinical impact, 60, 61
  culture, 61
  diagnosis, 61–63
  disease epidemiology, 60
  fungal metabolite testing, 63
  galactomannan (GM), 61
  infections, 106, 107
  management, 63–64
  Pneumocystis jiroveci pneumonia (PJP), 64
  prophylaxis, 63
  radiographic studies, 62
  treatment, 63
Molecular diagnostics
  on blood specimen, 1548–1549
  clinical outcomes, in transplant populations, 1549–1550
  on positive blood culture bottles, 1537–1548
Molecular identification techniques, 1120
Molecular tests, 729–731
Monoclonal antibodies, 1087–1088
Montenegro skin test, 1365
Motavizumab, 736
Mouth infection, 1516
mTOR inhibitors, 1621
Mucorales, *see* Mucormycosis
Mucormycosis, 63
  adjunctive treatments, 1130
  antifungal (*see* Antifungal therapy)
  causative pathogens, 1111
  clinical features, 1113
  culture, 1117
  genera and species, 1111
  histopathology, 1116, 1117
  imaging, 1114, 1116
  microscopy-based diagnostic methods, 1117
  molecular-based methods, 1118–1120
  next generation sequencing (NGS), 1120
  risk factors, 1112, 1113
  serology and antigen testing, 1118
  surgery, 1128, 1129
  susceptibility data, 1121
Mucosal infection, 1516–1519
Multi-drug resistant (MDR), 1536
  bacteria, 1488
  gram-negative bacterial infections, 53
  P. aeruginosa, 53
Multi-drug-resistant *Enterobacteriaceae* (MRE), 88
Multi-drug resistant organisms (MDRO), 88, 100, 111, 136, 157, 324, 1496–1497, 1721
  after transplantation, 288
Multiplex gastrointestinal panels, 1645
Multiplex polymerase chain reaction, 1643

CNS infections, 1564
gastrointestinal infections, 1565–1568
respiratory infection, 1560
Muscle infection, 1520–1521
MycAssay, 1554
Mycobacteria, 1687
infections, 177–179, 1607
prophylaxis, 162
Mycobacterium abscessus, 426, 438
infections, 1502–1504
pulmonary infection, 440
soft tissue infection, 441
Mycobacterium avium complex (MAC), 460, 473–475
Mycobacterium chelonae, 441
Mycobacterium chimaera, 475
Mycobacterium fortuitum, 442
Mycobacterium kansasii, 475
Mycobacterium tuberculosis, 162, 410–418, 1585
MycoGENIE assay, 1554, 1559
Mycophenolate mofetil (MMF), 19, 993, 1460
Mycoplasma infections
disease diagnostics and clinical evaluation, 510–511
epidemiology, 505–506
hematopoietic stem cell transplantation, 509–510
kidney-pancreas transplantation, 509
kidney transplantation, 508–509
liver transplantation, 508
lung and lung/heart transplantation, 506–508
management, 511–513
Venn diagram on antibiotic susceptibility of, 512
Myocarditis, 1300

N

Naegleria fowleri
aggressive management, 1466
amphotericin B deoxycholate, 1466
antimicrobial therapy, 1465
cerebral edema and absent ventricles, 1458
cerebrospinal fluid (CSF) studies, 1457
drugs and doses, 1465
ecology and pathobiology, 1454
lung, thyroid, heart and spleen tissue, 1461
primary amebic meningoencephalitis (PAM), 1457
risk factors and incidence, 1454
transplantation, 1460, 1462
National Respiratory and Enteric Virus Surveillance System (NREVSS), 726
Natural killer (NK) cell, 16, 46, 1209
Necator americanus, 1428
Neisseria gonorrhea, 1524
Nephrotoxicity, 68
Nerve infection, 1521–1522
Neuraminidase inhibitor (NAI) therapy, 751
Neuroimaging, 1669
Neutropenia, 46, 50
Neutropenic colitis, 1640
Neutrophil function, 348
New Delhi metallo-β-lactamase, 317, 327, 332
Next generation sequencing (NGS), 1120, 1164
Nifurtimox, 1325, 1326
Nitazoxanide, 1410, 1421, 1657
Nitroxoline, 1467
Nocardia, 1519, 1583, 1585
Nocardia infections
adjunctive surgery, 499
central nervous system manifestations, 492–493
clinical impact, 490–491
cutaneous manifestations, 492
diagnosis of, 493–495
disease diagnostics and clinical evaluation, 491–495
drug toxicity monitoring, 499
duration of therapy, 496–498
empiric therapy, 495–496
graft outcomes, 491
in humans, 488
immunosuppression reduction and immune based therapy, 499
incidence and risk factors in hematopoietic stem cell transplant recipients, 489
incidence and risk factors in solid organ transplant recipients, 489–490
management, 495–500
mortality, 490
oxazolidinone, 500
prevention, 500
routes of, 488
treatment-associated morbidity, 491
Non-*Aspergillus* fungal infections
clinical disease, 1143–1144
diagnosis, 1141–1142
epidemiology, 1140–1141
infection control, 1148
prophylaxis, 1148
therapy, 1144–1148

