

Cytomegalovirus Infections in Solid Organ Transplantation: A Review

Poornima Ramanan, and Raymund R Razonable

Division of Infectious Diseases, Department of Medicine and the William J von Liebig Transplant Center
Mayo Clinic, Rochester, Minnesota 55905, USA

Cytomegalovirus (CMV) continues to have a tremendous impact in solid organ transplantation despite remarkable advances in its diagnosis, prevention and treatment. It can affect allograft function and increase patient morbidity and mortality through a number of direct and indirect effects. Patients may develop asymptomatic viremia, CMV syndrome or tissue-invasive disease. Late-onset CMV disease continues to be a major problem in high-risk patients after completion of antiviral prophylaxis. Emerging data suggests that immunologic monitoring may be useful in predicting the risk of late onset CMV disease. There is now increasing interest in the development of an effective vaccine for prevention. Novel antiviral drugs with unique mechanisms of action and lesser toxicity are being developed. Viral load quantification is now undergoing standardization, and this will permit the generation of clinically relevant viral thresholds for the management of patients. This article provides a brief overview of the contemporary epidemiology, clinical presentation, diagnosis, prevention and treatment of CMV infection in solid organ transplant recipients.

Key Words: Cytomegalovirus, Transplant, Diagnosis, Prevention, Treatment

Introduction

Human cytomegalovirus (CMV) is a member of the *Beta-herpesvirinae* subfamily under the *Herpesviridae* family [1]. Discovered in the 1950s [2, 3], CMV is one of the largest known human viruses [4]. While most infections in immunocompetent individuals are benign and self-limited, CMV is an important cause of morbidity and mortality in individuals with underdeveloped or compromised immune function, in-

cluding transplant recipients. In order to reduce the impact of CMV on transplant outcomes, there have been remarkable efforts to improving its diagnosis, prevention, and treatment. Despite these significant advances in its diagnosis and therapy, CMV continues to have a major impact on patient and allograft survival among solid organ transplant (SOT) recipients through a variety of direct and indirect effects.

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Corresponding Author : Raymund R Razonable

Division of Infectious Diseases, Department of Medicine and the William J von Liebig

Transplant Center, Mayo Clinic, 200 First Street SW Rochester, Minnesota 55905, USA

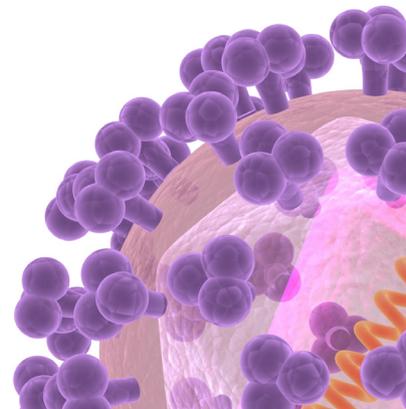
Tel: +01-507-284-3747, Fax: +01-507-255-7767

E-mail: razonable.raymund@mayo.edu

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Epidemiology

CMV is a ubiquitous virus, with a worldwide distribution. Infection is usually acquired early in life through contact with infected body fluids such as saliva. The seroprevalence of CMV varies geographically and is higher in developing countries, with rates reaching up to 100%, likely resulting from poor socio-economic status and over-crowding which facilitate viral transmission through close contacts [5]. In the United States, the CMV seroprevalence is about 50% among adults, although it is higher in women, older individuals, and those with lower household income [6].

