Strategies for Preventing Late-Onset Cytomegalovirus Disease in Organ Transplant Recipients

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Abstract

Objective: ***The incidence of late-onset cytomegalovirus disease (i.e. disease appearing after discontinuation of antiviral prophylaxis) in solid-organ transplant recipients remains excessively high. This review will focus on describing the several strategies that could potentially reduce the incidence of late-onset cytomegalovirus disease.***

Methods: ***We reviewed the literature and presented our own clinical experience in the field.***

Results: ***The incidence of late-onset cytomegalovirus disease in recent trials can be as high as 36% in high-risk patients (donor positive/recipient negative for cytomegalovirus). The extension of antiviral prophylaxis to six months has recently proven in a prospective randomized controlled trial to be effective for reducing late-onset cytomegalovirus disease. The monitoring of cytomegalovirus viral load by PCR after the discontinuation of prophylaxis seems to be of moderate usefulness in low/intermediate-risk patients. The use of low-dose valganciclovir could reduce drug toxicity and costs while maintaining similar efficacy, but further studies are needed. A potentially interesting approach to predict the individual risk for development of cytomegalovirus disease appears to be the assessment of specific cell-mediated immune response. If cell-mediated immunity assays become widely available in transplant centers in the future, these assays may possibly be used to tailor the cytomegalovirus preventive strategy on an individual basis. Finally, recent prospective trials have evaluated novel cytomegalovirus vaccines that merit further evaluation in the transplant setting, although currently there is no cytomegalovirus vaccine that has been approved for routine clinical use.***

Conclusions: ***Several studies have recently evaluated novel strategies to reduce the incidence of late-onset cytomegalovirus disease. It is therefore expected that this improvement in preventive strategies will allow to further reduce the negative effects of cytomegalovirus disease after transplantation.*** **(Trends in Transplant. 2010;4:36-44)**

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Key words

Antiviral prophylaxis. Valganciclovir. Cellular immunity. Indirect effects.

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Introduction

Cytomegalovirus (CMV) has been recognized as the most significant viral pathogen after solid-organ transplantation[1]. During the first era of transplantation, CMV infection was associated with significant mortality, graft loss, and an increased incidence of invasive bacterial and fungal infection[2]. The introduction of routine strategies for prevention of CMV infection and disease and the improvement of antiviral therapies have led to a considerable reduction in CMV-associated morbidity. In addition, CMV is nowadays a very rare cause of mortality after solid-organ transplantation. Despite these major advances, some challenges remain in the management of CMV infection. Cytomegalovirus disease appearing after the discontinuation of antiviral prophylaxis, i.e. "late-onset CMV disease", is relatively common and is associated with some morbidity after solid-organ transplantation (Fig. 1)[3,4].

In this article, we will describe the clinical manifestations and risk factors for late-onset CMV disease. We will then review the preventive strategies for reducing the incidence of CMV disease.

Clinical manifestations and risk factors for late-onset cytomegalovirus disease

The incidence of late-onset CMV disease depends on the CMV serostatus of donor and recipient, the type of organ transplant, and the immunosuppressive regimen used for induction and maintenance therapy. In transplant centers using universal prophylaxis for all patients at risk for CMV, overall rates of CMV disease vary between 5-15%[5]. If only CMV donor positive/recipient negative (D^+/R^-) patients are analyzed, then the incidence can be as high as 30-40% (Table 1)[5-10]. Lung transplant

