Cytomegalovirus in immunocompromised children

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Purpose of review
The purpose of this study is to explore the latest developments in the risk factors, prevention and treatment of cytomegalovirus (CMV) infection in immunocompromised children, including those with congenital immunodeficiency or iatrogenic immune suppression related to solid organ transplantation (SOT) or haematopoietic cell transplantation (HCT).

Recent findings
CMV viral load measurements now have international standards, allowing for more reliable comparison across sites and within individuals. Preemptive and prophylactic therapy with routine CMV monitoring in transplant patients has yielded significant reduction in CMV morbidity and mortality in these patients. The majority of U.S. states have adopted routine newborn screening for severe combined immunodeficiency (SCID). Viral infections, including CMV, are a major obstacle preventing optimal curative transplantation in these patients. Several new antiviral agents are currently being investigated for CMV infection in immunocompromised patients. Knowledge on CMV drug resistance in children is emerging and requires further study.

Summary
Conditions that diminish cell-mediated immunity impact the development of CMV infection and disease. These conditions include certain congenital immunodeficiencies and SOT and HCT. Infants identified as having SCID should be screened for CMV risk factors. A preemptive or prophylactic strategy should be chosen for CMV management in children who are high risk posttransplantation. In those who develop disease, viral loads should be monitored and resistance testing considered if response is not deemed adequate. Oral valganciclovir is being used as an alternative to ganciclovir in children, although pharmacokinetic data are limited. Other oral antiviral agents under development are promising future options for paediatric CMV therapy.

Keywords
antiviral resistance, antiviral therapy, cytomegalovirus, paediatric, severe combined immunodeficiency

INTRODUCTION

Cytomegalovirus (CMV) infection causes significant morbidity and mortality in immunocompromised children. Infections can be primary or occur as a result of reactivation of latent virus. Compromised cell-mediated immunity and lymphocyte function, due to haematopoietic cell transplantation (HCT), solid organ transplantation (SOT) or immunodeficiency, result in increased vulnerability to severe viral infections. New concepts in prevention and treatment of CMV infection in these hosts will be discussed in this review. Children vary significantly from adults in their immune response, underlying diagnoses leading to transplants and pharmacokinetic response to antiviral therapy. These differences must be considered in the treatment of paediatric CMV. This review will not cover congenital CMV, infection in premature infants or HIV-positive children.

LABORATORY METHODS

Diagnostic tests for CMV include culture, rapid viral assays, serology, histopathology, pp65 antigen detection and PCR. CMV in infected tissues or secretions can be detected by isolation in human fibroblasts. Rapid or shell vial assays combining culture and antigen detection can speed the time to detection of CMV to 2–3 days [1]. Serology is useful in determining the risk of CMV infection posttransplantation, but may be difficult to

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KEY POINTS

- Paediatric data are limited for almost all aspects of CMV diagnosis, prevention and treatment.
- With the advent of early diagnosis of SCID, prevention of CMV infection in these highly vulnerable infants is vital for timeliness and efficacy of curative transplantation.
- Further study of the dosing of oral valganciclovir is warranted, especially in young children.
- Resistance should be suspected in patients with prolonged antiviral exposure and assessed by evaluation for UL97 and UL54 mutations. Sequence variation must be interpreted in combination with available genotype information.
- New antiviral agents need to be studied in children urgently to provide options for those whom resistance to or toxicity with currently available agent prevents optimal therapy.

interpret in the setting of transfusion or immunosuppression. IgG positivity may represent the presence of maternal antibodies in children less than 12 months old [2▪]. Histopathology is useful for confirming CMV organ disease but generally requires an invasive procedure. The pp65 antigen assay has been used frequently in the setting of transplant as a marker of CMV infection. Blood volume requirements, the technical expertise required and the inability to perform the assay in the setting of leukopenia limit its utility and this technique has often been replaced by nucleic acid-based technologies [3].

