TRANSPLANTATION RÉNALE / KIDNEY TRANSPLANTATION

CYTOMEGALOVIRUS INFECTION POST KIDNEY TRANSPLANT
What Should We Know Now

Samir MALLAT[1], Maroun MOUKARZEL[2], David ATALLAH[3], Rima ABOU ARKOUB[4]
Chebl MOURANI[5]

ABSTRACT • Cytomegalovirus (CMV) remains one of the most important pathogen responsible for the morbidity and mortality of transplantation patients. The impact on recipients depends on the form of CMV infection knowing that 10% to 50% develop symptomatic disease while solid organ involvement if presumed (e.g. CMV nephritis) may have deleterious outcome and requires histopathology testing. Treatment with antivirals IV ganciclovir and valganciclovir is managed according to early diagnostic tools with quantitative nucleic acid testing (QNAT) and antigenemia that will indicate the extent of disease and monitor response to treatment. CMV prevention in particular conditions of high risk patients has proven to be beneficial, resistance to antivirals and CMV vaccines along with novel therapies are thoroughly discussed in this review describing the new perspectives of CMV infection management.

Keywords: CMV, transplant, review, diagnosis, treatment, ganciclovir, resistance

RÉSUMÉ • Le cytomégalovirus (CMV) est le plus important pathogène post transplantation rénale et responsable de morbidité et mortalité chez les patients transplantés. L'impact sur les receveurs dépend de la forme clinique de l'infection. Ainsi, 10%-50% développent une infection symptomatique et l'atteinte du rein (néphrite à CMV) peut avoir des conséquences délétères et nécessite un diagnostic histopathologique.

Le traitement par les antiviraux ganciclovir intraveineux ou valganciclovir par voie orale dépend d'un diagnostic précoce basé sur la quantité de réplication virale et l'antigénémie. Le traitement prophylactique dans les situations de malades à haut risque est bénéfique. Une discussion concernant la résistance aux antiviraux ainsi que de nouvelles modalités de traitements est proposée dans cette revue.

INTRODUCTION

One of the most common complications following solid organ transplantation is infection with cytomegalovirus (CMV), a member of the genus Herpesvirus of the family *Herpesviridae* [1].

The first exposure to CMV occurs in the majority of humans during the first two decades of life. The main host defense against CMV is cell-mediated immunity; however, virus-specific antibodies may also modify the disease caused by this virus.

Following primary infection, CMV is maintained in a latent state by integration within the host cell chromosome or by persistent low-level viral replication that is adequately controlled by a well functioning immune system. Consequently, any dysfunction of the immune system will allow for increased levels of CMV replication; a phenomenon present following solid organ transplantation [2].

CMV can be transmitted via saliva, sexual contact, placental transfer, breast-feeding, blood transfusion, solid-organ transplantation (SOT), or hematopoietic stem cell transplantation [3].

CMV was first isolated in a renal transplant recipient in 1965 [1]. It is evident now that human cytomegalovirus (CMV) is the most frequent opportunistic infection after renal transplantation [4].

More than 50% of solid organ transplant (SOT) recipients show evidence of CMV infection, with 10 to 50% of patients developing symptomatic disease, depending on the serostatus of the recipient (R) and donor (D) [5].

In an immunocompetent host, primary CMV infection often is asymptomatic, although it can manifest as a mononucleosis-like syndrome. In contrast, in immunocompromised hosts, primary CMV infection, reactivation of latent infection, or reinfection with a different strain usually causes CMVdisease [3].

VARIABLE FORMS OF INFECTION

There are three forms of CMV, as determined by prior and current experience with the virus :

1. *Primary CMV disease*, which occurs when an allograft (or a transfusion product) is obtained from a seropositive donor and is transplanted into a seronegative recipient. If no antiviral protocol utilized, approximately 60% of these D+/R− patients will become clinically ill, usually at around 4 weeks posttransplant.

[1]Nephrology Department, American University of Beirut Medical Center, Beirut, Lebanon.
[2]Urology, [3]Gynecology-Obstetrics & [5]Pediatrics Depts, CHU Hôtel-Dieu de France, Saint-Joseph University, Beirut, Lebanon.
[4]Division of Nephrology, University of Ottawa, Canada.
Correspondence: *Samir Mallat, MD*.
e-mail: sm104@aub.edu.lb

2. *Reactivation disease*. This occurs when a seropositive individual reactivates endogenous virus which then has the potential for producing clinical disease.
3. *Superinfection*. This occurs when an allograft from a seropositive donor is transplanted into a seropositive recipient and the virus that is reactivated is of donor origin [5].

