Late Cytomegalovirus Infection in Kidney Transplant Recipients after a Six-Month Prevention Protocol

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ABSTRACT

Background: Despite a reduction in the incidence of cytomegalovirus (CMV) infections after kidney transplantation, less is known about late CMV infection in kidney transplant recipients.

Objective: To assess incidence of CMV infection in a cohort of patients under a high surveillance CMV prevention protocol and identify factors associated with late CMV infection.

Methods: Analysis of a consecutive cohort of 181 kidney allograft recipients between January 2012 and Aug 2015. CMV prevention-protocol consisted of 6-month universal prophylaxis and pre-emptive therapy for high-risk group (D+/R– or patients submitted to lymphocyte-depleting agent for induction or rejection treatment) and pre-emptive therapy for standard-risk group (D±/R+). Stopping valganciclovir was followed by CMV screening in the next two appointments.

Results: CMV infection was identified in 73 of 181 patients; the rate in high-risk group and standard-risk group was similar (p=0.443). However, in the latter group, the infection occurred mostly in the first 6 months. Late CMV infection occurred in 25 of 181 patients (5 of standard-risk group and 20 of high-risk group), after a median (IQR) of 253 (230.3–312.3) days after transplantation and 55 (41–89.5) days after the protocol period. Screening for CMV after valganciclovir discontinuation revealed 56% of late CMV infections. In high-risk group, D+/R– was associated with late CMV infection (HR 2.7, p=0.039) and in standard-risk group; lower age was associated with late CMV infection (HR 0.89, p=0.02).

Conclusion: The incidence of CMV infection was similar to that reported in the literature. In high-risk patients, antigenemia surveillance during prophylaxis did not appear to reduce late CMV infections. Antigenemia screening after valganciclovir had limited results in the diagnosis of late CMV infection. D+/R– was associated to late CMV infection in high-risk group. Lower age appeared to influence late CMV infection in standard-risk group.

KEY WORDS: Late cytomegalovirus infection; Renal transplantation; Risk factor

INTRODUCTION

Cytomegalovirus (CMV) infection is a common complication after renal transplantation [1]. In addition to direct effects of CMV, its indirect effects that further contribute to the morbidity associated with CMV infection have been identified [2]. Patients with CMV infection are at increased risk of renal graft loss, cardiovascular events and mortality [3, 4]. Prevention of CMV infection with pharmacological prophylaxis or pre-emptive treatment led to considerable reduction in the incidence of the infection and its seriousness [1, 5-7]. The recommendations of the Spanish Societies of Transplantation and Infectious Diseases [7] propose universal prophylaxis for 3–6 months in high-risk patients (recipients with prior negative IgG serology for CMV who received grafts from positive

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donors [D+/R–] or induction therapy with lymphocyte-depleting agents), followed by an undefined screening period [7]. This recommendation highlights the risk of late-onset CMV disease, occurring in up to 37% of D+/R– kidney transplant recipients after the end of six months prophylaxis [8]. Screening for the viral load eight weeks after stopping prophylaxis was found to be of limited benefit because a great proportion of late onset disease in solid organ transplants was diagnosed after that period [9]. Therefore, it is important to identify patients at greater risk of developing late CMV infection, since CMV D+/R– is the only well-established risk factor [10].

A cohort of patients received a CMV prevention protocol that used universal prophylaxis and pre-emptive therapy in high-risk patients and pre-emptive therapy for the standard-risk patients, followed by a screening period after valganciclovir therapy. The objective of this study was to assess the effect of using this prevention protocol on the incidence of CMV infection, including late CMV infection (after the prevention protocol period) and identify factors associated with late CMV infection.

MATERIAL AND METHODS

Study Population

We analyzed all consecutive adult allograft recipients followed (or transplanted) in our unit between January 2012 and August 2015. The exclusion criteria were follow-up less than six months (n=14) and recipients with prior negative serology for CMV who received grafts from negative donors (n=4). We included 181 patients who underwent the same maintenance immunosuppressive protocol and CMV preventive protocol.

The standard immunosuppression was a triple-drug regimen with tacrolimus, mycophenolate mofetil and steroids. According to immunological risk, patients received induction therapy with basiliximab (n=99) and antithymocyte globulin (ATG) or thymoglobulin (TG) (n=82). A subgroup of patients was also treated with intravenous immunoglobulin and rituximab (n=16). Recent rejection treatment was defined as additional immunosuppressive therapy given three months prior to the diagnosis of CMV infection.