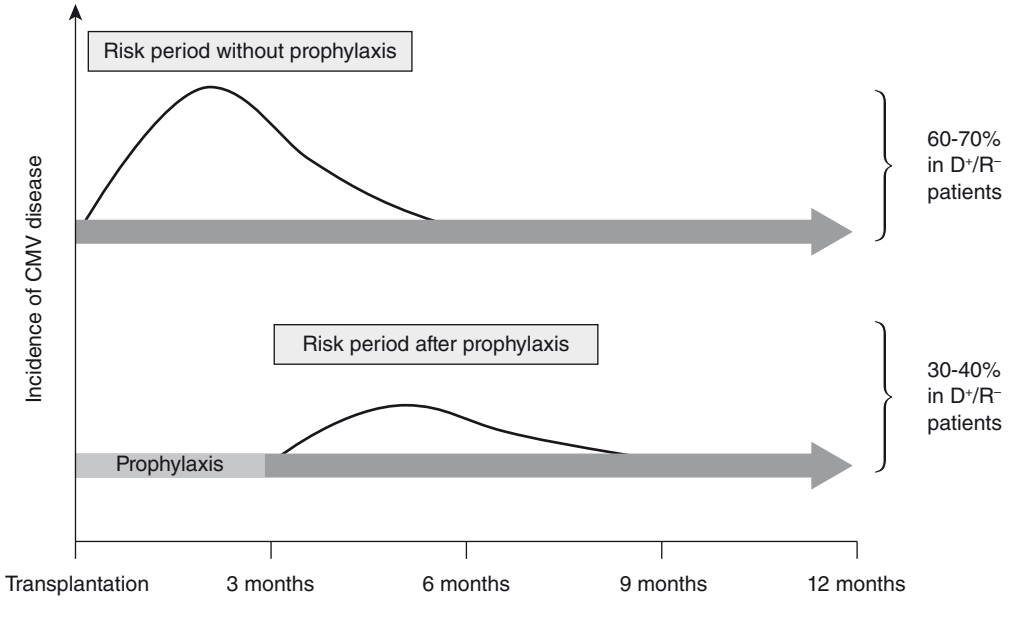

Figure 1. *Cytomegalovirus (CMV) disease without prophylaxis (upper part) and late-onset CMV disease following antiviral prophylaxis (lower part). A standard three-month period of antiviral prophylaxis can reduce by half the incidence of CMV disease, also delaying the onset of the infection (late-onset CMV disease).* Adapted with permission from Manuel, et al.[3].

Table 1. Incidence of late-onset cytomegalovirus disease in selected randomized controlled clinical trials using antiviral drug prophylaxis

Study	Date of publication	Organ transplant	CMV serostatus	Antiviral drug	Duration of prophylaxis	Incidence of CMV disease	p value
Lowance, et al.[9]	1999	Kidney	D^+/R^-	Valacyclovir Placebo	90 days 90 days	6 months: 16% 6 months: 45%	< 0.001
Lowance, et al.[9]	1999	Kidney	R^+	Valacyclovir Placebo	90 days 90 days	6 months: 1% 6 months: 6%	0.03
Paya, et al.[10]	2004	Kidney, liver, heart	D^+/R^-	Valganciclovir Ganciclovir	100 days 100 days	12 months: 17% 12 months: 18%	NS
Kliem, et al.[7]	2008	Kidney	D^+/R^- and R^+	Ganciclovir Preemptive approach	90 days 90 days	12 months: 5.5% 12 months: 26.5%	0.02/0.03*
Humar, et al.[6]	2009 (abstract)	Kidney	D^+/R^-	Valganciclovir Valganciclovir	100 days 200 days	12 months: 36% 12 months: 16%	< 0.0001

CMV: cytomegalovirus; D: donor; R: recipient; NS: not significant.
*p = 0.02 when comparing the incidence of viral syndrome (0 vs. 7.7%) and p = 0.03 when comparing the incidence of tissue-invasive disease (5.5 vs. 18.5%) using prophylaxis vs. preemptive approach, respectively.

recipients are generally at higher risk for developing CMV disease than other organ transplant recipients, although most of these patients now receive longer periods of prophylaxis, and therefore the incidence is lower than previously observed[11,12]. Although patients receiving induction therapy with antilymphocyte globulins are at higher risk for CMV, these patients generally receive universal antiviral prophylaxis, so that the risk for the development of CMV disease is attenuated at the time of prophylaxis discontinuation[13].

It is generally accepted that late-onset CMV disease is seen only in patients receiving antiviral prophylaxis after its discontinuation. Since most centers are using antiviral prophylaxis for D^+/R^- patients and this population is at the highest risk for developing late-onset CMV disease, the experience with late-onset CMV disease in D^+/R^- using a preemptive approach is less well defined. Some centers have reported low rates of CMV disease in high-risk patients followed by a preemptive approach[14]. This is in contrast with our own previous experience, when all organ transplant recipients were followed by a preemptive approach[15]. Indeed, D^+/R^- patients were at high risk for developing recurrent or protracted (> 30 days of viremia) CMV disease. For example, in the D^+/R^- group, 19/26 patients (76%) developed CMV infection, and 18/19 of those (95%) had a protracted course that generally required multiple courses of antiviral therapy[15].