The following definitions are commonly used in the transplant literature and are consistent with the American Society of Transplantation (AST) recommendations for use in clinical trials [6]:

1. *CMV infection*: evidence of CMV replication regardless of symptoms (differs from latent CMV).
2. *CMV disease*: evidence of CMV infection with attributable symptoms. CMV disease can be further categorized as either a viral syndrome with fever and/or malaise, leukopenia, thrombocytopenia or as tissue invasive disease (e.g. pneumonitis, hepatitis, nephritis, gastrointestinal disease).

Nephritis

"CMV nephritis" can be defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of histological features of CMV infection in a kidney biopsy specimen obtained from a patient with renal dysfunction. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV nephritis. Furthermore, detection of CMV in the urine of a patient with kidney dysfunction does not fulfill the definition of CMV nephritis [7].

OTHER EFFECTS OF CYTOMEGALOVIRUS IN TRANSPLANT RECIPIENTS

CMV infection suppresses host defenses, predisposing to secondary invasion by such pathogens as *Pneumocystis jiroveci*, *Candida*, and *Aspergillus* species, and some bacterial infections [8]. CMV may contributes to the risk of EBV-mediated posttransplant lymphoproliferative disorder (PTLD) [9], and increased risk of graft rejection [10]. The mechanisms for these effects include altered T cell subsets and synthesis and display of major histocompatibility antigens, and elaboration of the array of proinflammatory cytokines, chemokines, and growth factors.

DIAGNOSIS

Diagnostic tests for CMV include serology, quantitative nucleic acid testing (QNAT), antigenemia, culture, and histopathology.

Serology
CMV serology reflects prior exposure. Serologic methods are useful in defining donor and recipient serostatus and post-transplant risk of reactivation [4] but it has no role in the diagnosis of active CMV disease posttransplantation [11].

Antigenemia
The antigenemia assay is a rapid quantitative method that detects CMV antigens by directly immunostaining polymorphonuclear leukocytes (PMN) from blood specimens with monoclonal antibodies directed against the CMV lower-matrix protein (pp65) [2].

This is a semiquantitative test that has been shown to be helpful in initiating preemptive therapy, the diagnosis of clinical disease, and monitoring response to therapy.

Quantitative nucleic acid testing (QNAT) or polymerase chain reaction (PCR)
QNAT has higher sensitivity and similar specificity than the pp65 antigenemia assay, resulting in high negative predictive values, but lower positive predictive values [12].

The precision of QNAT viral load tests are such that changes in values should be at least 3-fold (0.5 log10 copies/mL) to represent biologically important changes in viral replication [13]. Generally, the highest viral loads are associated with tissue-invasive disease, while the lowest are seen with asymptomatic CMV infection, and the intermediate-range viral loads seen in patients with CMV syndrome [14].

This assay may not be possible to perform when the absolute neutrophils count is less than 1000. The test is labor intensive, and the blood specimen has limited stability and should be processed within 6 to 8 hr of collection to avoid a decrease in test sensitivity [11].

Both the pp65 antigenemia assay and QNAT are suitable for monitoring the occurrence of CMV infection, diagnosing CMV disease in symptomatic solid-organ transplant recipients, and monitoring response to antiviral therapy [11,15].

Viral cultures
Recovery of replicating CMV by cell culture (conventional tube and shell vial assay) has traditionally been the standard method for the diagnosis of CMV infection [2]. Viral culture of blood for CMV has limited clinical utility for diagnosis of disease due to poor sensitivity. There is no role for CMV urine culture in the diagnosis of disease due to poor specificity [11].

Culture of tissue specimens remains an important option for diagnosis of tissue invasive disease, particularly for gastrointestinal samples (i.e. colonic biopsies), where antigenemia or polymerase chain reaction (PCR) testing on blood may not always be positive even with invasive disease [16].

Histopathologic examination
Histopathologic examination of tissue is important in diagnosing tissue invasive disease and morphologic analysis is made more sensitive by the use of immunohistochemistry and/or in situ hybridization to identify CMV-infected cells [4].

There are two main methods for CMV prevention: The universal prophylaxis and preemptive therapy.

Universal prophylaxis involves the administration of antiviral medication to all patients or a subset of "at-risk" patients.