CMV establishes lifelong latency in a variety of cells following primary infection, which may lead to reactivation and intermittent viral shedding. Prior to implementation of widespread routine CMV prophylaxis among SOT recipients, CMV disease typically occurs during the first three months after transplantation. The epidemiology has changed, as late-onset CMV disease has emerged in high-risk CMV donor-positive/recipient-negative (D^+/R^-) patients after the completion of antiviral prophylaxis. The incidence of CMV infection and disease varies by the type of organ transplant, the serostatus of donor and recipient, and the prevention strategies used. In a recent study involving kidney and liver transplant recipients who received valganciclovir prophylaxis, the 1-year incidence of CMV disease was 19.2% and 31.3%, respectively in D^+/R^- group, but was only 2.5% and 3.2%, respectively, in D^-/R^- group [7]. In heart recipients who received universal antiviral prophylaxis in the first month after transplant followed by preemptive therapy, the cumulative incidence of CMV infection and disease during the first year was 47% and 7.5% (3.6% in low risk and 25% in high risk group), respectively [8]. The incidence of CMV disease among lung transplant recipients who received antiviral prophylaxis for 6 to 12 months was 14.9%, with a higher incidence (26.6%) in D^+/R^- group [9]. Most cases of CMV disease in patients who received antiviral prophylaxis occur after cessation of antiviral drug administration, hence the term “late-onset CMV disease”, and they occur predominantly in CMV mismatch (D^+/R^-) SOT recipients. Late-onset CMV disease remains associated with allograft failure and mortality [10, 11].

Risk factors

The risk of acquiring CMV disease in SOT recipients depends on a number of factors such as the serostatus of the do-

nor and recipient, the type of organ transplanted, the net state of the host immunosuppression and viral factors. Patients without immunity against CMV-seronegative (R^-) who receive an organ transplant from CMV seropositive donor (D^+) are at highest risk of primary CMV disease resulting from the reactivation of latent virus transmitted in the allograft [12]. The “high-risk” D^+/R^- patients lack the ability to mount an effective immune response against CMV due to pharmacological immunosuppression and therefore, carry the highest risk of acquiring CMV infection and disease after solid organ transplantation.

Lung and small bowel transplant recipients carry a higher risk of acquiring CMV disease compared to kidney and liver transplant recipients; this may be explained by the intensity of immunosuppression and the amount of lymphoid tissue transplanted [13, 14].

Transplant recipients who are severely immunocompromised are at higher risk of CMV disease. The net state of immunosuppression is a dynamic entity that is influenced by many determinants such as the dose, duration and type of immunosuppressive agents, innate and adaptive host immune defects, age, and underlying comorbidities [15]. The use of lymphocyte depleting agents such as anti-lymphocyte globulin (ALG), anti-thymocyte globulin (ATG), OKT3 (anti CD3 antibody) and alemtuzumab (anti-CD52 antibody) inhibit CMV specific immune reconstitution and have been associated with increased risk of CMV disease [12, 16, 17], especially if they are used for the treatment of acute allograft rejection [18].

There is a bidirectional relationship between CMV and allograft rejection [12]. Allograft rejection creates a pro-inflammatory environment that can reactivate CMV, and the treatment for allograft rejection severely impairs the ability to mount an immune response to control viral replication. Allograft rejection was strongly associated with the occurrence of late onset CMV disease in CMV D^+/R^- liver and kidney transplant patients [18]. Conversely, CMV upregulates antigens, and this results in alloreactivity and facilitates allograft rejection.

Some newer immunosuppressive drugs have been associated with a lower risk of CMV infection. In particular, the use of mTOR inhibitors such as everolimus has been associated with a lower risk of CMV infection and disease [19]. Infection with other herpes viruses (HHV-6 and HHV-7) may predispose to CMV disease, but does not have any effect on clinical outcome [20].

Defects in innate immune responses have been associated

with increased risk of CMV infection and disease in SOT recipients, whose adaptive immune responses are rendered ineffective by immunosuppressive drugs. Examples of innate or CMV-specific immune defects include Toll-like receptor (TLR) gene polymorphisms, mannose binding lectin (MBL) deficiency or polymorphism [21, 22], chemokine and cytokine defects including increased IL-10 expression [23], deficiency in CMV-specific CD4+ and CD8+ T cells [24, 25], programmed cell death 1 [23] and immune evasion genes expression [26]. TLRs are part of innate immunity that detect a broad range of pathogens. TLR2 recognizes CMV surface glycoprotein gB and gH, leading to activation of intracellular signal transduction pathway and the production of antiviral peptides and cytokines [26, 27]. Polymorphisms in TLR2 which render them ineffective therefore may predispose to CMV disease [28, 29]. Homozygosity for TLR2 R753Q single nucleotide polymorphism has been associated with tissue-invasive CMV disease in liver recipients [29].