The consequences of late-onset CMV disease are not completely established[4,16]. It is clear that without any preventive strategy in place, CMV can be associated with acute rejection, graft loss, opportunistic infections, and even a higher mortality[2]. Several meta-analyses have shown that antiviral prophylaxis reduces not only the incidence of CMV disease, but also the incidence of acute rejection and the all-causes mortality[16-19]. On the contrary, the potential advantages of the preemptive approach to decrease the indirect effects of CMV are less strongly proven, although the majority of recent studies using this approach showed a low rate of adverse outcomes[20,21]. Therefore, it appears that CMV disease occurring after any appropriate preventive strategy (prophylaxis or preemptive) should be associated with less morbidity. This is indeed consistent with our experience as we

did not find any differences in terms of outcomes (including allograft function) in kidney transplant recipients with or without late-onset CMV disease[8]. However, other studies have found an association between late-onset CMV disease and a higher mortality in kidney and liver transplant recipients[22,23]. In a cohort of 176 D^+/R^- kidney transplant recipients receiving three months of oral ganciclovir (GCV) or valganciclovir (VGC), the occurrence of tissue-invasive CMV disease after prophylaxis was associated with allograft loss or mortality (HR: 2.85; p = 0.016)[22]. Limaye, et al. also found a relationship between CMV disease and mortality at one year (HR: 5.1; p = 0.002), especially in case of infection-associated mortality (HR: 11; p = 0.002) in 437 liver transplant recipients[23]. The lack of association between late-onset CMV disease and mortality in our series could perhaps be explained by the fact that patients were promptly diagnosed and treated, thus avoiding the occurrence of CMV-associated indirect effects[8].

Valganciclovir as antiviral drug for cytomegalovirus prophylaxis

Valganciclovir has become the drug of choice for CMV prophylaxis, and it is used in the majority of centers in the USA and Europe[24]. The advantage of VGC compared to oral GCV is its enhanced bioavailability, allowing having a higher GCV exposure with a once-a-day medication. Valganciclovir was compared to oral GCV (the PV16000 study, a multicenter trial) in more than 300 solid-organ transplant recipients (mainly kidney and liver)[10]. There were no differences in terms of late-onset CMV disease between the two groups at 12 months (17.2% in the VGC group vs. 18.4% in the GCV group), although there was a higher incidence of tissue-invasive disease in the VGC group in the subgroup of liver transplant recipients. Second, there was a trend towards more neutropenia in the VGC group[25].

Recently, some studies have somewhat challenged the preferential use of VGC as the drug of choice for CMV prophylaxis. First, Shiley, et al. retrospectively compared the incidence of CMV disease occurring after VGC prophylaxis with an historical cohort of patients receiving oral GCV in liver transplant recipients[26]. Concordant with the PV16000 study, patients who received VGC had a higher risk for developing late-onset CMV disease than those receiving oral GCV. A recently published meta-analysis also assessed the efficacy of safety of VGC as prophylaxis for CMV[27]. In this analysis, the risk of late-onset CMV disease was similar in patients using VGC and GCV, but surprisingly it was higher compared to patients using other antiviral therapies (basically acyclovir and valacyclovir). Again, the risk of late-onset CMV disease was higher in liver transplant recipients receiving VGC, and the risk of neutropenia was also higher in patients on VGC. Although the consequences of neutropenia were not discussed in this study, our experience indicates that neutropenia in patients receiving VGC can be resolved without complications if VGC is temporarily discontinued[5].

Despite these two studies[26,27], most transplant physicians in charge of solid-organ transplant patients acknowledge the major improvements achieved in the management of CMV infection since the introduction of VGC[16,28]. Valganciclovir remains a very convenient and efficacious drug for the prevention of CMV disease after organ transplantation.

Strategies for preventing late-onset cytomegalovirus disease

Several strategies exist to reduce the incidence of late-onset CMV disease in solid-organ transplant recipients. Since both antiviral prophylaxis and preemptive therapy efficiently reduce the incidence of CMV disease

in low/intermediate-risk seropositive recipients (to levels of less than 5%), this section will focus on the population at the highest risk for CMV-related complications, i.e. D^+/R^- patients.

Prolonging antiviral prophylaxis

One possible strategy to reduce the incidence of late-onset CMV disease is to extend the duration of antiviral prophylaxis beyond the "standard" three-month period. Some data based on retrospective studies suggest a benefit of this strategy in kidney and lung transplant recipients. For example, Zamora, et al. showed that lung transplant recipients who received less than six months of VGC had a higher risk of developing CMV disease (6.9%; n = 29) than patients who received longer period of antiviral prophylaxis (0%; n = 61)[12]. Recently, a small retrospective study in D^+/R^- lung transplant recipients showed very similar results, with 44 vs. 13% of CMV disease in patients receiving three vs. 12 months of antiviral prophylaxis, respectively[11]. As a consequence of these studies, the majority of centers are now using at least six months of VGC as the standard duration of prophylaxis in lung transplant recipients. Interestingly, some rare centers have used lifelong antiviral prophylaxis, seeing a reduction on the incidence of obliterative bronchiolitis, although the cost-effectiveness of this strategy has not been evaluated[29]. Regarding kidney transplant recipients, a retrospective comparison of two historical cohorts of patients receiving three vs. six months of prophylaxis indicated also a benefit of prolonging prophylaxis[30]. Again, no higher rate of adverse events was seen in the six-month group of patients.

