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Viral load, CMV-specific T-cell immune response and cytomegalovirus disease in solid organ transplant recipients at higher risk for cytomegalovirus infection during preemptive therapy

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Summary

Despite advances in prevention, cytomegalovirus (CMV) recurrence is an important challenge in high-risk organ recipients. The present study prospectively evaluates the impact of CMV-specific T-cell immune response and secondary prophylaxis on the risk of recurrence in a cohort of CMV high-risk organ recipients and whether it is possible to determine a safe standardized viral load value below which CMV disease is unlikely. Thirty-nine recipients were included. Thirty-six had primary infections, and 88.9% recurred. Rate and duration of recurrent CMV infection was similar in patients with and without secondary prophylaxis: 57.9% vs. 53.6%, $P = 0.770$ and 16 vs. 15 days, $P = 0.786$, respectively. The only factor independently associated with no episodes of CMV recurrence was the acquisition of CMV-specific T-cell immune response (OR: 0.151, 95% CI: 0.028–0.815; $P = 0.028$). Cytomegalovirus diseases ($N = 5$) occurred in patients with CMV viral load above 1500 IU/ml who did not follow the planned monitorization schedule. Our observations suggest that episodes of recurrent CMV infection are common after preemptive therapy despite secondary prophylaxis and that CMV-specific T-cell immune response is associated with a decreased risk of recurrent infections. Preemptive therapy may be safe in patients at high risk for CMV infection with strict close monitoring of the CMV viral load.

Introduction

Cytomegalovirus (CMV) infection continues to be an important cause of morbidity in solid organ transplant (SOT) recipients. Overall, higher peak CMV viral loads are correlated with clinical symptoms of CMV infection and CMV-related complications [1,2]. In some studies, CMV primary infection that occurs in seronegative recipients

with graft from seropositive donors (D+R−) has been associated with higher viral loads compared with those for CMV recurrence [3,4]. The decline in CMV viral load during antiviral treatment has been correlated with treatment outcomes, thus viral load kinetics has been proposed as predictor of CMV disease and as a tool to establish the optimal duration of antiviral therapy [5]. For the diagnosis of tissue-invasive disease, the American Society for Transplantation recommended correlating viral loads with immunohistopathology and clinical outcomes. Some studies have identified cut-offs for predicting CMV disease in series of patients at low risk [6,7], while no viral load cut-off has been determined in SOT recipients at higher risk for CMV disease in the absence of symptoms.

The acquisition of a CMV-specific T-cell immune response has been associated with spontaneous clearance of CMV viremia in patients at high risk for CMV infection [8]. Some studies have reported that this determination before and after the transplant may predict CMV disease and CMV infection [9–11]. However, other studies have reported that specific $CD4^+$ and $CD8^+$ T cells correlated with concurrent but not subsequent CMV viremia and therefore would not be useful to predict CMV recurrence [12–15].

Secondary prophylaxis has been suggested in consensus documents for patients at high risk for CMV infection in order to prevent recurrent infections [16]; nevertheless, there are not prospective studies that address this issue with a high level of evidence.

The aims of this study were to prospectively evaluate whether it is possible to determine a safe standardized viral load value below which CMV disease is unlikely in a cohort of SOT patients at high risk for CMV infection, and to analyze the influence of CMV-specific T-cell immune response and secondary prophylaxis on the risk of recurrence in this group of patients.