Patients with CMV D/R status carry the lowest risk of CMV disease. Current data suggests that their incidence of CMV disease is at 1-2% at one year after transplantation. These patients may acquire the infection through natural transmission in the community settings. Blood may transmit the virus, and hence, these patients should receive leuco-depleted or CMV negative blood products, if they require blood supplementation.

Clinical features

The direct effects of CMV are the clinical manifestations occurring as a result of CMV replication, dissemination and tissue invasion of specific organs [14, 30]. CMV disease denotes the presence of CMV infection (indicated by a positive antigenemia, culture, biopsy or viral load) accompanied by clinical symptoms and signs. CMV disease can be further categorized into CMV syndrome and tissue-invasive CMV disease (or end-organ disease). CMV syndrome manifests generally as flu-like illness, fever and malaise frequently associated with leucopenia or thrombocytopenia. Tissue-invasive CMV disease is associated with specific organ involvement (gastro-intestinal, pneumonitis, hepatitis, nephritis, myocarditis and retinitis, among others). Among these, the gastro-intestinal tract is the commonest organ to be involved [31].

CMV tends to involve the allograft because of altered immune mechanism locally within the allograft and the presence of the virus within latent cells of the allograft tissue obtained from seropositive donors. Depending on the type of

organ transplanted, CMV disease can manifest as hepatitis, nephritis, pneumonitis, myocarditis and pancreatitis in liver, kidney, lung, heart and pancreas transplant recipients, respectively [12].

CMV is known to cause a number of indirect effects due to its immunomodulatory properties [32, 33]. This property has been implicated in the association between CMV and the increased risk of bacteremia [34], invasive fungal infections [35], recurrent hepatitis C after liver transplant [36] and malignancies such as Epstein-Barr virus (EBV) associated post-transplant lymphoproliferative disorder [37]. CMV has also been associated with increased vascular thrombosis [38], probably related to infection of the endothelial cells. CMV has been associated with acute [39] and chronic allograft rejection, and with allograft failure [40]. Some of the transplant-specific indirect effects of CMV include chronic allograft nephropathy after renal transplantation, hepatic artery thrombosis after liver transplantation [38], coronary vasculopathy after cardiac transplantation [41], and bronchiolitis obliterans after lung transplantation [42]. Recently, CMV has been associated with the occurrence of new onset diabetes mellitus after transplant [43].

Laboratory diagnosis

The laboratory tests that are available for screening and diagnosis of CMV include histopathology, viral culture, pp65 antigenemia, and nucleic acid tests (NAT). Measures for immunity to CMV such as serology and novel immunology assays detecting CMV specific cellular immunity may be used to assess the risk of CMV infection in SOT recipients [33].

1. Nucleic acid testing

NAT, which detects and quantifies CMV nucleic acid in clinical samples, is the preferred test for the diagnosis of CMV infection in the solid organ transplant recipient. The test may detect CMV RNA (which is generally indicative of an actively replicating virus) or CMV DNA. There is a wide array of NAT that has been developed for clinical use. However, only one assay has been approved by the US FDA for monitoring transplant patients with CMV disease.