Non-melanoma skin cancer, 843
Non-relapse mortality (NRM), 4
Non-tuberculous mycobacteria (NTM), 106, 108, 110, 178, 427–429, 1584, 1607
  infections, 162
Norovirus, 1647, 1648, 1655
Nose infection, 1516
Nosocomial transmission, 507
Nucleic acid amplification testing (NAAT), 54, 530, 715, 729, 1645, 1720
Nucleic acid detection, 663–664
Nucleic acid tests (NAT), 549, 865, 930, 995–997, 1001, 1648

O
Obinutuzumab, 28–29
Occupation, 172, 175, 181
  exposures, 242
Ocular infections
  after organ transplantation, 1596
  anatomy, 1596
  bacterial infections, 1606–1607
  fungal infections, 1602–1606
  herpesviruses, 1598–1601
  microbiologic testing, 1598
  parasitic infections, 1608
  transplant recipient, 1598
Ocular toxoplasmosis, 1257, 1259, 1265
  See also Toxoplasma chorioretinitis
OligoG, 403
Opportunistic infection, 863, 864, 871–873
Opportunistic premise plumbing pathogens (OPPP), 108
Oral vancomycin, 55
Organ Procurement and Transplantation Network (OPTN), 87, 930, 1215
Organ procurement organization (OPO), 87, 1518
Organ transplantation, 444, 1145, 1220
Orthotopic liver transplantation, 1596
Oseltamivir, 715, 716
Osteomyelitis, 1143, 1145
Outcome of EBV+ PTLD after HSCT, 662
Oxacillinase (OXA)-48-like β-lactamases, 317
Oxazolidinone, 500

P
Palivizumab, 731, 734–736
Pan American Health Organization (PAHO), 991, 1296, 1355
Panel reactive antibody, 14
Panuveitis, 1597
Paracoccidioidomycosis, 180, 1226, 1231, 1240
  clinical impact in transplant patients, 1238
  diagnosis, 1239–1240
  disease epidemiology, 1237–1238
  pathogenesis and clinical manifestations, 1238–1239
  prophylaxis, 1241–1242
  treatment, 1240–1241
Parainfluenza virus, 758–760
  treatment and prevention, 759–760
Parasite lactate dehydrogenase (pLDH), 1384
Parasitemia, 1309, 1310, 1314
Parasitic infections, 184–187, 1282
  Strongyloides, 69
  toxoplasmosis, 68
Parasitic prophylaxis, 165–167
Paromomycin, 1657
Parvovirus, 1679–1681
Passenger lymphocyte syndrome, 1693–1694
Passive immunoprophylaxis, 673
PCR-based diagnostic methods
  blood and body fluids, 1119
  fresh and formalin fixed tissue, 1118
  mucormycosis, 1119
Pediatric exclusivity (PE), 1721
Pediatric organ transplant recipients, 549
Pediatric transplant recipients
  acute flaccid myelitis (AFM), 1719
  adverse event assessment, limitations in, 1722–1723
  age-specific considerations, 1712–1713
  arboviruses, 1719
  Clostridioides difficile infection (CDI), 1720–1721
  cytomegalovirus (CMV) infection, 1716–1717
  Epstein-Barr virus (EBV) infection, 1717–1718
  EV-D68, 1718–1719
  human papillomavirus (HPV) vaccination, 1711–1712
  influenza vaccination, 1712
  lack of antimicrobial dosing data and indication, 1721
  measles, mumps, and rubella (MMR) vaccination, 1711
  pediatric pharmacokinetics, variations in, 1721–1722
  respiratory viral infection (RVI), 1713–1716
  rotavirus vaccination, 1710–1711
  route of drug administration, limitations in, 1722
  serostatus, determination of, 1719–1720
  varicella vaccination, 1711
Penetrating keratoplasty, 1145
Penicillin allergies, 137