According to the latest International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation, the duration of prophylaxis in D+/R- patients should be generally between 3 months and 6 months. The decision to use 3 vs. 6 months may depend on degree of immunosuppression, including the use of antilymphocyte antibodies for induction [11]. A randomized controlled trial compared the efficacy and safety of 200 days' versus 100 days' valganciclovir prophylaxis (900 mg once daily) in 326 high-risk (D+/R-) kidney allograft recipients. The prolonged prophylaxis of 200 days reduced significantly the CMV disease at 12 months compared to 100 days prophylaxis (16.1% vs. 36.8%; $p < 0.0001$) [17]. The choice of immunosuppressive regimen may also affect the risk of CMV disease. A recent meta-analysis concluded that mTOR-inhibitor treatment either alone or in combination with CNIs reduces significantly the CMV incidence after organ transplantation [18]. When a prophylaxis strategy is used for prevention in D+/R- kidney transplant patients, the following antiviral medications are recommended: valganciclovir IV or oral ganciclovir, or valacyclovir [11].

Preemptive therapy is defined as serial testing done weekly or biweekly for the first few months after transplant or after treatment of rejection, with therapy initiated once a certain defined positive threshold is reached [16]. The advantages of preemptive therapy include more selective drug targeting, decreased drug cost, and associated toxicities. However, it is more difficult to coordinate because it requires weekly laboratory monitoring. In addition, optimal threshold values for antigenemia or viral load are assay dependent and have not been well established [11]. A systematic review concluded that late-onset CMV disease is a complication observed uniquely with valganciclovir prophylaxis, but not with preemptive therapy [19].

On the other hand, in a prospective, randomized study comparing oral ganciclovir prophylaxis to preemptive IV ganciclovir in kidney transplant patients, prophylaxis strategy reduced CMV infection by 65%, and graft survival was improved at 4 years post transplant [20].

In summary: Both universal prophylaxis and preemptive strategies are viable approaches for prevention of CMV disease. For the highest risk patients (D+/R-), prophylaxis may have some advantages over preemptive therapy [11].

A dose adjustment for the creatinine clearance should be taken into consideration as proposed in table I.

TABLE I
DOSAGE of ANTIVIRAL DRUGS ADJUSTED for KIDNEY FUNCTION*

Antiviral (Administration)	CCr (mL/min)	Dosage
Antivirals Used for Universal Prophylaxis		
IV ganciclovir (slow infusion over at least 1 h)	≥70	5 mg/kg every 24 h
	50-69	2.5 mg/kg every 24 h
	25-49	1.25 mg/kg every 24 h
	10-24	0.625 mg/kg every 24 h
	<10	0.625 mg/kg 3×/wk after HD
Oral ganciclovir (intake with food)	≥70	1,000 mg 3×/d
	50-69	1,500 mg 1×/d or 500 mg 3×/d
	25-49	1,000 mg 1×/d or 500 mg 2×/d
	10-24	500 mg 1×/d
	<10	500 mg 3×/wk after HD
Valganciclovir (intake with food)	≥60	900 mg 1×/d
	40-59	450 mg 1×/d
	25-39	450 mg every other day
	10-24	450 mg 2×/wk
	<10	NR
Valacyclovir (intake without regard to meals)	≥75	2,000 mg 4×/d
	50-74	1,500 mg 4×/d
	25-49	1,500 mg 3×/d
	10-24	1,500 mg 2×/d
	<10	1,500 mg 1×/d
Antivirals Used for Treatment of Established CMV disease and in Pre-Emptive Therapy		
IV ganciclovir (slow infusion over at least 1 h)	≥70	5 mg/kg every 12 h
	50-69	2.5 mg/kg every 12 h
	25-49	2.5 mg/kg every 24 h
	10-24	1.25 mg/kg every 24 h
	<10	1.25 mg/kg 3×/wk after HD
Valganciclovir (intake with food)	≥60	900 mg 2×/d
	40-59	450 mg 2×/d
	25-39	450 mg 1×/d
	10-24	450 mg every other day
	<10	NR

Abbreviations: CCr, creatinine clearance; CMV, cytomegalovirus; HD, hemodialysis; IV, intravenous; NR, not recommended.

*Adapted from: Human cytomegalovirus and kidney transplantation: a clinician's update. *American Journal of Kidney Diseases* (De Keyzer K *et al.*, 2011) [15].

VACCINATION

Several CMV vaccines are under development; including live attenuated, DNA-subunit, and recombinant viral vaccines.