The biggest drawback of CMV NAT has been, until recently, the variability of test results across laboratories due to the lack of assay standardization. There are differences in commercial detection reagents, calibration, nucleic acid extraction methods and the selection of primers and probes targeting differ-

ent genes, among others, which contribute to significant differences in viral load reporting [44, 45]. In a study that systematically looked into the variability in CMV NAT reporting, there was an up to a 3 log₁₀ difference in viral load values across different laboratories [46]. Also, significant variations in viral load may be due to sample type. Whole blood samples is more sensitive and yields higher viral load and earlier time to viral detection compared to plasma samples [33]. In a study which compared plasma versus whole blood for monitoring of CMV levels during treatment of CMV disease, there was a higher rate of detectable virus at day 21 in the whole blood samples when compared to the plasma samples (70% versus 52%). This difference has strong clinical implications for the diagnosis of infection (especially at low viral load levels) and in assessing the duration of treatment (longer course is anticipated with a more sensitive assay, if no detectable virus is the end of treatment goal) [47].

Because of the lack of assay standardization, there have only been a limited number of well-defined viral load threshold to guide physicians with regards to pre-emptive monitoring, prognostication and therapeutic monitoring for CMV disease. In 2010, the World Health Organization (WHO) released the first international reference for quantification of CMV nucleic acid, which will allow assay calibration and standardization among laboratories. The standardization of QNAT assays will assure uniform test reporting and facilitate the development of relevant viral threshold for clinical decision making. In a recent multinational study, the only FDA approved assay, COBAS AmpliPrep/COBAS TaqMan CMV test, was found to have high interlaboratory agreement and precision of test results across five different laboratories [48]. The same assay was used to define viral load threshold for prognostication and therapeutic monitoring. Patients with a pretreatment CMV DNA of <18,200 IU/mL were found to be 1.5 times more likely to have resolution of CMV disease. Moreover, virological suppression, as defined by a viral load copy of <137 IU/mL was predictive of resolution of clinical symptoms during antiviral treatment of CMV disease [49]. It is anticipated that, as more laboratories are optimizing their assays according to the new standard, various thresholds will be defined among different solid organ transplant recipients and risk groups. We anticipate different viral load thresholds among CMV D⁺/R⁻ compared to CMV R⁺ patients, and among various solid organ transplant types.

2. Serology

The role of CMV serology in the post-transplant period for diagnosis of CMV infection is very limited, owing to impaired

ability of SOT recipients to mount an adequate antibody response [50]. Indeed, its use is not recommended for diagnosis of CMV disease after transplantation. The main utility of serology is the risk stratification of patients in the pre-transplant screening phase. In this regard, transplant candidates and donors are tested for CMV IgG antibodies, which is an indicator of latent infection. Depending on the presence or absence of CMV IgG in the donor or recipient, the risk of CMV disease after transplantation varies, with the D⁺/R⁻ group having the highest risk.

3. Histopathology

Histopathology is used to confirm tissue-invasive CMV disease. However, its invasive nature has limited its use in certain clinical settings. For example, in a patient with gastrointestinal CMV disease, a biopsy may not be done if the patient's blood contains high levels of CMV. Certain situations that would warrant biopsy and histopathology are (1) when allograft rejection is suspected (which requires more immunosuppression, whereas treatment of CMV disease requires a reduction in immunosuppression), (2) when co-infection with other pathogens is suspected (when symptoms do not resolve with treatment), and (3) when "compartmentalized" disease is suspected due to the absence of detectable virus in the blood.

4. Culture

Viral culture is highly specific for the detection of CMV, but it has low sensitivity and the assay has a long turn-around time [51]. As a result, the use of viral culture for the diagnosis of CMV in the transplant setting has been supplanted by antigenemia and NAT. The main utility of culture is in the isolation of CMV from tissue specimens since NAT are not yet optimized for these samples.

5. Antigenemia

The antigenemia assay, which detects CMV pp65 antigen in infected peripheral blood leucocytes, and has been used for the rapid diagnosis of CMV infection in transplant recipients [51]. While it has a higher sensitivity than viral culture, it may have limited clinical utility in leucopenic patients, and the test requires a quick sample processing time for accuracy (4 to 6 hours) [51].