Penile transplants, 1524
Pentamidine, 1463, 1464, 1466
Pentavalent antimonials (PAs), 1357
Peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH), 354
Pericardial effusion, 1300
Periodic acid-Schiff (PAS), 1116
Peri-operative antibacterial prophylaxis, in solid organ transplantation, 156–158
Peripheral nervous system, 1304
Pets, 172, 179
Phaeohyphomycosis, 1140, 1141, 1143, 1146
Phage therapy, 403
Phosphatidylinositol-3-kinase delta (PI3Kδ) inhibitor, 1190
Plaque reduction neutralization test (PRNT), 976, 1013
Plasmodium
  life cycle, 1374
  P. falciparum, 1373, 1385, 1389, 1390
  P. knowlesi, 1374, 1390
  P. malariae, 1374, 1389
  P. ovale, 1374, 1385, 1389
  P. vivax, 1373, 1385, 1389, 1391
Plate method, 1284
Plazomicin, 332, 362
Pleuropulmonary disease, 466
PNA FISH BC assays, 1538, 1539
Pneumococcal disease, 259, 260
Pneumocystis, 1520
  P. carinii, 144, 1184
  P. jirovecii, 64, 1268–1269
Pneumocystis jiroveci pneumonia
  beta-glucan testing, 1195
  clinical findings, 1192–1193
  direct microscopic examination, 1193–1194
  epidemiology and prevention, 1186–1192
  factors predisposing to, 1187
  host/pathogen interactions, 1185–1186
  immunofluorescent staining, 1200
  microbiology, 1184–1185
  molecular methods, 1194
  prevention, 1188
  radiographic manifestations, 1193
  resistance issues, 1198
  response to therapy, 1199
  steroids, 1198–1199
  treatment, 1196–1198
Pneumonia, 578, 579, 1143, 1146, 1155, 1158, 1172, 1617–1619, 1623
Polyclonal antibody therapies, 21
Polyenes, 1097
Polymerase chain reaction (PCR), 791, 792, 1085–1087, 1117, 1142, 1227, 1231, 1236, 1240, 1260, 1262, 1273, 1306, 1459, 1647
  cytomegalovirus (CMV), 583–585
Polymyxins, 139, 379–380
Polyomavirus-associated hemorrhagic cystitis, *see* Hemorrhagic cystitis
Polyomavirus-associated nephropathy
  clinical evaluation and diagnosis, 813–815
  diagnosis and evaluation of, 812
  epidemiology and risk factors, 815
  management, 815–817
  pathology and clinical impact, 808–813
Posaconazole, 63, 1080, 1081, 1092, 1095, 1096, 1100–1102, 1122, 1126, 1128, 1131, 1145, 1146, 1149, 1232, 1325, 1466, 1605
Positron emission tomography (PET), 1089–1090
Posterior reversible encephalopathy syndrome (PRES), 1662
Posterior uveitis, 1597
Post-exposure prophylaxis (PEP), 896, 1031
Post-kala-azar dermal leishmaniasis (PKDL), 1344, 1361
Post-prescriptive review, 136
  and feedback, 146
Post-transplant lymphoproliferative disorder (PTLD), 67, 656, 1600, 1640, 1684–1685, 1717, 1718
  Epstein-Barr virus driven (*see* Epstein-Barr virus driven post-transplant lymphoproliferative disorder)
  histological classification of, 657
Post-transplant malaria, 1374, 1375
Post-transplant management
  positive donor/recipient, 1322, 1323
  preemptive therapy, 1324
  seronegative recipients, 1323
  seropositive recipients, 1324
Preauthorization, 136
Prednisone, 20, 1199, 1460
Pre-emptive management approach, 67
Preemptive therapy, 556
Preexposure prophylaxis, 888
Pre-prescription authorization, 136
Presatovir, 736, 757
Pre-transplant consultation, 7
Pre-transplant evaluation, 240, 248
Pre-transplant risk assessment
  endemic and non-endemic areas, 1320
  lysates, 1320
  positive donor, 1320, 1321
  positive recipients, 1321