Knowing that although cytomegalovirus disease occurs in the context of suppressed cell-mediated immunity posttransplantation, humoral immunity still has a role in reduction of cytomegalovirus viraemia; the cytomegalovirus glycoprotein-B vaccine was tested in a phase-2 randomized placebo controlled trial in adults awaiting kidney or liver transplantation. In the R-/D+, the duration of viraemia and number of days of ganciclovir treatment were significantly reduced in vaccine recipients [21].

The latest 2010 international consensus guidelines on the management of cytomegalovirus in solid organ transplantation stated that until further evidence is available, no recommendation can be made with regards to type of CMV vaccine or timing of vaccination [11].

CYTOMEGALOVIRUS TREATMENT

In any solid transplant recipient, CMV disease should be treated with either intravenous ganciclovir (5 mg/kg two times a day) or oral valganciclovir (900 mg two times a day) until the following criteria are met:

a. Clinical resolution of symptoms AND
b. Virologic clearance below the threshold negative value (test specific; see text); monitor patients with viral load or pp65 antigenemia once a week AND
c. Minimum 2 weeks of treatment [11].

In a randomized controlled trial comparing oral valganciclovir and intravenous ganciclovir for the treatment of CMV disease in SOT recipients, 321 SOT recipients (around 72% were kidney transplant) received either twice daily intravenous ganciclovir or oral valganciclovir (for 21 days) followed by once daily valganciclovir until day 49. The long-term follow-up revealed comparable treatment success rates, CMV recurrence rates and a low incidence of ganciclovir resistance [22].

Intravenous ganciclovir is preferable to oral valganciclovir in patients with severe or life-threatening disease, or in patients who may have a problem with gastrointestinal absorption of oral drug (as significant diarrhea). After completion of treatment, a 1-3 month course of secondary prophylaxis may be considered depending on the clinical situation [11,14].

Dose reduction of antiviral treatment due to side effects such as leukopenia should be avoided as much as possible. Granulocyte colony-stimulating factor (G-CSF) may be considered for severe leukopenia, especially if the absolute neutrophil count is less than 1000/mm^3 [11].

Dose reduction of the immunosuppressive therapy should be individualized but should be considered in severe CMV disease, in non-responding patients, in patients with high viral loads, and with leukopenia [11].

The role of CMV immunoglobulin in the treatment of CMV disease is unclear. It may be considered as adjunctive therapy for severe forms of CMV disease such as pneumonitis [11,14].

CYTOMEGALOVIRUS RESISTANCE

In 2000, Limaye *et al.* described the emergence of ganciclovir-resistant CMV in kidney, liver, and pancreas recipients after oral ganciclovir prophylaxis, occurring in 7% of
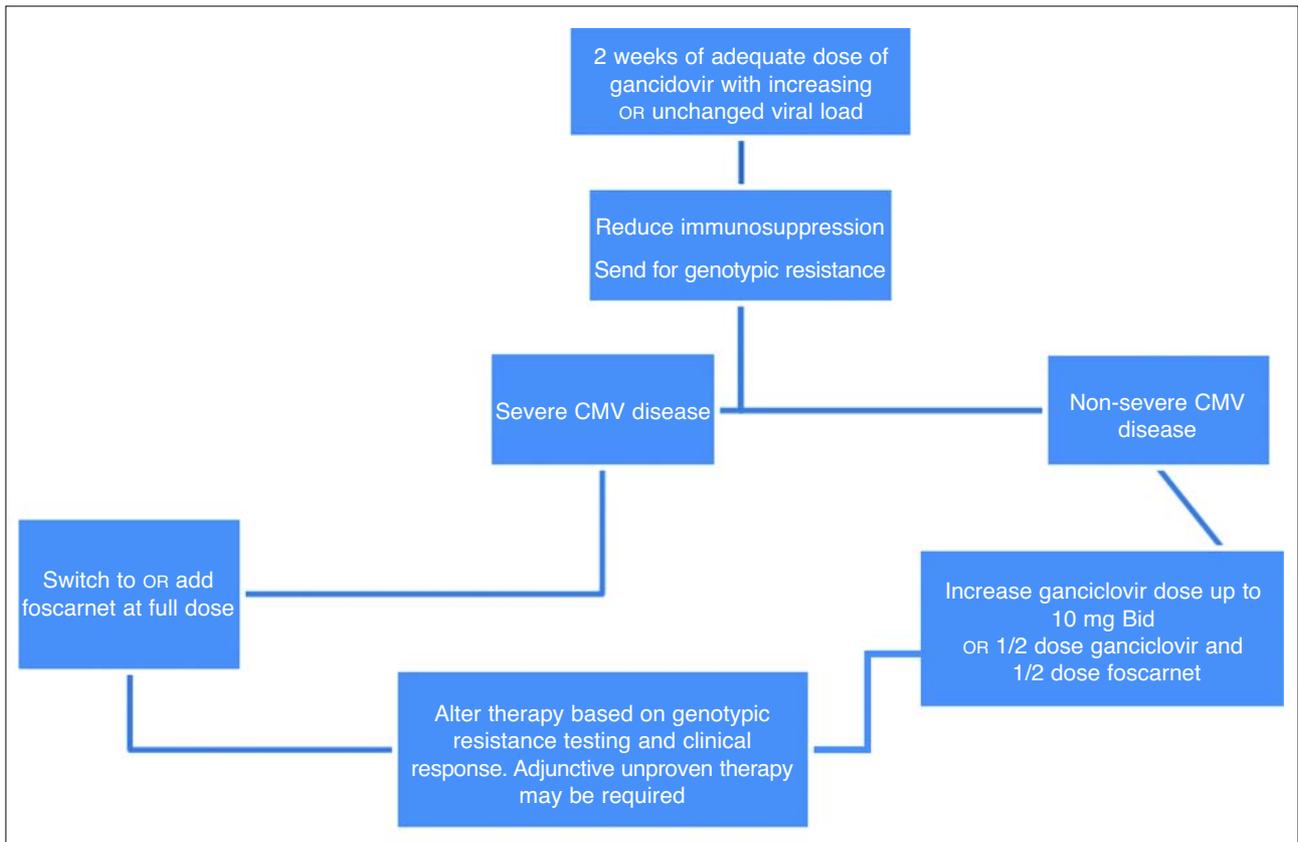