6. Immunologic assays

In addition to serology (discussed above), there are a number of studies trying to co-relate the patient's cellular immunity against CMV as a predictor of risk of developing subsequent CMV disease [52, 53]. In a recent study, an assay that mea-

measures interferon-gamma levels after in-vitro stimulation in high-risk CMV D⁺/R⁻ patients was correlated with risk of CMV disease after completion of antiviral prophylaxis. Patients with a positive test had a lower incidence of subsequent CMV disease when compared to patients with negative and indeterminate results (6.4% vs 22.2% vs 58.3%, respectively; $P < 0.001$) [53]. This study supported previous work correlating CD8⁺ T-cell immunity with CMV disease in high-risk SOT recipients. The incidence of late-onset CMV disease was lower in patients with a detectable interferon-gamma response when compared to those with a negative response (5.3% versus 22.9%, respectively) and the same pattern was reflected in the D⁺/R⁻ subgroup of patients (10% versus 40%) [54]. These studies suggest that immune monitoring may complement viral load measures in recognizing patients who have a strong probability of developing late CMV disease. Various other assays have been developed, and are being optimized, to measure cellular immune response against CMV [55].

Prevention of CMV disease

Advances in CMV prevention strategies have resulted in a decrease in CMV related mortality, tissue-invasive disease and detrimental indirect effects in solid organ transplant recipients. There are two major strategies used to prevent CMV disease in SOT recipients – (1) antiviral prophylaxis and (2) preemptive therapy. Table 1 lists the pros and cons of both strategies. In some centers, a hybrid approach is used, wherein antiviral prophylaxis is used during the highest-risk period, and then transitioned to preemptive therapy during the peri-

ods of modest risk.

Antiviral prophylaxis involves administering antiviral drug to all at-risk patients, starting shortly after transplant (usually during the first 10 days), and given up to a pre-defined period of time, usually 3 to 6 months (and even for longer periods after lung transplantation). The advantages of antiviral prophylaxis are ease of medication administration, protection from infections caused by other herpes viruses (HSV, VZV, EBV, HHV-6) and a decreased incidence of CMV related “indirect” effects such as allograft rejection, opportunistic infections and mortality (Table 1). The main disadvantages of antiviral prophylaxis are drug toxicities (mainly leukopenia and neutropenia from ganciclovir or valganciclovir) and late-onset CMV disease (CMV disease occurring after the completion of antiviral prophylaxis) [56]. The drugs used for antiviral prophylaxis are valganciclovir (most common), oral ganciclovir, intravenous ganciclovir, or valaciclovir (in kidney transplant recipients only) (Table 2). Valganciclovir is preferred over oral ganciclovir due to higher oral bioavailability and lower pill burden, and is comparable to oral ganciclovir in preventing CMV disease in solid organ transplant recipients [31]. Valganciclovir was associated with a higher rate of tissue invasive disease in liver transplant recipients compared to oral ganciclovir [31], but it is still the preferred drug used in liver transplant recipients. Duration of antiviral prophylaxis depends on the serostatus of the donor and recipient as well as the type of organ transplanted. In the IMPACT trial, which compared the efficacy of 200 days versus 100 days of valganciclovir prophylaxis in D⁺/R⁻ kidney transplant recipients, late onset CMV disease was significantly lower in the 200 days’ group [56]. This trial resulted in the recommendation of extending valganciclovir pro-

Table 1. Pros and cons of pre-emptive versus antiviral prophylaxis

Parameters	Pre-emptive therapy	Antiviral prophylaxis
Cost	Increased laboratory cost	Increased drug related cost
Ease of coordination	Difficult to coordinate lab draw, follow up of results and time-appropriate action	Easier to coordinate, however drug toxicity needs to be monitored
Drug toxicities	Lower	Higher
Protection against other Herpes viruses	None	Yes
Protection against “Indirect” effects	Less	Yes
Development of CMV specific immunity	+	-
Incidence of late onset CMV	Low	High in D ⁺ /R ⁻
Antiviral resistance	+	+
“Escape” infections	Can occur due to rapidly replicating virus	No (breakthrough infections may occur in patients receiving suboptimal dosing)