Pre-transplant screening, 172
Aspergillus and molds, 183–184
bacterial infections, 172–177
blastomycosis, 180
bloodborne pathogens, 187–188
Chagas disease, 184–186
Chikungunya virus (CHIKV), 191
coccidioidomycosis, 180–182
cryptococcosis, 182–183
dengue virus (DENV), 191
endemic human herpes viruses, 188–189
HHV-8, 189
histoplasmosis, 179–180
HTLV-1, 189–190
leishmaniasis, 186
M. abscessus, 178
malaria, 186–187
non-M.*abscessus* NTM, 178–179
non-tuberculous mycobacteria (NTM), 178
paracoccidioidomycosis, 180
schistosomiasis, 186
strongyloidiasis, 186
toxoplasmosis, 187
tuberculosis, 177–178
West Nile virus (WNV), 190
Zika virus (ZIKV), 191
Preventative health, 249
Primaquine, 1389, 1391
Primary amebic meningoencephalitis (PAM), 1452, 1454, 1457, 1458
Primary effusion lymphoma, 695
Primary sclerosing cholangitis, 347
Probable disease, 785
Progressive outer retinal necrosis (PORN), 1596, 1600
Progressive outer variant, 1600
Prophylactic therapy, 872
Prophylaxis, 534, 689, 716
antibiotic, 158–159
antifungal, 159–162
antiviral for HSV and VZV, 642
BK polyomavirus, 165
cytomegalovirus (CMV), 163–165, 595–599
herpes simplex virus and varicella zoster virus, 163
influenza, 162–163
mycobacterial, 162
parasitic, 165–167
peri-operative antibacterial, 156–158
post-transplant post-exposure, 639
Prospective antifungal therapy (PATH) alliance, 1055
Prospective audit and feedback (PAF), 136
Protective environment (PE), 106
Protist, 1453
Proton pump inhibitors, 521
Protozoa, 1399
Pseudomonas aeruginosa, 158, 299–306
Pseudomonas aeruginosa infection
antimicrobial resistance, 354
aztreonam-avibactam, 362
cefiderocol, 361
ceftazidime-avibactam, 360–361
ceftolozane-tazobactam, 360
environmental factors in transplant recipients, 353
epidemiology, 344–352
fosfomycin, 363
hematopoietic stem cell transplantation, 349–352
imipenem-relebactam, 362
meropenem-vaborbactam, 362
microbiology, 352–353
pathogenesis of infection, 352–354
plazomicin, 362
solid organ transplant recipients, 344–349
Public Health Agency of Canada (PHAC), 730
Pulmonary aspergillosis, 1114
Pulmonary syndromes
chest imaging, 1630
diagnostic evaluation, 1627–1628
empiric antimicrobial therapy, 1631–1632
infectious syndromes in HSCT, 1622–1625
infectious syndromes in SOT, 1616–1619
noninfectious syndromes in HSCT, 1625–1626
noninfectious syndromes in SOT, 1619–1622
patient evaluation, 1626–1631
Pulmonary toxoplasmosis, 1257, 1260
Punicalagin, 1170
Pyridoxine, 414
Pyrimethamine, 1463
Pyrimethamine-sulfadiazine (PYR/SDZ), 1265, 1272

Q

Qualitative-PCR, 1306
Quantiferon-CMV assay, 555
Quantitative nucleic acid testing (QNAT), CMV, 583–585
Quantitative PCR (qPCR), 1142, 1308
QuickFISH BC assays, 1538, 1539
Quinine dihydrochloride, 1390
Quinine sulfate, 1391
Quinopristin/dalfopristin, 294