Patients and methods

We performed an observational prospective cohort study of consecutive patients at high risk for CMV infection (D+R−) undergoing SOT (liver, kidney, heart). Patients were included from October 2008 to May 2012. CMV viral load was monitored in all patients during the 12 months of follow-up. Blood samples were collected weekly for the first 100 days after the transplant, every other week between day 100 and 180 and monthly to complete the follow-up period. During the episodes of replication, patients were monitored every week until 2 weeks after the end of treatment. CMV viral load and CMV-specific T-cell immune response were determined in patient's samples at each time point during the follow-up. CMV viral load was determined in plasma using the Quant CMV LightCycler 2.0 real-time PCR system from October 2008 to April 2012 and using the Cobas Ampliprep/Cobas Taqman CMV test (Roche Applied Science Roche Molecular System, Branchburg, New Yersey, EE.UU) from April 2012 to June 2013. Results were standardized to international units per milliliter (IU/ml) using the World Health Organization (WHO) International Standard for Human CMV for Nucleic Acid Amplification Technique (National Institute for Biological Standards and Controls, NIBSC 09/162). One copy of CMV DNA using the COBAS Ampliprep/COBAS Taqman CMV test was equivalent to 0.91 International Unit (IU), while for the Quant CMV LightCycler 2.0 real-time PCR assay was equivalent to 1.53 as previously described [7]. CMV-specific T-cell immune response was determined as previously described [8]. Briefly, peripheral blood mononuclear cells were stimulated with 1 μg/ml of each of the peptides PepMix HCMV pp65 and PepMix HCMV IE-1 (JPT Peptides Technologies GmbH, Berlin, Germany). Unstimulated blood was used as a negative control, and for positive control, blood was stimulated with 1.5 mg/ml of *Streptomyces conglobatus* ionomycin and 25 ng/ml of PMA (4-alpha-phorbol 12-myristate 13-acetate, Sigma Aldrich, Buchs, Switzerland). All samples were co-stimulated with 1 mg/ml of CD28/CD49d (Beckton Dickinson, San Jose, CA, USA), and 10 mg/ml Brefeldin A (Becton Dickinson) was used to prevent cytokine secretion. Samples were incubated 4 h at 37 °C and 5% CO2, followed by 15 min incubation at room temperature with FACS Lysis solution (Beckton Dickinson). Samples were washed with PBS and processed for flow cytometry analysis. Cells were incubated in the dark for 20 min at room temperature with the monoclonal antibodies: 0.04 mg/ml anti-human CD69 PE, 0.1 mg/ml anti-human CD4 PerCP/Cy5.5, 0.1 mg/ml APC/Cy7 anti-human CD8, and 0.5 mg/ml Alexa Fluor® 700 anti-human CD3 (Biolegend). Cells were fixed by adding 50 μl of reagent IntraPrep 1 (Beckman Coulter, Fullerton, CA, USA) to each tube and incubating for 15 min. After washing with PBS, cells were permeabilized by adding 50 μl of permeabilization reagent IntraPrep 2 and incubating for 1 min. For intracellular cytokine, staining samples were incubated in the dark for 15 min at RT with 0.025 mg/ml APC anti-human IL-2 and 0.05 mg/ml FITC anti-human IFN-γ. Cells were washed and resuspended in 250 μl of PBS. Thirty thousand $CD3^+$ cells were analyzed on the LSRFortessa cytometer (Becton Dickinson) using the FACSDIVA software (version 6.2; Becton Dickinson, Biosciences). The percentage of activated $CD4^+$, $CD8^+$, and $CD3^+$ T cells that produced IFN-γ, IL-2, and CD69 were normalized to the negative control. Samples were considered positive when CD69 expression was more than 1% and IFN-γ secretion was more than 0.25%, normalized to the total number of $CD3^+$ T cells.

Cytomegalovirus infection and disease were defined according to the GESITRA-SEIMC/REIPI recommendations for the management of CMV infection in SOT recipients that were based on the definitions published by Ljungman *et al.* and The International Consensus Guidelines [17–19]. Preemptive therapy was initiated when a positive result was obtained by real-time PCR or if evidence of symptoms of CMV disease. Patients received 900 mg valganciclovir twice daily during 21 days and/or until two consecutive negative viral loads. In order to prevent recurrent CMV replication episodes, some patients received secondary prophylaxis (valganciclovir 900 mg daily) during 2 or 3 weeks following preemptive therapy. The decision of instituting secondary prophylaxis was left to the criteria of the clinician in charge of the patient. The study was approved by the local Ethics Committee for Clinical Research, and patients gave written informed consent. Data were recorded in a standardized computer-assisted protocol. Data related to viral loads and epidemiology such as date and type of transplantation, age, sex, blood collection date, type of donor, immunosuppressive regimens, type of CMV disease, were collected. Data recorded related to CMV infection were valganciclovir dose and length, concomitant medication, duration of viremia and outcomes of infection. Data related to hematological parameters such as hemoglobin, neutrophils, and platelet cell counts at baseline and during preemptive treatment were collected. Grade ≥3 that refers to the severity of the adverse events defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) guidelines (version 4.0) were recorded. A descriptive analysis of episodes of CMV infection was performed. For categorical variables, the chi-square or Fisher's exact tests were used, and for continuous variables, the Student's *t*-test or Mann–Whitney *U*-test. Statistical and quantitative variables were expressed as median and interquartile range (IQR). The relative risk was expressed as odds ratios (OR) and 95% confidence intervals (CI). A linear regression model was performed to establish the association between the time for viremia clearance and the peak viral load. A bivariate analysis was performed to determine differences between CMV primary infection and CMV recurrence and for the prognosis according to the use of secondary prophylaxis. To estimate risk factors for recurrent CMV infections, a multivariate analysis was performed. All analyses were performed using SPSS statistical package (15.0 version SPSS Inc., Chicago, Illinois, EE.UU). Statistical significance was established for $P < 0.05$.