All patients were followed in our unit and laboratory tests were performed weekly in the first month, 1–3-week intervals until six months, 2–6-week intervals until 12 months, and every two months during the second year.

CMV Infection Prevention Protocol

Patients were divided into two groups based on CMV infection risk—high-risk group comprising of D+/R–, patients who received lymphocyte-depleting agents as induction therapy or for acute rejection episode. This group received universal prophylaxis with oral valganciclovir (900 mg/day and adjusted for CrCl < 60 min/min) for six months. In the same period, all patients were screened for CMV antigenemia (CMVpp65 antigenemia test) at every clinic visit and on the two subsequent visits after valganciclovir discontinuation. Those with clinically significant CMV antigenemia were treated. Prophylaxis and screening were also maintained up to six months after rejection treatment. The standard-risk group included recipients with prior positive IgG serology for CMV who received grafts from positive or negative donors (D±/R+); no lymphocyte-depleting agent was used as induction therapy or anti-rejection treatment. This group was submitted to pre-emptive therapy. CMV antigenemia was screened for six months in every clinical visit; valganciclovir was started if clinically significant CMV antigenemia occurred.

In both groups, clinically significant CMV antigenemia were treated based on physician's opinion who considered patient's risk factors (cumulative immunosuppression, recipient CMV IgG negative) and presence of CMV infection-related symptoms. In the remaining cases, immunosuppression was reduced whenever possible.

The treatment consisted of valganciclovir

(900 mg bid, adjusted for CrCl < 60 min/min) until a negative CMV antigenemia result was attained followed by a variable sustained treatment period. All patients were screened for CMV antigenemia in two subsequent visits after discontinuation of the treatment.

CMV Infection Definition

CMV infection was defined as evidence of CMV replication regardless of symptoms [7, 11], i.e., at least one positive CMV antigenemia with one or more positive cells per 2×10^5 cells examined. Positive antigenemia were treated according to the caring physician's opinion. Severe CMV disease was considered in patients with CMV infection accompanied by clinical signs and symptoms that required hospitalization.

Early CMV infection was defined as all the positive CMV antigenemia occurring during the protocol period (up to six months). Late CMV infection was defined as all the positive CMV antigenemia after the protocol period (after six months). These infections were classified as reactivations or primary late infections.

Statistical Analysis

The data collected from patients' medical charts were reviewed. The baseline data included age, sex, race, primary renal disease, diabetes mellitus, renal replacement therapy prior to transplantation, length of pretransplant dialysis, donor source, CMV status prior to transplantation (CMV IgG donor/recipient), cold ischemia time, HLA mismatch, and induction therapy. Follow-up data included CMV infection, severe CMV disease, early CMV infection, late CMV infection, time until CMV infection, time until late CMV infection, time until late CMV infection after the protocol, rejection treatment (and recent rejection treatment), estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration formula at the 1st, 6th, 12th, and 24th months, mortality, and graft loss.

Data were expressed as number (%) or median (IQR). Statistical significances between the groups were measured by Mann-Whitney U test for continuous variables without normal distribution, and by χ^2 or Fisher exact test for categorical variables. Cox regression analysis was used to identify independent factors associated with late CMV antigenemia using time to late CMV antigenemia after the protocol. Variables with p value <0.1 in univariate analysis were included in the multivariate model. Age, sex and CMV-prevention protocol group were forced into the multivariate model. Cold ischemic time was defined as being 20 min for living-donor recipients for Cox regression analysis. SPSS® for Windows® *ver* 17.0 (SPSS Inc, Chicago, IL, USA) was used for data analysis. A p value <0.05 was considered statistically significant.

RESULTS

We studied 181 kidney (110 male, 60.8%) recipients with a median (IQR) age of 54 (41.5–61) years (Table 1). During a median (IQR) follow-up of 29.3 (17–40.3) months, three patients died from sepsis, H_1N_1 infection, and unknown cause; and five lost their grafts. Reasons for graft loss were acute rejection, chronic rejection, two relapses of primary renal disease, and urologic complication. Deaths or graft losses were not associated with valganciclovir or CMV infection. Acute rejection was diagnosed in 18 (9.9%) patients.