Quantitative PCR has become a widespread tool for monitoring CMV infection and predicting disease in immunocompromised patients. Until recently, PCR assays varied from centre to centre, limiting the ability to compare results. In 2010, the WHO adopted an international standard for CMV nucleic acid amplification quantification [4]. This allows more uniform representation of viral loads, advantageous for both clinical and research purposes.

CYTOMEGALOVIRUS AND PRIMARY IMMUNODEFICIENCY

CMV transmission to neonates occurs via the transplacental route, oral (p.o.) and/or cervical secretions, and breast milk. Transplacental transmission can result in congenital CMV. Although postnatal transmission of CMV can result in significant disease in premature infants, it is generally not problematic in otherwise healthy term infants, given the presence of maternal antibody. Children with primary immunodeficiencies, specifically those with severe combined immunodeficiency (SCID), are at a substantial risk of morbidity and mortality due to CMV disease. Several past studies demonstrated that CMV-seropositive mothers have high rates of reactivation in their breast milk with viral detection of up to 40% and infant infection up to 70%; the presence of CMV in breast milk may be intermittent and therefore a single screening is not sufficient to determine the potential for transmission [5,6]. A recent U.S. prospective multicentre study of CMV transmission in very low birth weight infants found maternal seroprevalence to be 76.2%. The cumulative incidence of CMV infection in these infants by 12 weeks of age was 6.9%. Of these, 27 out of 28 babies were fed CMV-positive breast milk [7].

The development of TREC (T-cell recombinant excision circle) screening has been beneficial in diagnosing congenital immunodeficiencies characterized by low or absent lymphocyte counts, including SCID. This rapid and relatively inexpensive test detects whether normal numbers of T cells are present (although functionality is not assessed) and can be performed on dried blood spots already collected from newborns for the detection of other congenital diseases. This test is now being performed or will begin soon in 42 U.S. states [8]. This allows the opportunity for early diagnosis and treatment of this otherwise fatal condition.

Pai *et al.* [9▪] retrospectively reviewed 240 infants with SCID who received HCT; 7% were diagnosed with CMV infection. Five-year survival in infants aged more than 3.5 months with active infection at the time of transplant was only 50 compared with 90% of those who had no history of infection; even resolved infection was detrimental (82% survival). Infants aged less than 3.5 months without infection had the highest probability of survival (94%). Although bacteria and *Pneumocystis jirovecii* were the most common causes of infection, viral infections were more likely to be active at the time of transplant. In the setting of SCID and active viral infection, the authors recommend consideration of HCT performed without conditioning [9▪].

As the presence of infection can delay HCT and predicts poor outcome, prevention of CMV in children with SCID is of paramount importance. Treatment of active infection is likely more complicated in this population, as they appear to be at a high risk for the development of resistance during antiviral therapy [10]. More data on optimal treatment of CMV in SCID are necessary. Discontinuation of breastfeeding to prevent CMV transmission should be a consideration. Pasteurization is logistically challenging and expensive, but this approach may

be an option for families that wish to continue breast milk feeding.

CYTOMEGALOVIRUS IN PAEDIATRIC TRANSPLANT PATIENTS

CMV disease can take many forms, including pneumonitis, hepatitis, encephalitis, colitis, cytopenias and retinitis. In the current era, the presentation is often asymptomatic or nonspecific. Active CMV infection can contribute to graft rejection and GVHD via the virus' own effect on T-cell function. Data on these latter, indirect effects of the virus are much more limited in children than adults [2▪]. One recent study [11] demonstrated a surprisingly frequent rate of CMV retinitis in paediatric HCT patients, suggesting that routine ophthalmologic screening may be beneficial particularly in those with primary immunodeficiencies who have pretransplant viremia.

Nonuniform definitions of infection, monitoring schedules and approaches to treatment pose a challenge to CMV management in paediatric transplant patients. CMV can be detected as a result of reactivation of latent disease, primary infection or reinfection with a new strain. Children are at an increased risk for primary infection, as they are more likely to be CMV-naive at the time of transplantation. In HCT, the patients at highest risk to develop CMV disease are those who are donor−/recipient+ (D−/R+), leading to a scenario in which the recipient's latent CMV can no longer be controlled by a depleted immune system and yet do not receive any protection from experienced donor T cells [12,13]. In contrast, D+/R− patients undergoing SOT may be more likely to develop CMV-related complications from a primary infection [14–17].