R
Rabies virus infection, 1028
diagnosis, 1030
signs and symptoms, 1029–1030
treatment, 1031
Radioimmunoprecipitation assay (RIPA), 1306
Rapid diagnostics tests (RDTs), 51, 1384
Rapidly-growing mycobacteria (RGM) infections, 426
antibiotics, 437
antimicrobial susceptibilities for, 436
clinical impact in transplant, 448–449
community and healthcare facility surveys and outbreak investigations, 430
diagnosis, 433–435
epidemiology, 426–433
management, 435–448
types, 428
Recipients
post-transplant surveillance, 1322, 1323
pre-transplant treatment, 1321
seronegative prophylaxis, 1323
seropositive prophylaxis, 1324
Recombinant antigen methods, 1306
Recreational water, 243
Rehydration, 1651
Renal transplant, human adenovirus (HAdV) infection in, 787–788
Renal transplant recipients (RTRs), 1578
RenDx Fungiplex assay, 1558
Respiratory infections, emerging diagnostics, 1552
Aspergillus polymerase chain reaction, 1553–1559
gas-chromatography mass spectrometry, of exhaled metabolites, 1559–1560
metagenomic sequencing, 1560–1563
multiplex polymerase chain reaction, 1560
Respiratory syncytial virus (RSV), 113, 115, 754
infection management, 144, 755–757
prevention, 757
Respiratory syncytial virus (RSV), in transplant
ALN-RSV01, 735
ALS-8112, 736
ALS-8176, 736
clinical impact, 728–729
disease diagnostics and clinical evaluation, 729–730
disease epidemiology, 726–728
immunoglobulin, 734–735
infection control, 730–731
JNJ-53718678, 736
PC786, 736
presatovir, 736
ribavirin, 732–734
vaccine, 731–732
Respiratory tract infection (RTI), 144
Respiratory viral infection (RVI), pediatric transplant recipients
hematopoietic stem cell transplantation (HSCT), 1714–1715
preventative strategies, 1715–1716
solid organ transplantation (SOT), 1713–1714
Respiratory viral pathogens in transplant recipients
adenovirus, 761–763
bocavirus, 763
clinical presentations, 746–747
coronavirus, 760–761
diagnostic overview, 748–750
disease progression and complications, 747–748
donor and recipient issues, 765–766
epidemiology, 745
infection control practices, 763–766
influenza, 750–754
parainfluenza virus, 758–760
rhinovirus, 760
syncytial virus, 754–757
uman metapneumovirus, 757–758
Respiratory viruses, 113–114
Reticuloendothelial system, 1344
Retinitis, 578, 580, 1597, 1599
Retinochoroiditis, 1261
Reverse transcriptase polymerase chain reaction (RT-PCR), 882–884, 1013, 1648
Rezafungin, 1097
Rhinocerebral mucormycosis, 1605
Rhino-orbital-cerebral mucormycosis (ROCM), 1113, 1128, 1129
Rhinovirus, 760
Ribavirin, 144, 732–734, 736, 756, 914, 916–918, 920
for human adenovirus (HAdV) infection, 794
Ribosomal DNA, 1142
Rifabutin, 472
Rifampin, 415, 472, 1464, 1466
Rifamycins, 1463
Rituximab, 28, 668
Roche SeptiFast assay, 1558
Roseolovirus, 684, 692

Rotavirus, 1648, 1655
vaccination, 1710–1711
Ruxolitinib, 32, 65

S
Safe living, 242
Safe water, 245
Salivary glands, 1522
Sapovirus, 1648
Scedosporium
clinical impact in transplant, 1499
diagnosis and clinical evaluation for, 1500–1501
epidemiology, 1499
Lomentospora prolificans, 1502
management, 1501
Scedosporium apiospermum, 1501
Scedosporium spp., 1140–1142, 1144–1146
Schistosomiasis, 186
Screening HHV-8 antibodies, 697
Secondary prophylaxis, 1328
Seizures, 1664
Sensitivity, 1538, 1549–1554, 1558–1560, 1564, 1565, 1569
SepsiTest, 1548
Septic arthritis, 1143, 1145
Sequence-based identification, 471
Serological tests, 663, 1649
conventional methods, 1306
non-conventional methods, 1306
Seropositive organ donors, 961
Seroprevalence, 695
Sexual health, 244–245
Shiga-like toxin producing *E. coli* (STEC), 1647
Sinus, 1516
Sinusoidal obstruction syndrome (SOS), 891
Skin infection, 1519–1520
Skin lesions, 466
Slow growing nontuberculous mycobacteria infections in transplant recipients
advantages and disadvantages of molecular diagnostics for, 471
antibiotics in treatment of, 476–478
and associated clinical syndromes, 461
clinical impact, 464–466
clinical manifestations, 466–468
diagnosis, 468–472
duration of therapy, 476–478
epidemiology, 460–464
incidence, 462
M. chimaera, 475
M. kansasii, 475
management principles, 472–473
mode of transmission, 460–462
Mycobacterium avium complex (MAC), 473–475
prevention, 478–479
risk factors, 463–464
timing of infection, 463
Sofosbuvir, 918
Solid organ transplantation (SOT), 132, 253, 1015–1017, 1021, 1110, 1209, 1213, 1214, 1254, 1256, 1257, 1270, 1283, 1287, 1315, 1343, 1375, 1452, 1639, 1640, 1713–1714
acute Chagas disease (ChD), 1312, 1313
antiviral medication, 641
Chagas disease (ChD) reactivation, 1315, 1317
clinical impact, 290
clinical implications, 296, 297
cryptococcosis, 1157
disease epidemiology, 295
donors, 1320, 1321
EBV infection and PTLD, 657–658
epidemiology, 289–290
human adenovirus (HAdV) infection, 785–789
outcome of PTLD after, 662
patients, 5
post-transplant immunization, 638
pre-transplant immunization, 637
recombinant zoster vaccine, 644
respiratory viral pathogens (*see* Respiratory viral pathogens in transplant recipients)
risk factors for PTLD after, 658–660
treatment for EBV disease and PTLD in, 668–670
varicella zoster virus (VZV) immunization in seronegative, 637
varicella zoster virus (VZV) immunization in seropositive, 644
Solid organ transplant recipients (SOTRs), 1582, 1586
altered mental status (*see* Altered mental status)
Aspergillus in (*see Aspergillus* spp.)
blastomycosis (*see* Blastomycosis)
histoplasmosis (*see* Histoplasmosis)
paracoccidioidomycosis (*see* Paracoccidioidomycosis)
posttransplant HAV vaccination, 892–893