Results

A total of 39 D+R− SOT (23 kidney, 15 liver, 1 heart) recipients were included. The median age was 50 years (IQR 42–58), and 71.8% were male. Baseline characteristics of the patients are listed in Table 1.

Table 1. Baseline characteristics of the patients included in the study.

Variables	Values $N = 39$
Sex, Male/Female, *n* (%)	28/11 (71.8/28.2)
Recipient age, median (IQR)	50 (42–58)
Solid organ transplant, *n* (%)	
Kidney	23 (59.0)
Liver	15 (38.5)
Heart	1 (2.6)
Type of donor, *n* (%)	
Deceased donor	33 (84.6)
Living related	3 (7.7)
Living unrelated	3 (7.7)
Immunosuppressive regimens, *n* (%)	
Induction Therapy	
Basilixumab	10 (25.6)
Daclizumab	2 (5.1)
Maintenance	
Steroids	39 (100)
MMF/MPA	38 (97.4)
Tacrolimus	32 (82.1)
Cyclosporin	6 (15.4)
mTOR inhibitors	1 (2.6)
Others	1 (2.6)

A total of 94 CMV replication episodes were analyzed, 36 of which were primary infection that occurred at a median of 34 days (26–49) post-transplantation. Thirty-two primary infections (88.9%) were followed by at least one episode of recurrent CMV infection. Seventy-six CMV infections received antiviral therapy, all cases of primary infection and 40 cases of recurrences ($P < 0.001$). The 18 untreated CMV replication episodes corresponded to episodes of recurrent infection that occurred more than 90 days post-transplantation. All of them had detectable acquired CMV-specific T-cell immune response, and a watch and wait approach under clinical supervision was adopted. Primary infections were treated 39 days (25–48) vs. 27 days (22–34) in cases of recurrence ($P = 0.083$), with a median time for viremia clearance of 23 days (20–31) and 16 days (7–27), $P = 0.106$, respectively. In a linear logistic regression model, higher peak viral load tended to have longer viral clearance (β standardized coefficient 0.206, $P = 0.075$).

There were no statistical significant differences between primary infection and recurrent infection regarding to type of transplant, duration of viremia and treatment, peak viral load, antiviral used and CMV disease and mortality (Table 2).

After 19 episodes of CMV replication, secondary prophylaxis following preemptive therapy was established for a median of 16 days (14–24). Secondary prophylaxis was

used only in kidney recipients. When considering only kidney recipients, there were no statistical differences between episodes with or without secondary prophylaxis regarding to time since transplantation, induction therapy, rate of severe neutropenia (<1000 cells/μl), duration of viremia, peak viral load, and previous CMV recurrence (Table 3). Rate and duration of recurrent CMV infection was similar in patients with and without prophylaxis: 57.9% vs. 53.6%, $P = 0.770$ and 16 days (10–24) vs. 15 days (9–20), $P = 0.786$, respectively. No patient developed CMV infection during secondary prophylaxis. Duration of prophylaxis was not associated with risk of recurrence ($P = 0.400$) or time to recurrence ($P = 0.229$). A higher rate of severe neutropenia was not observed in patients with secondary prophylaxis, and no patient discontinued antiviral treatment because of hematological toxicity or other adverse effect. When adjusting for confounding factors in a multivariate analysis, secondary prophylaxis was not a protector factor of CMV recurrence (Table 4). The only factor independently associated with a decreased risk of CMV recurrence was the acquisition of CMV-specific T-cell immune response (OR: 0.151, 95% CI: 0.028–0.815; $P = 0.028$).