At the American Transplant Congress (Boston, 2009), Humar, et al. presented the results of the IMPACT clinical trial, which compared 100 vs. 200 days of antiviral prophylaxis with VGC in high-risk (i.e. D^+/R^-) kidney transplant recipients[6]. The IMPACT trial showed a significant reduction of CMV disease in the 200-days group (16 vs. 36% in the 100-days group), without a higher incidence of drug-related adverse events. Incidence of acute rejection and graft loss was overall low in both groups, and no significant differences were observed. No data is available regarding long-term outcomes in patients with or without CMV disease. The relatively high incidence of CMV disease in the 100-days group (36%, higher than for example 18% in the PV16000 study) could be explained by the new definitions used for CMV disease, e.g. fever > 1 day was not a necessary criteria for diagnosing CMV disease.

Preemptive therapy after antiviral prophylaxis ("hybrid" approach)

Since the onset of CMV disease correlates with the slope of viral load in blood[31], the monitoring of CMV viremia after the discontinuation of antiviral prophylaxis using a quantitative method (generally measuring of CMV DNA by PCR) could potentially identify patients with low-grade viremia before they eventually develop CMV-associated symptoms. This preemptive approach after prophylaxis, or "hybrid" approach according to some authors[32], has not been extensively studied due to the difficulty of organizing regular monitoring in patients a long time after transplantation (> 3-6 months). Humar, et al. evaluated the usefulness of the monitoring of PCR every 2-4 weeks after three months of GCV or VGC prophylaxis in D^+/R^- organ transplant recipients[33]. According to their study, approximately one-third of late-onset CMV disease could have been avoided using this strategy. Our own recent experience with regular monitoring after prophylaxis indicates that most of the low/intermediate-risk patients (R^+ recipients) present either with no or low-grade viremia without consequences, whereas

D^+/R^- patients often present with simultaneous viremia and CMV disease, which can be difficult to prevent even with appropriate PCR monitoring (Boillat, et al., manuscript in preparation).

Low-dose valganciclovir and delayed prophylaxis

Some pharmacokinetic studies have shown that the GCV exposure of daily oral GCV (3 g) is similar than that of half a dose of VGC (i.e. 450 mg daily instead of 900 mg for normal kidney function)[34]. Because oral GCV is an equally effective drug as compared to VGC[10], and oral GCV is associated with slightly less neutropenia, low-dose VGC may be an attractive strategy to reduce adverse events and costs, while effectively preventing tissue-invasive CMV disease. Some studies using VGC 450 mg instead of 900 mg have shown a similar efficacy than that obtained with VGC 900 mg, although most of these studies have been retrospective[35,36]. Indeed, in the majority of these studies, a "low dose" was actually a "fixed dose", i.e., the dose of 450 mg was after not adapted to kidney function[37]. In a prospective pharmacokinetic study from our institution, we found that GCV exposure after administration of VGC 450 mg daily in recipients with normal kidney function was comparable to that reported with oral GCV in the PV16000 study (29.6 vs. 28.0 mg·h/l, respectively)[38]. No major differences in adverse events according to GCV exposure were observed. In the meta-analysis by Kalil, et al. the rate of adverse events was also similar between low-dose vs. standard-dose VGC[27].

Recently, an original study evaluated a new strategy for reducing the incidence of late-onset CMV disease, consisting in initiating antiviral prophylaxis between 14-21 days after transplantation in D^+/R^- organ transplant recipients[39]. Only 5.5% of the patients (1/18) developed CMV disease compared to 27% (7/26) of patients in the conventional group ($p = 0.07$) in which prophylaxis was initiated at a median of 14 days after transplant. Of note, no patients received thymoglobulin in the delayed-prophylaxis group. According to the authors, this strategy might allow the transplant recipient to be exposed to CMV and, therefore, to start mounting a specific immune response against CMV, although no detailed assessment of cell-mediated immune response was described to confirm this hypothesis. Although potentially interesting, these strategies need confirmation in larger studies.

Monitoring of cytomegalovirus-specific cell-mediated immunity

Both B-cell and T-cell mediated immune response play a key role in the control of CMV infection in solid-organ transplant recipients[40]. Theoretically, the assessment of the humoral and cellular immune response at the time of discontinuation of antiviral prophylaxis could identify the patients at highest risk for the development of CMV disease[41]. These patients could thus benefit from a reduction of immunosuppression or from an extension of antiviral prophylaxis.