During the follow-up, 73 (40.3%) patients had positive CMV antigenemia after a median (IQR) of 60 (39.5–154.5) days of transplantation (Fig 1). In CMV-positive patients, the median (IQR) maximal antigenemia was 7 (3–35.5) cells/2×10^5. Fifty-nine (32.6%) patients were treated (Table 2). The median (IQR) maximal antigenemia in treated patients was 15.5 (5–65) cells/2×10^5. Five (2.8%) had severe CMV infection—3 happened after the protocol period. There were no significant difference between patients who developed CMV infection compared with those who did not in terms of graft loss (p=0.43) and mortality (p=0.59).

Considering CMV-prevention protocol, 98

Table 1: Characteristics of patients with late CMV infection *vs.* no late CMV infection. Values are either median (IQR) or n (%).

Parameters	Total (n=181)	No late CMV infection (n=156)	Late CMV infection (n=25)	p value
Age	54 (41.5–61)	56 (43.3–62)	45 (33–52.5)	0.001
Male	110 (60.8)	96 (61.5)	14 (56)	0.599
Caucasian	148 (81.8)	129 (82.7)	19 (76)	0.411
Diabetes	20 (11.0)	20 (12.8)	0 (0)	0.081
Primary renal disease				
DM	15 (8.3)	14 (9.0)	1 (4)	
GN	33 (18.2)	27 (17.3)	6 (24)	
ADPKD	22 (12.2)	20 (12.8)	2 (8)	0.536
Others	63 (34.8)	51 (32.7)	12 (48)	
Unknown	48 (26.5)	44 (28.2)	4 (16)	
HD/PD	143 (79.0)/29 (16.0)	126 (80,8)/17 (9.2)	17 (80)/5 (20)	0.137
Preemptive	9 (5.0)	6 (3.8)	3 (12)	
Pretransplant dialysis (months)	68.39 (41.17–94.47) n=170	71.3 (42.3–95.6) n=148	57.6 (31.2–92) n=22	0.263
CMV status prior to KT				
D–/R+	15 (8.3)	14 (9)	1 (4)	
D+/R+	143 (79.0)	127 (81.4)	16 (64)	0.013
D+/R–	23 (12.7)	15 (9.6)	8 (32)	
CMV-prevention protocol				
Standard risk group	83 (45.9)	78 (50)	5 (20)	0.005
High risk group	98 (54.1)	78 (50)	20 (80)	
Deceased donor	155 (85.6)	136 (87.2)	19 (76)	0.214
Living donor	26 (14.4)	20 (13.8)	6 (24)	
HLA-A.B. DR mismatch	4 (3–4) n=180	4 (3–5) n=155	4 (3–4)	0.825
Cold ischemic time (min)*	990 (742.8–1223.8) n=146	1078 (900–1325) n=126	983 (799–1177) n=23	0.231
Induction therapy on KT				
Basiliximab	99 (54.7)	87 (55.8)	12 (48)	0.358
ATG/TG	82 (45.3)	65 (44.2)	13 (52)	
Rejection treatment at 6 months	10 (5.5)	8 (5.1)	2 (8)	0.631
Recent rejection treatment	5 (2.8)	4 (2.6)	1 (4)	0.529
eGFR after				
1 month	54 (40–67)	53 (43–71.8)	58 (40.7-69.3)	0.912
6 months	57 (46–70.5)	55.5 (46–68.8)	64.5 (50.5–73.5)	0.503
12 months	57 (46–70) n=159	56.6 (46–69.8)	60 (50.8–77.8)	0.333
24 months	60 (46–71.8) n=112	60 (46–72.8)	60 (43.75–66.5)	0.848

Continued

Table 1: Characteristics of patients with late CMV infection *vs.* no late CMV infection. Values are either median (IQR) or n (%).

Parameters	Total (n=181)	No late CMV infection (n=156)	Late CMV infection (n=25)	p value
Protocol period (days)†	190 (159.5–220.5)	194 (161.5–200.5)	189.5 (159.3–222.8)	0.897
Follow-up (months)	29.3 (17–40.3)	29.7 (16.7–40.9)	26.6 (19.4–37.3)	0.844

*Living donors were not included; †Includes prophylactic and screening period. CMV: cytomegalovirus; DM: diabetes mellitus; GN: glomerulonephritis; ADPKD: autosomal dominant polycystic kidney disease; HD: hemodialysis; PD: peritoneal dialysis; KT: kidney transplantation; D+/R–: CMV IgG donor positive, recipient negative; D+/R+: CMV IgG donor positive, recipient positive; D–/R+: CMV IgG donor negative, recipient positive; ATG/TG: antithymocyte globulin/thymoglobulin; eGFR: estimated glomerular filtration rate