The acquisition of the CMV-specific T-cell immune response was characterized at several time points during the first year after transplantation. Two patients (5.1%) had no CMV-specific immune response and both died at 22 and 30 weeks, respectively, after transplantation for causes not related with CMV infection, while 37 (94.9%) acquired a CMV-specific T-cell immune response. The acquisition of immunity occurs at a median of 14.7 weeks (range 7–26) after the transplant with a median of 0.75% (range 0.3–3.55) of $CD8^+$ T cell expressing IFN-γ value that was significantly higher compared with 2 weeks after transplantation (median 0%, range 0–0.5; $P < 0.0001$; Fig. 1a). At the end of follow-up, patients also had significantly higher percentage of $CD8^+$ T cells expressing IFN-γ (median

Table 2. Bivariate analysis of episodes of cytomegalovirus (CMV) primary infection and CMV recurrent infection.

	CMV primary infection, *N* = 36	CMV recurrent infection, *N* = 58	*P*-value
Solid organ transplant, *n* (%)			
Kidney	20 (55.6)	38 (65.5)	0.335
Others	16 (44.4)	20 (34.5)	
Duration of viremia, days, median (IQR)	23 (21–32)	21 (11–30)	0.597
Median peak viral load, UI/ml, median (IQR)	4536 (1981–15 946)	3267 (1576–8484)	0.354
Preemptive therapy, *n* (%)	36 (100)	40 (69)	<0.001
Duration of treatment, days, median (IQR)	39 (25–48)	27 (22–34)	0.083
Preemptive therapy drugs, *n* (%)			
Valganciclovir	30 (83.3)	35 (87.5)	0.856
Ganciclovir iv–valganciclovir	5 (13.9)	4 (10.0)	
Ganciclovir iv	1 (2.8)	0 (0)	
Foscarnet	0 (0)	1 (2.5)	
Clearance time of viremia after treatment initiation, days, median (IQR)	23 (20–31)	16 (7–27)	0.106
Clinical infection, *n* (%)			
Asymptomatic	33 (91.7)	56 (96.6)	0.320
Symptomatic	3 (8.3)	2 (3.4)	
Viral syndrome	1 (2.8)	1 (1.7)	
Organ disease	2 (5.6)	1 (1.7)	
Outcome, *n* (%)			
Cure	36 (100)	56 (96.6)	0.999
Death	0 (0)	2 (3.4)	

Table 3. Bivariate analysis of cytomegalovirus (CMV) replication episodes according to the use of secondary prophylaxis in kidney recipients.

	Secondary prophylaxis vs. no prophylaxis (19 vs. 28)		*P*-value
Time post-transplant, days, median (IQR)	76 (42–108)	106 (47–140)	0.574
Induction therapy, *n* (%)	8 (42.1)	10 (35.7)	0.659
Neutropenia, *n* (%)	3 (15.8)	8 (28.6)	0.310
Peak viral load, IU/ml, median (IQR)	8645 (2708–16 524)	5447 (2873–26 469)	0.271
Viremia duration, days, median (IQR)	30 (23–35)	25 (13–34)	0.655
CMV Recurrence episode, *n* (%)	11 (57.9)	15 (53.6)	0.770

0.46%, range 0.01–2.24; $P < 0.001$). We found similar kinetic regarding the CMV-specific $CD4^+$ T-cell subpopulation, with a significantly higher percentage of $CD4^+$ T cells secreting IFN-γ at the moment of acquisition of immunity (median 0.3%, range 0–5.4, $P < 0.001$; Fig. 1b) versus 2 weeks after transplantation (median 0%, range 0–0.8). We found no differences between the percentage of $CD8^+$ versus $CD4^+$ T cells secreting IFN-γ at 2 weeks or at the acquisition of the immune response, while at 12 months after the transplant, the percentage of $CD4^+$ T cell secreting IFN-γ was significantly lower compared with the percentage of $CD8^+$ T cell expressing IFN-γ (median 0.46% vs. 0.048%, respectively; $P = 0.007$).

We characterized whether the incidence of episodes of CMV replication was related with the acquisition of the CMV-specific T-cell immunity. The incidence of CMV replication increased rapidly after the third week of transplantation, and by 8 weeks, 35 (89.7%) patients had experienced CMV replication (Fig. 1c). Three patients never experienced CMV replication episodes, and the other had his first replication episode by week 34. After week 8, the incidence of replication decreased progressively until the end of the follow-up. No incidence of CMV disease or CMV-related mortality was reported in these patients. The decline in the incidence of CMV replication episodes inversely correlated with the acquisition of the CMV-specific

Table 4. Multivariate logistic regression of the risk of cytomegalovirus (CMV) recurrence.