Table 2. Preferred and alternative drugs active against CMV

Preferred Drugs	Antiviral prophylaxis	Treatment	Side effects/Remarks
Valganciclovir	900 mg PO once daily	900 mg PO twice daily	Bone marrow suppression - Leucopenia
Ganciclovir IV	5 mg/kg once daily	5 mg/kg twice daily	Bone marrow suppression - Leucopenia
Alternative drugs	Antiviral prophylaxis	Treatment	Side effects/Remarks
Oral ganciclovir	1 g PO thrice daily	Not recommended	Leucopenia, high pill burden Induction of resistance
Valaciclovir	2 g PO four times daily	Not recommended	Only in kidney recipients Second line even in kidney SOT High pill burden
Foscarnet	Not recommended	60 mg/kg IV every 8 h or 90 mg/kg every 12 h	Used in high level UL97 mutant ganciclovir resistance Nephrotoxic
Cidofovir	Not recommended	5 mg/kg once weekly × 2, followed by q 2 weeks thereafter.	Used as alternative drug in UL97 mutant ganciclovir resistance Nephrotoxic

CMV, cytomegalovirus.; PO, per oral; SOT, solid organ transplantation..

phylaxis to 200 days in high risk (D^+/R^-) kidney recipients. This has also been adapted by the liver, heart, pancreas transplant programs, even if systematic studies have not been performed in these organ recipients. A multicenter trial compared the incidence of late onset CMV disease and viremia in high risk lung transplant recipients receiving 3 months versus 12 months of valganciclovir prophylaxis. Patients who received 12 months of antiviral therapy had significantly lower rates of CMV disease and viremia [57] and had a durable, long-term CMV protective benefit [58]. As a result, many centers have adopted 12 months of antiviral prophylaxis in CMV D^+/R^- and CMV R^+ lung transplant recipients, while some centers have even extended the duration for longer period due to anecdotal experience of continued occurrence of CMV disease despite a year of prophylaxis [59, 60]. Some have given adjunctive CMV-specific immunoglobulin or intravenous immunoglobulin (IVIg), in addition to antiviral prophylaxis, in high-risk lung and heart transplant recipients [61-63].

Preemptive therapy involves monitoring asymptomatic patients with quantitative assays (either with pp65 antigenemia or QNAT) at regular intervals (usually once a week) for a predefined period of time (usually 3 months after transplantation) and treating those patients who have a positive assay, essentially, catching them in the early phase of CMV infection and preventing progression to disease. The advantages of preemptive therapy are decreased drug related toxicities and costs. There is also the theoretical advantage of allowing the patient to develop CMV specific cellular immunity during exposure to low level CMV viremia [64], thereby leading to lower

incidence of late onset CMV disease. The disadvantages of preemptive therapy include higher laboratory costs, the difficulty in coordinating multiple laboratory and clinic visits, follow up of results and acting in a time-appropriate manner. Preemptive strategy does not protect against other herpes viral infections (hence the need to provide acyclovir prophylaxis to prevent herpes simplex infection) and this may not reduce the “indirect” effects of CMV to the extent that has been demonstrated for antiviral prophylaxis [65]. There is also a concern for rapid progression of tissue-invasive disease (“escape” infections) in high risk patients (D^+/R^-) due to rapidly replicating virus which may be missed by weekly (or less frequent) laboratory monitoring [12]. In addition, there has not been an established viral load threshold to guide pre-emptive therapy due to, until recently, the lack of standardization of assays. One of the recent studies using a standardized assay suggested a viral load of 3,983 IU/mL as cut-off for starting pre-emptive therapy in CMV-seropositive patients [66]. Once viral load is above a predefined threshold, patients are treated with either oral valganciclovir 900 mg twice daily or intravenous ganciclovir (5 mg/kg) twice daily, and the treatment is continued until the viral load becomes “negative” or below the lowest viral load threshold.