(54.1%) patients were included in high-risk group and 83 (45.9%) in the standard risk-group (Table 2). In standard-risk group, the majority of CMV infection occurred during the protocol period; in high-risk group, approximately half of the incidents occurred during the protocol period and the other half happened thereafter (Table 2). There were no differences between the two groups in terms of the number of CMV infected patients (p=0.443) or treated patients (0.897) during the follow-up. Nor was a significant (p=0.358) difference in the incidence of severe CMV infections between the high-risk and standard-risk group.

Late CMV infection occurred in 13.8% of patients (n=25, 5 in the standard-risk group and 20 in high-risk group) (Table 2). In the standard-risk group, the majority of late CMV infections corresponded to reactivation of the virus; in the high-risk group it attributed to primary infections. In the high-risk group, lymphocyte-depleting treated patients had lower incidence of late CMV infection compared with D+/R– patients (16% *vs.* 34.7%, respectively) (Table 2). Late CMV infection patients were younger and more likely D+/R– (Table 1).

The median (IQR) time to late CMV infection was 253 (230.3–312.3) days after transplantation, corresponding to a median (IQR) of 55 (41–89.5) days after the protocol period. Screening for CMV antigenemia in the next two appointments after stopping valganciclovir treatment or prophylaxis period only allowed for the diagnosis of 56% of late infections. A screening period of 100 days after valganciclovir could have allowed for the diagnosis of 83.3% of late CMV infections. In the high-risk group, the screening after prophylaxis identified 12 (60%) of 20 positive patients; in the standard-risk group the screening after

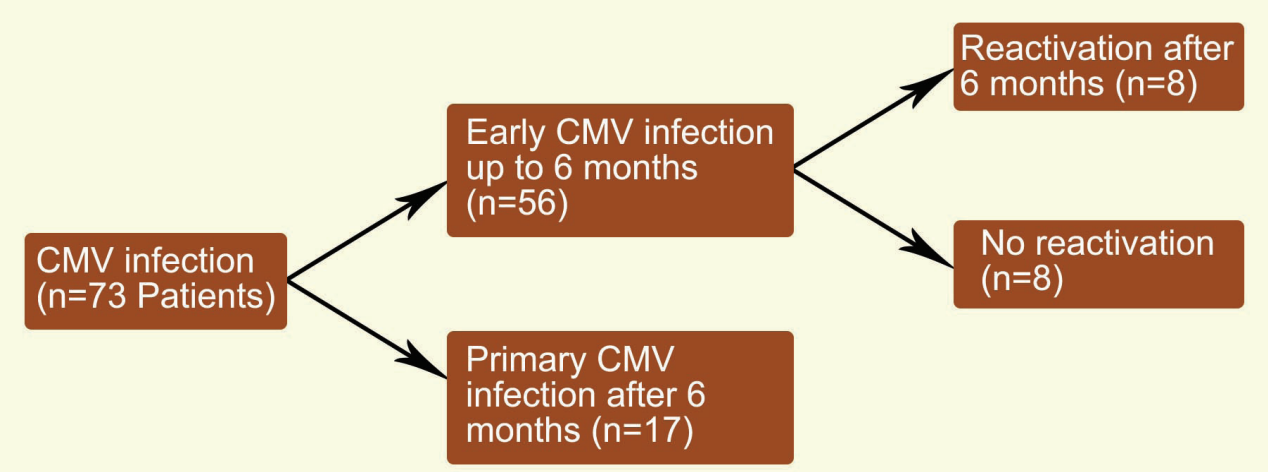

Figure 1: CMV infection classification

Table 2: Results of CMV-prevention protocol stratified by patients' risk. Values are either median (IQR) or n (%).