	Recurrence vs. No recurrence (58 vs. 36)		Odds ratio (CI 95%)	*P*-value
Time from transplantation, days, median (IQR)	59 (31–107)	150 (97–188)	0.993 (0.982–1.004)	0.208
Kidney transplant	38 (65.5)	20 (55.6)	4.662 (0.988–21.997)	0.052
Positive cellular immune response	12 (20.7)	25 (69.4)	0.151 (0.028–0.815)	0.028
Peak viral load, IU/ml, median (IQR)	5386 (1951–16 409)	2861 (1622–6162)	1	0.401
Viremia duration, days, median (IQR)	25 (17–32)	20 (8–30)	1.006 (0.978–1.035)	0.692
Treatment duration, days, median (IQR)	32 (22–44)	21 (0–27)	1.029 (0.993–1.067)	0.118
Use of secondary prophylaxis, *n* (%)	14 (24.1)	5 (13.9)	0.532 (0.103–2.745)	0.451

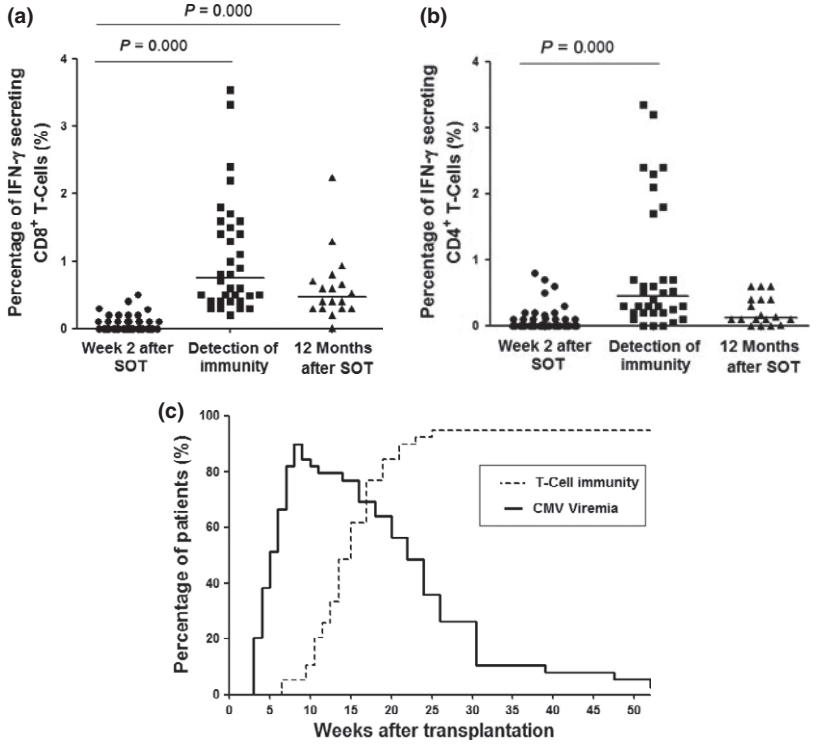

Figure 1 Cytokine kinetics, cytomegalovirus (CMV) replication, and CMV-specific T-cell response. CMV-specific immune response was evaluated by detecting intracellular secretion of interferon (IFN-γ) at the indicated times after transplantation in $CD4^+$ and $CD8^+$ T cells by flow cytometry. (a) IFN-γ secretion in $CD4^+$ T cells; (b) IFN-γ secretion in $CD8^+$ T cells. Percentages were calculated referred to the total number of CD3-positive cells analyzed. (c) Evolution of CMV infection measured by real-time PCR compared with detection of the CMV-specific immune response detected by intracellular cytokine staining using flow cytometry.

Table 5. Clinical, immunological, and virological findings and outcome of patients with cytomegalovirus (CMV) disease.