There were no significant differences among patients getting antiviral prophylaxis versus pre-emptive therapy in preventing CMV disease [67], however, long-term graft survival was higher with antiviral prophylaxis [68]. Drug resistant CMV has been observed in patients receiving antiviral prophylaxis or preemptive therapy [69-73]. The incidence of CMV drug resistance in D^+/R^- kidney recipients was similar if they received

100 days or 200 days of valganciclovir prophylaxis (1.8 and 1.9%, respectively) [73]. Although prophylaxis should theoretically induce more ganciclovir resistance in CMV than preemptive therapy, this has not been observed in a study wherein the incidence of drug resistant CMV infection was higher in the preemptive therapy group when compared to the antiviral prophylaxis group (16% versus 3%) in D⁺/R⁻ kidney recipients [72]. In another study, the incidence of ganciclovir-resistant CMV infection was 2.2% in a large cohort of 1244 kidney recipients undergoing preemptive therapy [71].

There are currently no CMV vaccines available though many are in various stages of research. In a recent study, the administration of glycoprotein B/MF 59 vaccine to adults awaiting kidney or liver transplantation resulted in (1) significantly increased glycoprotein B antibody titers in both seronegative and seropositive patients, (2) shorter duration of viremia and (3) lessened antiviral drug use [74].

Prevention of late-onset CMV disease

The major drawback of antiviral prophylaxis is late onset CMV disease. This is most commonly observed among CMV D⁺/R⁻ solid organ transplant recipients after completion of antiviral prophylaxis. These high-risk patients should therefore be educated about the various symptoms of CMV syndrome and tissue-invasive disease and be advised to seek early medical attention. To reduce this risk, some have recommended a hybrid approach wherein the CMV D⁺/R⁻ patients are monitored by pp65 antigenemia or PCR after they complete the standard prophylaxis program. However, data have suggested that this is of limited value [75]. In one study that evaluated this approach, weekly viral loads were performed for 8 weeks in 71 D⁺/R⁻ patients after completion of antiviral prophylaxis. Among the 29 patients who developed CMV disease, more than half occurred after the 8 week surveillance period, suggesting that a longer duration of surveillance may be needed. In addition, only 15.8% (3 of 19) of viremic patients during the 8 week surveillance period required preemptive antiviral therapy; the other either spontaneously cleared low level viremia (and did not require treatment) or developed CMV disease (at the time of viremia detection) [75].

Treatment of CMV disease

Treatment of CMV in solid organ transplant recipients re-

duces the risk of allograft injury and death [76, 77]. The two main drugs used for treating CMV disease are intravenous (IV) ganciclovir (5-mg/kg every 12 hours) and oral valganciclovir (900-mg twice daily)(Table 2) [14]. Oral valganciclovir achieves comparable blood levels to IV ganciclovir and is recommended for the treatment of mild to moderate CMV disease in solid organ transplant recipients [78]. In a study of 321 adult solid organ transplant recipients with CMV disease, the clinical and virologic outcomes were not significantly different between those patients who received oral valganciclovir or IV ganciclovir. The rate of viremia eradication for valganciclovir group and IV ganciclovir group were comparable – 45.1% versus 48.4% at day 21, and 67.1% versus 70.1% at day 49, respectively. The median time of viremia eradication (21 versus 19 days), side effect profiles, and treatment outcomes were also comparable between the two groups [78].

IV ganciclovir is preferred drug for treatment of severe or life-threatening CMV disease or in those with questionable gastrointestinal absorption [14]. IV ganciclovir is also recommended for those with very high viral load. Oral ganciclovir should never be used in the treatment of CMV disease because of poor oral-bioavailability leading to sub-therapeutic blood levels. In addition to the antiviral therapy, it is strongly emphasized that a cautious reduction in immunosuppression will help in the clearance of infection. CMV occurs as a result of an over-immunocompromised state, hence, the reduction in immunosuppression will allow for the recovery or the generation of CMV-specific immunity that will allow longer-lasting control of the virus infection.