Several approaches have emerged to manage CMV disease in transplant patients. The preemptive strategy involves frequent monitoring of CMV viral load and treating if and when that value reaches a certain threshold. The prophylactic strategy, on the contrary, recommends presumptive therapy of all patients for a certain time period. A hybrid strategy combines these approaches by administering prophylaxis for 2–12 weeks followed by viral load monitoring. All three strategies seem to have comparable efficacy on the basis of retrospective data [2▪]. Antiviral prophylaxis for SOT consists of either intravenous (i.v.) ganciclovir or p.o. valganciclovir; the duration of prophylaxis varies between centres and with the type of organ transplanted. The prophylactic approach proves somewhat difficult in the HCT population prior to engraftment given the myelotoxicity of ganciclovir. If ongoing prophylaxis is chosen, an alternative agent such as acyclovir, foscarnet or cidofivir may be considered. With the widespread utilization of these strategies, morbidity and mortality related to CMV in posttransplant patients have declined.

A European survey of paediatric HCT centres revealed that all 56 respondents were using a preemptive strategy. Twenty-one out of 56 centres were also using prophylactic measures for either all or only high-risk patients, as defined by D/R serostatus. Quantitative PCR was the most common monitoring tool, with only four out of 56 sites using pp65 antigenemia alone. Prophylactic agents were varied and included acyclovir, ganciclovir and foscarnet. The vast majority used ganciclovir as their first-line agent of choice for therapy. There was no uniform strategy for detecting resistance, second-line therapy or other therapies such as directed T cells [18]. A cost-effective analysis of preemptive screening for CMV and Epstein–Barr virus in paediatric HCT patients found a significant cost saving compared with deferring treatment until symptoms emerged, although there was no comparison to a prophylactic approach [19].

There is increasing recognition of late-onset CMV disease following the completion of prophylaxis. Late CMV infection and disease are independent predictors of death after HCT [20]. Boeckh *et al.* [20] recently published a multicentre randomized controlled trial of valganciclovir prophylaxis compared with PCR-guided preemptive therapy for the prevention of late-onset CMV infection in adult HCT recipients. Patients were randomized at 3 months posttransplant and prophylaxis was administered for 6 months. A viral load threshold of 1000 copies/ml or greater than five-fold increase over baseline was used in the preemptive therapy group. There was no difference between the groups by 640 days after HCT in death, CMV disease or other invasive infection. The incidence of CMV viremia was significantly reduced in the prophylaxis group; however, they did require more haematopoietic growth factors. The researchers concluded that both prolonged prophylaxis and preemptive strategies were useful in the prevention of late complications from CMV in HCT patients [21▪].

ANTIVIRAL AGENTS FOR THE TREATMENT OF CYTOMEGALOVIRUS

Antiviral prophylaxis and treatment options for CMV remain limited. Ganciclovir has been used primarily. Foscarnet and cidofivir also have activity, but their use is limited by toxicity. The advent of the pro-drug valganciclovir has allowed for a p.o. alternative. In adults, the efficacy of valganciclovir

for prophylaxis has been well documented [22,23]. It has also been demonstrated as noninferior to ganciclovir for CMV disease in adult SOT patients [23,24]. No direct comparison between i.v. ganciclovir and p.o. valganciclovir treatment in controlled paediatric studies has been conducted, but pharmacokinetic data suggest that p.o. valganciclovir can reach a similar AUC as i.v. ganciclovir in neonates with congenital CMV with appropriate dosing [25]. Prolonged p.o. valganciclovir therapy for up to 6 months appears well tolerated and effective in infants with congenital CMV at a dose of 16 mg/kg given twice daily [26▪].