CMV disease	Organ transplanted	Time from transplantation (days)	CMV primary infection/recurrence	CMV viral load at diagnostic of infection (IU/ml)	Clearance time of viremia after treatment initiation (days)	CMV-specific T-cell immune response	Outcome
Viral syndrome	Liver	103	Recurrence	11 8728	5	Positive	Cure
Viral syndrome	Liver	38	Primary infection	2601	24	Negative	Cure
Typhlitis	Kidney	252	Primary infection	12 179	23	Positive	Cure
Gastritis	Liver	99	Recurrence	1556	25	Negative	Cure
Gastritis	Kidney	45	Primary infection	10 588	30	Negative	Cure

T-cell response (linear regression $r^2 = 0.775$, Pearson correlation=0.88; $P < 0.001$; Fig. 1c).

Patients with detectable CMV-specific T-cell immune response were treated during 27 days (22–34) compared with 31 days (22–41) in those without detectable CMV-specific T-cell immune response ($P = 0.528$).

Length of previous valganciclovir treatment was related with having recurrence in the bivariate analysis (OR: 1.062, 95% CI: 1.014–1.114; $P = 0.012$) but not in the multivariate analysis (OR: 1.029, 95% CI: 0.993–1.067; $P = 0.118$).

Except for one case, viremia was cleared in all CMV replication episodes after completing antiviral treatment. Five patients (three primary infections and two recurrences) were diagnosed of CMV disease (two viral syndromes and three digestive diseases). Viral loads at diagnosis of CMV disease ranged from 1556 to 11 8728 IU/ml (Table 5). In the five patients, the median time from a previous negative viral load result to the onset of CMV disease was 12 days (range 10–21). A patient with a peak viral load of 11 8728 IU/ml cleared the viremia after 5 days of treatment. This episode corresponded to a recurrent infection manifested as a viral syndrome in a patient with acquisition of CMV-specific T-cell response. Two patients died unrelated to CMV infection, one patient (with detectable CMV viral load) because of biliary septic shock produced by a multiresistant *Acinetobacter baumannii* and the second patient (with undetectable CMV viral load) because of intestinal ischemia.

Discussion

The present study shows that in SOT recipients at high risk for CMV infection, all symptomatic CMV infection occurred with viral loads in plasma above 1500 IU/ml, with no differences between primary or recurrent infections. It also shows that the acquisition of CMV-specific T-cell immune response prevents from episodes of CMV recurrence in this group of patients, while secondary prophylaxis did not avoid CMV relapse.

Previous studies performed in patients at low risk described higher CMV viral loads in the absence of symptoms. One of these studies established a cut-off between 2000 and 5000 copies/ml for predicting disease in CMV seropositive liver transplant recipients [6]. In our group, we performed a prospective cohort study of consecutive SOT recipients at low risk establishing by receiver operating characteristic (ROC) plots a standardized cut-off value of 3983 UI/ml (2600 copies/ml) in plasma samples [7]. Although it is well-known that CMV infection is more frequent and severe in SOT patients at high risk, a CMV viral load value to safely initiate preemptive therapy in this population has not been determined. Based on antigenemia results, a cut-off of 1000 copies/ml was proposed; however, it was not validated in the clinical practice [20]. Most of the studies in SOT recipients have proposed nonstandardized viral load values to initiate antiviral therapy ranging from 1000 to 3000 copies/ml; however, these values were based on unpublished previous local experiences, and no differences were established between patients at low and high risk [1,3,4]. One study of a cohort of SOT recipients reported that a viral load of 2275 IU/ml adequately discriminated self-clearing infections from patients requiring therapy; however, only 3 D+R− patients were included in the study [21]. It has been previously reported that primary CMV infection has a different kinetic profile compared with recurrent CMV infection [3,5]. However, in our experience in SOT recipients at high risk for CMV infection, no statistical differences were found regarding viral load peaks, duration of viremia, and incidence of CMV primary infection and subsequent episodes of recurrent infection. The differences in patient characteristics of both studies may explain the discrepancies.