Regarding humoral immunity, a substudy of the PV16000 trial evaluated the usefulness of CMV serology in predicting the development of CMV disease in high-risk D^+/R^- solid-organ transplant recipients[42]. Overall, patients who seroconverted at the end of the prophylaxis period (three months) had a similar risk of developing CMV disease as patients who remained serologically CMV negative, thus showing that serology alone is of limited value for predicting CMV disease.

More recently, there has been an increasing interest in measuring specific cell-mediated immunity as a tool to identify the patients at higher risk for developing CMV

disease[43-46]. Examples of *ex vivo* assays measuring CMV-specific cell-mediated immunity include enzyme-linked ImmunoSpot (ELISPOT) and flow cytometric intracellular cytokine staining, which allow the detection of interferon-γ secreting cells in response to *in vitro* CMV antigen stimulation. Because most of these assays have been tested in experimental settings using in-house assays, it has been difficult so far to validate and incorporate them in the routine clinical practice.

A new assay called QuantiFERON®-CMV (Cellestis) has been recently evaluated as a test to predict CMV disease in transplant recipients at high risk for CMV disease (i.e. D^+/R^- patients, patients receiving thymoglobulin, and lung transplant recipients)[47]. This assay also measures the *in vitro* release of interferon-γ by secreting cells, and is very similar to the QuantiFERON®-TB in Tube assay[48], which is extensively used for the diagnosis of latent tuberculosis infection. Thus the main advantage of the QuantiFERON®-CMV assay is to use a standardized test that can be performed even in centers without research capability[49]. Kumar, et al. showed that patients with a detectable interferon-γ response at the time of prophylaxis discontinuation had a lower risk for developing CMV disease compared to those patients with a negative response (5.3 vs. 22.9% patients, respectively; $p = 0.038$)[47]. To confirm or not these results, an international multicenter trial in D^+/R^- organ transplant recipients is currently ongoing using the QuantiFERON®-CMV assay.

If confirmed, these assays could be used in the near future to tailor CMV preventive strategies on an individual basis according to the actual risk for the development of CMV disease, and not only based in general markers[50].

Vaccination

Finally, a paramount achievement in the prevention of CMV after transplantation would be the development of a protective vaccine against CMV. The challenges associated with CMV vaccination have been recently nicely reviewed by others[51,52]. Currently, there is no CMV vaccine that has been approved for use in the routine clinical setting. Two recent prospective trials have evaluated novel CMV vaccines that merit further evaluation in the transplant setting. First, Pass, et al. evaluated a vaccine in seronegative women consisting of recombinant CMV envelope glycoprotein B with MF59 adjuvant[53]. Vaccine efficacy was evaluated at approximately 50%, decreasing the subsequent incidence of maternal CMV infection. It is not known, however, whether this vaccine would be protective in solid-organ transplant recipients, since the glycoprotein B elicits predominantly a humoral response, and T-cell mediated immunity seems to play a more important role in the control of CMV infection in transplant recipients. Second, a bivalent CMV vaccine using DNA technology showed promising results in terms of safety and immunogenicity in healthy volunteers. This vaccine is composed by pp65 antigen and glycoprotein B in order to boost both cell-mediated and humoral immunity[54]. A trial using this vaccine in hematopoietic stem cell transplant recipients is ongoing.

Summary and conclusions

In parallel to the remarkable reduction in the incidence of acute rejection and graft loss over the last 10 years due to the improvement in the immunosuppressive regimens after solid-organ transplantation, the routine usage of universal preventive strategies has also significantly decreased the CMV infection incidence and the morbidity and mortality associated to viral infection. Cytomegalovirus is not anymore the feared virus that could jeopardize the kidney allograft or even the patient's life. The typical kidney transplant recipient with CMV disease generally presents now with mild-to-moderate disease (fatigue, low-grade fever,

diarrhea), and can be treated in an outpatient setting with oral VGC[55,56]. Cytomegalovirus-associated complications are seen only in a minority of patients, e.g. those severely immunosuppressed. Nevertheless, the incidence of late-onset CMV disease after prophylaxis discontinuation remains too high.

Over recent years, several studies have evaluated novel strategies to reduce the incidence of late-onset CMV disease. Some of these, such as the extension of the period of prophylaxis (IMPACT study), are already becoming the standard of care in the clinical setting for D^+/R^- patients. Other approaches that have shown promising results, such as the use of cell-mediated immune assays to predict CMV disease, are currently under evaluation in larger trials. It is therefore expected that over the coming years this new progress in delineating the optimal preventive strategies of CMV after transplantation will allow us to further reduce the negative effects of this important viral infection.

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