Valganciclovir dosing strategies for older infants and toddlers are not always reliable, remain somewhat controversial and typically involve a combination of renal function and body surface area or weight. A commonly used dosing algorithm developed by Pescovitzis was derived from a study of valganciclovir for CMV prophylaxis in paediatric SOT patients aged 4 months to 16 years. The formula suggests a dose seven times the body surface area (m^2) x creatinine clearance (CrCl) in mg/day. Maximum value for CrCl is 150 ml/min/1.73 m^2 and for daily dose is 900 mg [23,27,28]. Villaneuve *et al.* [29] recommended an alternate weight-based dosing approach to better standardize plasma exposures in infants and toddlers. They start with a dose of 14–16 mg/kg once daily for prophylaxis and twice daily for treatment. Doses are adjusted for CrCl less than 60 [29]. Asberg *et al.* [30▪] compared these algorithms and found that the Pescovitz formula overdoses almost all young children and underdoses older children. They suggest the Villanueve model performs better for young children but then propose their own calculation [30▪].

Given the complexity that exists, therapeutic drug monitoring (TDM) may be warranted to optimize valganciclovir exposure, avoid inadequate drug levels and assure safety [30▪]. More data on paediatric valganciclovir dosing and monitoring are needed. Concerns associated with prolonged exposure to ganciclovir (as well as cidofivir) have been raised due to a potential for carcinogenesis and altered spermatogenesis seen in mice and rats [31,32]. However, these hypothetical side effects have not been documented in humans. Of note, the potential for drug-related toxicity is substantially less with prophylactic dosing because of once-daily administration; therapeutic monitoring in this setting is generally not utilized.

Other antiviral drugs being investigated for the prophylaxis and treatment of CMV in immunocompromised patients include maribavir, letermovir and brincidofivir. Table 1 outlines the mechanism of action, formulation, licensing status and adverse effects of both current and future therapeutic agents for CMV [33,34▪▪,35–37,38▪]. Of note, there is uncertainty regarding the dosing used in the phase 3 maribavir trial and whether CMV disease is an appropriate endpoint [33]. As preemptive and prophylactic strategies have enabled strides in decreasing the rate of CMV end-organ disease, a viral marker of infection, such as PCR, may serve as a more appropriate marker of success. The phase 3 study of letermovir had a primary efficacy endpoint of all-cause prophylaxis failure, including detection of viral antigen or DNA, as well as evidence of end-organ disease. No obvious safety concerns were found [38▪]. Unlike cidofivir, brincidofivir is not concentrated in the renal proximal tubules and no renal or haematologic toxicity has been documented. Some limited data for children exist [39,40▪].

Riddell *et al.* [41] used CMV-infected fibroblasts to develop virus-specific T cells almost 20 years ago. The technology has been limited by the time and effort involved in developing the cells. Work continues to develop adoptive immunotherapy to restore cellular immunity against CMV more rapidly using autologous, donor or third-party T cells [42].

ANTIVIRAL RESISTANCE IN CYTOMEGALOVIRUS

The widespread use of ganciclovir and valganciclovir for CMV management posttransplantation has raised concerns for the development of antiviral resistance. Resistance rates of 1.5–2% have been reported in heart and abdominal organ transplant patients with even higher rates in lung transplant patients [43]. Although CMV resistance most commonly occurs during prolonged exposure to therapy, primary disease caused by resistant virus has also been reported [10,44,45]. CMV resistance in children is not well described; however, mutations developing during active antiviral therapy have been documented, resulting in serious outcomes [10,46]. The presence of resistance is suggested by a rising, persistently elevated or rebounding viral load in the setting of appropriate therapy. Persistent viral replication predisposing to resistance can result from low antiviral drug levels or suppressed immune status, such as with drug-induced cytopenias, T-cell depletion, congenital immunodeficiency or cord blood transplantation [47▪]. Mutations in the UL97-encoded CMV phosphotransferase are reported to occur first and confer resistance to ganciclovir. With continued ganciclovir exposure, alterations in the UL54-encoded viral DNA polymerase can arise, conferring high-level resistance and cross-resistance to cidofivir and possibly foscarnet [48–50]. All mutations in UL97 do not confer the