Parameters	Total (n=181)	High-Risk Group			Standard-Risk Group (D±/R+) (n=83)
		Total (n=98)	Lymphocyte-depleting agent (n=75)	D+/R– (n=23)	
CMV infection	73 (40.3)	37 (38)	24 (32)	12 (52)	36 (43)
Treated	59 (80.8)	31 (84)	20 (83)	11 (92)	28 (78)
Severe CMV disease	5 (2.8)	4 (4)	2 (3)	2 (9)	1 (1)
Early CMV infection	56 (30.9)	21 (21)	13 (17)	7 (30)	35 (42)
Time to early CMV infection (days)	50 (36–69)	42 (29–73)	60 (29.5–120)	40 (27–45)	52 (39–69)
Late CMV infection					
Time to late CMV infection (days)	25 (13.8)	20 (20)	12 (16)	8 (35)	5 (6)
Time to late CMV infection after protocol period (days)	253 (230–312)* 55 (41–90)*	258 (212–312) 55 (42–84)	257.5 (211.5–307.5) 73.5 (43.8–111.5)	260 (213.8–312.3) 52 (36.8–68.8)	251 (242–426)* 53 (26–199)*
Reactivation	8 (32)	4 (20)	2 (15)	2 (25)	4 (80)†
Primary late CMV infection	17 (68)	16 (80)	11 (85)	6(75)	1 (20)

The high-risk group was subdivided into ATG/TG induction therapy and D+/R– (with basiliximab induction therapy). *Excluded from this analysis was a patient with reactivation; †One case of reactivation after rejection treatment. CMV: cytomegalovirus; D+/R–: CMV IgG donor positive, recipient negative; D±/R+: CMV IgG donor positive or negative, recipient positive.

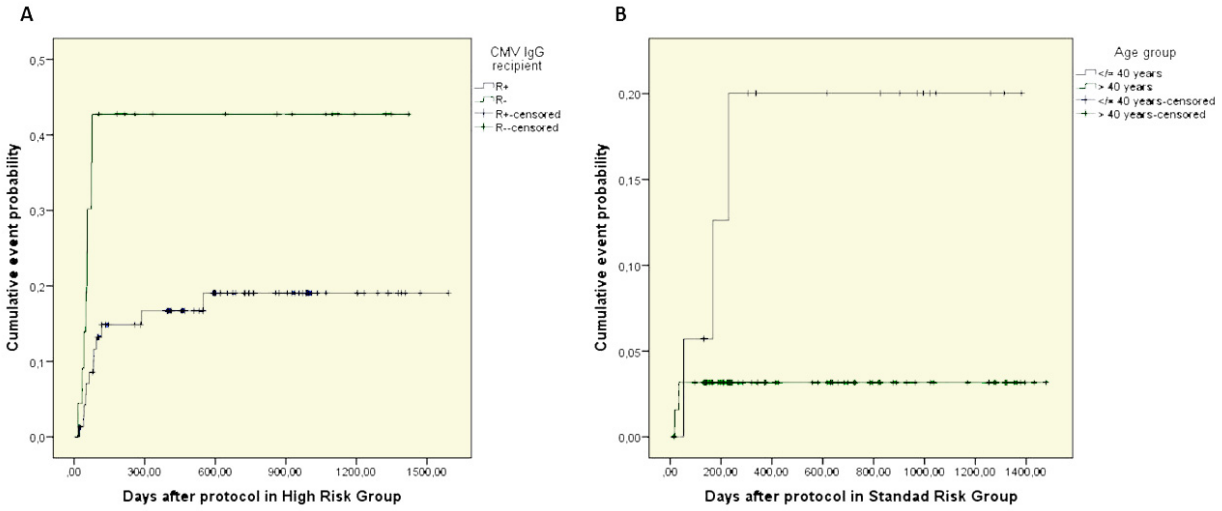

Figure 2: Cumulative probability of developing late CMV infection. A) The high-risk group according to CMV status (R+ vs. R–), Log Rank p=0.035; R+: 74 patients at risk with 12 events; R–: 23 patients at risk with 8 events. B) The standard-risk group according to age group (≤40 years vs. >40 years), Log Rank p=0.042. ≤40 years: 18 patients at risk with 3 events; >40 years: 65 patients at risk with 2 events.
CMV: cytomegalovirus; R+: CMV IgG recipient positive; R–: CMV IgG recipient negative

treatment identified 2 of 4 reactivations.