pretransplant HAV vaccination, 890–891
Solid organ transplant (SOT) recipients, influenza infection
adjuvanted vaccine, 719
fever, 713
graft dysfunction, 714
high dose vaccination, 718
hospital admission, 712
ICU admission, 712
immunosuppressive medications, 716
incidence, 710–711
intradermal *vs*. intramuscular vaccine, 718–719
lower respiratory tract infection, 713
prophylaxis, 716
repeated vaccination, 718
vaccination side effects, 719
Squamous intraepithelial lesions (SIL), 839–841
Staphylococcus aureus, 1518–1520, 1583
bacteremia, 4, 6
Stem cell transplantation
cryptococcosis, 1158
disease epidemiology, 296
Stem cell transplant recipients, 1452
posttransplant HAV vaccination, 893–895
pretransplant HAV vaccination, 891–892
Stool antigen detection, 1400
Streptococcus, 1518, 1519, 1521
S. pneumoniae, 159
Strongyloides, 69, 173, 175
S. stercoralis, 1433
Strongyloides stercoralis infection, *see* Strongyloidiasis
Strongyloidiasis, 69, 186, 1281, 1650
clinical impact on transplant, 1282–1283
diagnosis, 1283
epidemiology, 1282
management, 1285–1288
prevention, 1287
Strout method, 1306
Subcutaneous abscess, 1147
Sulbactam, 380
Sulfadiazine, 1463, 1464, 1466
Sulfhydryl variable (SHV), 314, 317, 329
Superficial infections, 1145
Surgical antibacterial prophylaxis (SAP), 156
Surgical site infections (SSI), 90, 105–106, 156, 506, 507
Susceptibility testing, 1501
Sustained virological response (SVR), 917, 918, 952
Syphilis, 1526

T
Tacrolimus, 1460
Taenia saginata, 1435
Taenia solium, 1436–1438
Tafenoquine, 1385
TandemHeart, 1477
Targeted antimicrobial therapy, 1651, 1656
T-cell(s), 46, 47
depleting therapy, 65
mediated rejection, 14
Tedizolid, 474
TEM, 314, 329
Tenofovir alafenamide (TAF), 935
Tenofovir disoproxil fumarate (TDF), 935
Terbinafine, 1147
Tezacaftor/ivacaftor, 1506
Therapeutic drug monitoring (TDM), 18, 1064–1065, 1128
Thermal desorption-gas chromatography/mass spectrometry technology, 63
Thrombotic microangiopathy, 1691–1692
Tigecycline, 381
T-lymphocytes, 15
Toll-like receptors (TLRs), 15
Torovirus, 1642
Toxoplasma, 1520, 1524, 1525
infection, 1262, 1272, 1273
T. gondii, 68, 167, 1254, 1608
Toxoplasma chorioretinitis, 1608
Toxoplasma-seronegative recipients, 1264
Toxoplasma-seropositive recipients, 1259, 1262, 1263, 1273
Toxoplasmosis, 68, 187, 1599
anti-*Toxoplasma* therapy (*see* Treatment)
clinical signs, 1257, 1258
factors, 1254
follow-up, 1273, 1274
histopathologic examination, 1260
hematopoietic stem cell transplantation (HSCT), 1256
hygienic recommendations, 1272
imaging, 1261
parasite detection, 1259, 1260
patient management, 1262, 1263
patients counseling, 1272
prophylaxis, 166
reactivation infection, 1254, 1259, 1271
serologic screening, 1268–1273
serology (IgG, IgM), 1259, 1262, 1268
solid organ transplant (SOT), 1256, 1257, 1270
treatment, 1264–1268
Transfusion-malaria transmission, 1384