FIGURE 1. Algorithm for treatment of ganciclovir resistance.
Adapted from: Cytomegalovirus in solid organ transplant recipients. *American Journal of Transplantation*. (Humar A & Snydman D, 2009).

all seronegative recipients of a CMV-seropositive organ, (D$^+$/R$^-$) and in 20% of those with CMV disease [23].

Currently, the incidence of ganciclovir-resistant cytomegalovirus in kidney transplant recipients is estimated to be 0.54-1% [24]

Antiviral drug resistance is suspected when increasing or high-level CMV viremia or progressive clinical disease is observed during prolonged antiviral therapy. Genotypic assays for rapid antiviral resistance testing are useful to identify characteristic viral mutations indicative of drug resistance.

One of the main causes of resistance is a mutation of UL97 protein, a viral protein kinase needed for antiviral activity. When a major mutation exists causing more than 5-fold increased ganciclovir resistance, therapy should be switched to intravenous foscarnet. If there is a minor UL97 mutation, high-dose intravenous ganciclovir can be continued but additional testing for mutation of the gene encoding UL54 (the viral DNA polymerase catalytic subunit) is advisable [11]. A practical approach is suggested in Figure 1.

Novel therapies for treatment of ganciclovir-resistant CMV

The most promising is Maribavir (MBV) which is an orally administered potent inhibitor of the CMV UL97 kinase (UL97 kinase phosphorylates a number of substrates, including both viral and host proteins.) [25].

A study on six transplant patients (one of them a kidney recipient) who had failed to respond to other therapies and/or had known ganciclovir-resistant CMV were treated with maribavir. Four of six patients had no detectable CMV DNA viremia within six weeks of starting MBV therapy [26].

Another agent, Leflunomide has a striking antiviral activity against CMV, and there are some case reports that proved success of treatment of multiresistant CMV infections by leflunomide [27].

Finally, a novel anti-CMV (AIC246) compound which targets the viral terminase complex and remains active against virus resistant to DNA polymerase inhibitors has been developed by Kaul *et al*. [28] who reported the first successful case of CMV treated with this compound.

All of these novel therapies deserve further systematic evaluation as treatment for CMV-resistant infection.

CONCLUSION

Despite the important progress that has been made with diagnosis, prevention, and treatment of CMV disease, it continues to be a common cause of morbidity and mortality in transplant recipients.

It is important to focus in the future on developing effective vaccination programs and to develop studies of molecular markers of disease activity to determine the optimal duration of therapy. Finally, randomized studies will be needed to compare new medications versus ganciclovir.

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