The duration of antiviral therapy should be individualized and be guided by resolution of clinical symptoms and viral load monitoring. Viral load kinetics that have shown to help predict clinical response to antiviral therapy include a lower pre-treatment viral load, a faster rate of viral load decline in response to therapy, and viral suppression at the end of treatment [79, 80]. In a recent study which used the WHO international standard for reporting, patients with a pretreatment CMV DNA < 18,200 IU/mL were more likely to have CMV disease resolution. Moreover, CMV suppression < 137 IU/mL was predictive of clinical response to therapy [49].

Treatment of ganciclovir resistant CMV

While still uncommon, ganciclovir resistance in CMV has been increasing in frequency. Infection with ganciclovir-resistant CMV has been associated with increased morbidity and

Table 3. Risk factors for development of ganciclovir resistant CMV

Risk factors for development of ganciclovir resistant CMV
D ⁺ /R ⁻ CMV serostatus
Lung transplantation
Increased intensity of immunosuppression
High pre-treatment CMV viral load
Prolonged subclinical viremia
Previous exposure to sub-therapeutic doses of valganciclovir or ganciclovir

mortality in SOT patients [81]. The incidence is highest among lung transplant recipients [82].

The major mutations conferring drug resistance in CMV are UL97 phosphotransferase and less commonly, UL54 DNA polymerase genes [83]. The active form of ganciclovir is ganciclovir-triphosphate. The first phosphorylation step is carried out by a viral kinase encoded by UL97 gene. Mutations in UL97 may, therefore, render low or high level resistance to ganciclovir, depending on the site of mutation [82]. Ganciclovir triphosphate prevents viral replication by competitively inhibiting DNA polymerase, encoded by UL54 gene. Mutations in UL54 are less common and usually occur after UL97 mutation. Combined UL54-UL97 mutations render high level resistance to ganciclovir [82].

The most consistent risk factor associated with drug resistant CMV is D⁺/R⁻ serostatus (Table 3) [71, 84]. Other risk factors include receipt of lung transplantation [69], high pre-treatment CMV viral load [72], intensity of immunosuppression [83], prolonged subclinical viremia [83] and exposure to sub-therapeutic levels of antiviral agents [81, 85], though increasing the duration of valganciclovir prophylaxis did not affect the incidence of ganciclovir resistance [57, 73]. Resistance should be suspected in a patient with any of the above risk factors with clinical or virological failure after three weeks of adequate therapy [83, 86].

Genotypic testing should be performed when resistance is suspected. In patients with low level resistance to ganciclovir conferred by UL97 mutation, increased dose of IV ganciclovir may be used (up to 10 mg/kg twice daily) [14]. Foscarnet is the preferred drug in high level ganciclovir resistance, though cidofovir has been used occasionally [14]. Reducing immunosuppression or switching from calcineurin to mTOR inhibitors such as sirolimus may be helpful [87]. Experimental antiviral drugs that are being developed or considered for use in CMV resistant to ganciclovir, foscarnet and cidofovir are letermovir, CMX-001 (orally available lipid prodrug of cidofovir), cyclo-

propavir, artesunate and leflunomide [83].

Conclusion

Despite significant advances in the prevention and management of CMV in SOT recipients, CMV remains at large and continues to have significant impact among solid organ transplant patients. Advances in the field of CMV and solid organ transplantation will be facilitated by the development of (1) optimized threshold for viral diagnosis, (2) effective vaccines for prevention, (3) diagnostic assays to stratify risk of late onset CMV disease by immunological monitoring, and (4) newer antiviral agents with unique mechanisms of action and ideally with much less toxicity.

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