Table 1. Antiviral agents for cytomegalovirus

Drug	Mechanism of action	Formulation	Status	Most frequent adverse effects
Ganciclovir	Phosphorylated by UL97, inhibits DNA synthesis	i.v. and p.o.	Licensed U.S.: adults with retinitis, prophylaxis in transplant patients	Neutropenia, anaemia, thrombocytopenia
Valganciclovir	Activated in gut and liver to ganciclovir, same mechanism	i.v. and p.o.	Licensed U.S.: retinitis, adults and children transplant patients for prophylaxis	Neutropenia, anaemia, thrombocytopenia
Cidofivir	Inhibits DNA polymerase	i.v.	Licensed U.S.: adults with retinitis	Nephrotoxicity, neutropenia, metabolic acidosis
Foscarnet	Inhibits DNA polymerase	i.v.	Licensed U.S.: adults with retinitis	Renal impairment, electrolyte abnormalities, seizures, anaemia, genital irritation
Maribavir	UL 97 inhibitor	p.o.	Phase 3 trials for prophylaxis in HCT and liver transplant failed to decrease CMV disease [33]	Dose-dependent taste disturbance, nausea, vomiting
Brincidofivir (CMX001)	Intracellular conversion to cidofivir	p.o.	Efficacy in cidofivir-resistant strains Phase 2 trial in seropositive adults after HCT reduced incidence of CMV Phase 3 underway, including children aged >12 years [34▪▪,35]	Dose-dependent diarrhoea (26% in children), ALT elevation
Letermovir	Targets viral terminase	p.o.	Phase 2 efficacy for prophylaxis in adult HCT patients. Phase 3 in progress [36,37,38▪]	No haematologic or renal effects seen

CMV, cytomegalovirus; HCT, haematopoietic cell transplantation; i.v., intravenous; p.o., orally.

same resistant phenotype, as many sequence variants of unknown relevance are being reported [10,51].

In a retrospective study of paediatric transplant patients with UL97 mutations, the authors suggest that high viral loads may predispose to the development of resistance [10]. A prospective study of 49 paediatric HSCT patients with CMV infection analysed the *UL97* and *UL54* genes at enrolment; 4.1% developed resistance in the *UL97* and 2% in the *UL54* gene. Mortality related to drug resistant-CMV was seen in one patient [47▪]. Ganciclovir resistance has also been associated with substantial morbidity and prolonged CMV-associated hospitalization [43]. The currently recommended strategy to confront resistance in the face of persistent CMV infection is to change therapy to an alternative agent with CMV activity, including foscarnet or cidofivir, and reduce immunosuppression when possible. Hopefully, the alternate antiviral agents discussed above will help address this situation in the near future.

Resistance may develop early in children with congenital immunodeficiency treated with antivirals for CMV. Wolf *et al.* [52] found that in children with combined immunodeficiency syndromes (two with SCID), resistance emerged within 10 days to 3 weeks following initiation of therapy. They hypothesized that this was due to the rapid selection of pretherapy-resistant strains in the presence of profound immunosuppression [52]. This emergence of early resistance may be an important consideration in managing CMV disease in newly diagnosed infants with SCID in whom CMV infection occurs in the peri-transplant period and argues for the potential use of TDM or innovative therapeutic strategies in this patient population.

CONCLUSION

CMV continues to contribute to morbidity and mortality in immunocompromised children. Early diagnosis of SCID allows the opportunity to prevent viral infection in these highly vulnerable children. p.o. valganciclovir has emerged as an attractive alternative for the prevention and treatment of CMV disease, but further study of dosing in children is still needed. Management strategies for CMV in

transplant patients vary and therefore development of standardized protocols based on risk and international standards for viral load monitoring are necessary. CMV resistance is an emerging issue with relatively limited data in the paediatric population. The future in CMV treatment includes a number of promising antiviral agents that require further study in children as well as adults. T-cell immunotherapy continues to evolve and may have a larger role to play in posttransplant patients. A major milestone in prevention of CMV in high-risk children would be the development of an effective CMV vaccine. A number of strategies are currently under investigation [53].

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Conflicts of interest

There are no conflicts of interest.

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