Transfusion-transmitted Chagas disease, 1296
Transfusion-transmitted (TT) dengue, 989
Transfusion-transmitted leishmaniasis (TTL), 1355
Transmission
  donor-derived, 1028
  Eastern Equine Encephalitis virus (EEEV) encephalitis, 1035
  prevention of donor association, 1036–1038
  rabies, 1029
  zoonotic, 1036
TRANSNET surveillance system, 57
Transplant-associated cytopenias
  adenovirus, 1681–1682
  autoimmune cytopenia, 1692–1693
  cytomegalovirus, 1678–1679
  differential diagnosis, 1696–1697
  drugs, 1687–1690
  EBV and post-transplant lymphproliferative disorder, 1684–1685
  graft failure, 1695–1696
  graft versus host disease, 1694
  hemophagocytic lymphohistiocytosis, 1690
  HHV-8, 1683–1684
  histoplasmosis, 1685–1686
  human herpes viruses 6 and 7, 1682–1683
  infectious etiologies, 1678–1687
  management, 1698–1699
  mycobacterial infections, 1687
  parvovirus, 1679–1681
  passenger lymphocyte syndrome, 1693–1694
  thrombotic microangiopathy, 1691–1692
Transplant-Associated Infection Surveillance Network (TRANSNET), 1078, 1228, 1230, 1234
Transplant infectious diseases, specialists, 3–8
Transplant tourism
  definition, 202
  and infectious complications, 202–204
Travel safety, 248
Triazoles, 139, 140, 1126, 1215
Trichomonas, 1524
Trichophyton rubrum, 1519
Trichuris trichiura, 1431–1433
Trimethoprim-sulfamethoxazole (TMP-SMX), 64, 158, 490, 491, 1190, 1197, 1258, 1265, 1272, 1464, 1499, 1586, 1617
Tropheryma whipplei, 1607
Trophozoite(s), 1185, 1454
Trypanosoma cruzi (*T. cruzi*), 166, 184, 1294
  amastigotes, 1323
  blood donors, 1298
  Chagas disease (ChD) reactivation, 1310, 1314–1317
  conventional serologic assays, 1306
  discrete typing units (DTUs), 1308
  donor-to-recipient transmission, 1294
  myocardial infection, 1301
  oral transmission, 1297
  parasite life cycle, 1299
  peripheral blood samples, 1308
  polymerase chain reaction (PCR) tests, 1308
  prevalence, 1296, 1297
  prophylaxis, 1324
  solid organ transplant (SOT) recipients, 1312
  strains, 1299
  transmission rates, 1312
  triatomine species, 1295
  trypamastigotes, 1314
  vector transmission, 1296
Trypomastigote(s), 1299
Trypomastigote excretory-secretory antigen (TESA), 1306
T-SPOT.CMV, 554
T-Track CMV assay, 554
Tuberculin skin test (TST), 177
Tuberculosis (TB), 177–178, 1607
  active, 414–416
  diagnosis, 412
  epidemiology, 410–411
  latent, 416–418
  mortality, 411
  screening, 412–414
  symptoms of, 411
  urine and serum tests, 414
Tumor necrosis factor α (TNF-α), 1208
Typical bacteria, 1606

U
Umbilical cord blood (UCB), 48
Umbilical cord blood transplantation (UCBT), 48, 49
Umbilical cord transplant, 686
Upper airways infection, 1516
Upper respiratory tract infection (URTI), 733
Ureaplasma infections, *see Mycoplasma* infections
Urinary antigen detection, 1088
Urinary tract infections (UTI), 143–145, 158, 345
Urogenital infections, 506
Urothelial cancers, 823–824
US Acute Liver Failure (US ALF) Study Group, 886

US stem cell transplant recipients, 4
US TRANSNET surveillance study, 1111
Uterine transplants, 1517, 1524