Reactivation in patients at high risk, without previous CMV-specific T-cell immunity may be comparable with primary infection and may be different to CMV reactivation episodes occurring in patients at low risk with previous exposure to CMV infection [3,22]. In a prospective study of CMV seropositive liver recipients, most of the episodes of CMV reactivation were asymptomatic, temporal,

self-limiting and with low-level DNAemias [23]. One study described that the median peak viral load, duration of viremia, and duration of treatment were higher during CMV primary infection than during the following episodes of reinfection and reactivation. In this study, the rate of increase in CMV viral load in whole blood was higher in D+R− patients with a median of doubling time of 1.45 days for the first episode and 2.10 days for the second episode ($P = 0.017$) [3]. Based on these results, patients at high risk might not have a safe lapse of time between viral load determinations to ensure the absence of CMV disease. In the present study, all cases of symptomatic disease occurred in patients with viral loads above 1500 IU/ml that did not accomplish the established schedule of CMV viral load monitoring. Therefore, in our experience, preemptive therapy strategy for preventing CMV disease with weekly CMV viral load monitoring may be safe in patients at high risk; however, it should be mandatory a strict adherence of the monitoring schedule, to avoid the risk CMV disease.

The acquisition of a CMV-specific T-cell immune response has been associated with spontaneous clearance of CMV viremia in patients at high risk for CMV infection [8]. Some studies have reported that this determination before and after the transplant may predict CMV infection and CMV disease [9–11]. In a recent study with 127 SOT recipients at high risk determined the utility of monitoring CMV-specific T-cell-mediated immunity to predict CMV disease after discontinuation of prophylaxis measuring the interferon (IFN)-γ response using the Quantiferon-CMV assay [24]. In the present study, CMV-specific T-cell immune response was prevented in patients at high risk for CMV infection. Only 20% of patients that had acquired a CMV-specific T-cell immune response recurred compared with 80% of patients that did not have immune response. Moreover, in the multivariate analysis, CMV-specific T-cell immune response was the only protector factor associated with episode of CMV recurrence.

The administration of secondary prophylaxis has been suggested in consensus documents in patients at high risk for CMV infection in order to prevent recurrent infections [16]; however, no conclusive studies have support this measure. In a retrospective study of 62 kidney transplant recipients, of whom only 11 were D+R−, no association was found between secondary prophylaxis and episodes of recurrence. However, the small number of patients at high risk for CMV infection precluded from driving firm conclusions [25]. Other authors in a retrospective study carried out in 26 D+R− SOT recipients, the authors reported that the use of prophylaxis after gastrointestinal CMV disease was not associated with CMV relapse [26]. In a randomized trial of 321 SOT recipients, the only independent factor predicting recurrent CMV disease was viral eradication at day 21 post-treatment. Secondary prophylaxis could not be evaluated as a variable because all patients received secondary prophylaxis [27]. To the best of our knowledge, this is the first study that has evaluated secondary prophylaxis prospectively in patients at high risk for CMV infection. Although this is not a randomized clinical trial and with the limited study sample, secondary prophylaxis did not prevent CMV relapse when adjusted for other confounding factors.

Some limitations of our study need to be highlighted. First, our results may not be applicable to other centers if different immunosuppresion regimens are administered. Second, since valganciclovir universal prophylaxis is administered in our hospital to patients with thymoglobulin regimen, they were not included in the study; thus, no conclusions can be made in this population. Third, as this was not a randomized trial, it was the clinician who decided the administration of secondary prophylaxis, and possible selection bias might have occurred. However, patient's baseline and viremia characteristics were similar in cases with and without secondary prophylaxis. And finally, the absence of differences between episodes of primary and recurrent infections may be related to the sample size.

In summary, our observations suggest that preemptive therapy may be safe in patients at high risk for CMV infection with strict close monitoring of the CMV viral load. In our experience, no episodes of symptomatic CMV disease were diagnosed in patients with viral loads below 1500 IU/ml. Because no relevant differences between CMV primary and recurrent infection were found in SOT recipients at high risk for CMV infection, the same clinical interventions may be applied. In addition, episodes of recurrent CMV infection occur commonly despite secondary prophylaxis, while CMV-specific T-cell immune response was associated with a decreased risk of recurrent infections. Further studies are warranted to confirm these data.

Authorship

CM-G: performed the clinical data collection, analyzed results, and generated all the tables and the manuscript. MS: performed the real-time PCR for CMV. PB-L and OJB-H: performed the determination of the CMV-specific T-cell immune response. MAG, CB and JMS: provided patient care. MJR-H: provided patient care. PP-R: designed and coordinated the work and participated in the writing of the paper. EC: designed and coordinated the work, provided patient care and was responsible for the project and the preparation of the paper. All authors reviewed the paper and had access to the primary clinical data.

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