V
Vaccination, 7, 69, 114, 115, 118, 119, 241
  cytomegalovirus (CMV), 604–605
  hepatitis B virus (HBV), 936
  yellow fever (YF), 1014–1018
Vaginal infection, 1518–1519
Valacyclovir, 163, 596–597, 632
Valganciclovir, 595–596
Valganciclovir prophylaxis, 693
Valley fever, 1208
Value, 5–8
Vancomycin, 140, 291, 1656
  febrile Neutropenia, 142
Vancomycin resistant *Enterococcus* (VRE), 52, 111, 288–289
Varicella vaccination, 1711
Varicella zoster virus (VZV), 64, 66, 118, 188, 1521, 1580, 1581, 1599
  acyclovir shortage, 647
  antiviral prophylaxis after transplant, 642
  clinical impact in transplant, 624–625
  clinical issues and risk factors for infections in transplant patients, 624
  disease diagnostics and clinical evaluation, 628–630
  disease epidemiology, 621–623
  immunization in seronegative SOT and HSCT, 637, 644
  infection control, 646
  laboratory methods for diagnosis of, 629–630
  post-transplant post-exposure prophylaxis, 639–640
  in pregnant transplant recipients, 647
  prevention of reactivation, 640
  primary, 269
  primary infection prevention, 636–637
  reactivation, 625
  recommendations for treatment in transplant patients, 634
  recurrent, 270
  treatment, 634
  vaccination, 643
  vaccination against, 269–271
Vascular anastomoses, 347
Vascularized composite allograft (VCA) transplants
  environmental exposures, 1527
  graft preparation, 1515, 1516
  immunosuppression, 1514–1515
  organ-specific infections, 1522–1524
  perioperative antibiotics, 1515
  rejection, 1515
  safe living practices, 1527
  systemic infections, 1525–1526
  tissue-specific infection, 1516–1522
  vaccinations, 1526
VCA transplants, *see* Vascularized composite allograft (VCA) transplants
Vector-borne disease, 1385
Vector-borne infections, 980
Veno-arterial extracorporeal membrane oxygenator, 1476
Ventilator-associated pneumonia (VAP), 379
Ventricular assist device (VAD), 174, 1477
  cerebrovascular accident, 1489
  clinical outcomes, 1485–1486
  incidence of infections, 1480–1481
  infection definitions, 1479
  management of infection, 1486–1488
  microbiology, 1481
  post-heart transplant infection, 1489
  prevention strategies for infecion, 1484–1485
  risk factors for infections, 1482–1484
Verigene enteric pathogens (Verigene EP), 1643
Verigene GN assay, 1542, 1547
Verigene GP assay, 1541, 1547
Verigene gram-negative blood culture (BC-GN) assay, 354
Verona integron-encoded metallo-β-lactamase, 317
Viral infections
  adenovirus (AdV), 67, 68
  BK virus, 68
  cytomegalovirus (CMV), 65–67
  Epstein-Barr virus (EBV), 67
  herpes simplex virus (HSV), 64
  varicella zoster virus (VZV), 64
Viral prophylaxis, 557
Viremia, 581, 1655
Visceral leishmaniasis (VL)
  diagnosis, 1353, 1354, 1356
  disease endemicity, 1344
  disease epidemiology, 1343, 1344
  donor transmission, 1355, 1356
  geographical distribution, 1344
  haematological parameters, 1359
  hematopoietic stem cell transplantation (HSCT) recipients, 1344, 1359
  recipient infection and reactivation, 1356, 1357
  secondary prophylaxis, 1358

Visceral leishmaniasis (VL) (*cont.*)
serological techniques, 1354
solid organ transplant (SOT) recipients, 1344, 1359
transplant recipients, 1345–1347, 1349, 1351, 1352
treatment, 1357, 1358
Volatile organic compounds, 1088
Voriconazole, 63, 1060, 1065, 1080, 1081, 1090–1096, 1098–1103, 1145–1149, 1232, 1464, 1580, 1587

W
Waterborne pathogens, 107, 108, 110, 111
Water safety, 245–247
Western blot, 1259, 1269
West Nile virus (WNV), 190
West Nile virus infection
blood donor screening, 979
diagnostic considerations and laboratory testing, 975–977
disease manifestation, 977–978
environmental prevention, 981
epidemiology, 971–974
management, 977–978
organ donor screening, 979–981
preventive strategies, 978–981
Whipple's disease, 1607
Whole genome next-generation sequencing (NGS) technologies, 472
World Health Organization (WHO), 1294, 1343
malaria terminology, 1388
Wound infections, 506–508

X
Xenodiagnosis, 1306, 1309
Xpert MRSA/SA blood culture assay, 1538

Y
Yeasts
blood cultures, 58
C. auris-specific diagnostics, 59
diagnosis, 58–59
disease epidemiology, 57, 58
fungal antigen testing, 58
management, 59–60
nanodiagnostics, 58
Yellow fever (YF), 1016
clinical manifestations, 1012–1013
diagnosis, 1013–1014
epidemiology, 1011–1012
hematological malignancies and HSCT candidates, 1017–1018
kidney transplant candidates, 1018
liver transplantation, 1019–1020
liver transplant candidates, 1018–1019
prevention, 1014–1016

Z
Zanamivir, 715
Ziehl-Neelsen staining, 1649
Zika virus (ZIKV), 191
blood supply safety, 1001
clinical impact on transplantation, 995
diagnosis, 996–997
disease epidemiology, 991–992
management, 998
nucleic acid tests (NAT) screening policy, 1001
vaccine, 1000
vector control, 999
Zoster ophthalmicus, 1600
Zygomycosis, 1110