Cox regression analysis showed a tendency to high-risk group and D+/R– patients to experience late CMV infection (Table 3). The same model was applied separately to the high-risk and standard-risk groups. In the high-risk group, D+/R– was associated with late CMV infection (HR 2.7, p=0.039). Cold ischemic time had a HR close to 1 (HR 0.999, p=0.013). In the standard-risk group the multivariable analysis was performed using CMV donor status (D+ *vs.* D–) instead of CMV recipient status since all recipients were CMV IgG positive. The analysis showed that although with small effect, age was associated with CMV infection (HR 0.89, p=0.02). The identified factors were then analyzed with Kaplan-Meier survival analysis. In the high-risk group, using time to late CMV infection after cessation of the protocol, D+/R– status showed a higher cumulative probability of CMV late infection (Figure 2A). In the standard-risk group, those aged ≤40 years had a higher probability of CMV infection (Figure 2B).

DISCUSSION

In this cohort of adult kidney transplant recipients receiving a high surveillance protocol, the incidence of CMV (and late CMV) infection was similar to what has been reported in previous studies. To better understand the clinical course of late CMV infection, we analyzed its potential risk factors. We found that in the high-risk group, D+/R– was an important risk factor and, with a small effect, in the standard-risk group, lower age was associated with late CMV infection. As part of a high surveillance protocol, screening of CMV antigenemia after stopping valganciclovir did not appear to be useful in the diagnosis of late CMV infection.

We observed a rate of 37.8% positive CMV antigenemia in the high-risk group; the rate was 43.5% in the standard-risk group. In a previous study using similar protocol but with a three-month protocol period, the incidence of CMV viremia was higher in the high-risk group (47% positive CMV PCR) and lower in the standard-risk group (30%) [12]. Our six-month protocol-period might contributed to the lower incidence of the infection observed in the high-risk group, probably because a six-month prophylaxis would be related to lower incidence of CMV infection compared with that a three-month period would [13]. Regarding the standard-risk group, we used anti-IL2 in the induction therapy, which could contribute to the higher incidence of CMV

Table 3: Univariate and Cox regression analyses of factors affecting late CMV infection. Multivariate model χ^2= 24.3, p<0.001.

Factors for late CMV infection	Univariate model*		Multivariate model†	
	HR (95% CI)	p value	HR (95% CI)	p value
Age	0.95 (0.92–0.98)	0.002	0.98 (0.94–1.02)	0.287
Male sex	0.81 (0.36–1.79)	0.609	0.98 (0.409–2.35)	0.963
Diabetes	0.04 (0–8.33)	0.239		
CMV status prior to KT				
D±/R+	1	—	1	—
D+/R–	3.82 (1.65–8.87)	0.002	2.29 (0.89–5.92)	0.086
CMV-prevention protocol				
Standard risk	1	—	1	—
High risk	3.62 (1.35–9.64)	0.010	2.04 (0.98–9.43)	0.054
Second transplant	0.99 (0.23–4.21)	0.993		
Living donor	1	—		
Deceased donor	0.49 (0.19–1.22)	0.126		
HLA-A.B. DR mismatch	0.98 (0.73–1.32)	0.915		
Cold ischemic time (min)	0.999 (0.998–1)	0.034	0.999 (0.998–1)	0.069
Induction therapy on KT				
Basiliximab	1	—		
ATG/TG	1.3 (0.6–2.87)	0.503		
Rejection treatment at 6 months	0.99 (0.98–1.0)	0.178		
Recent rejection treatment	0.71 (0.17–3.0)	0.641		
Protocol Period (days)†	1 (0.99–1.01)	0.623		

Factors included in multivariate model were those reported in multivariate column. *Recent rejection treatment as a time-dependent variable (Cox proportional hazards model); †Includes prophylaxis, treatment and pre-emptive period before late CMV infection. CMV: cytomegalovirus; KT: kidney transplantation; D+/R–: CMV IgG donor positive, recipient negative; D±/R+: CMV IgG donor positive/negative, recipient positive; ATG/TG: antithymocyte globulin/thymoglobulin

infection observed in the current study. In a large retrospective study with R+ patients, the use of pre-emptive strategy in the first six months was associated with a 48% asymptomatic CMV infection rate; the rate was 49% after 12 months, which is similar to our results [25]. Regarding the high-risk group, another study found 29% positive CMV viremia (≥2000 copies/mL) within 100 days prophylaxis therapy in a group of patients treated with thymoglobulin [14]. The higher rate of CMV positive patients in our series could be related to the lower cut-off value we used. Li, *et al*, reported that a cut-off of 1000 CMV copies/mL corresponds to antigenemia levels of one positive cell per 2×10^5 [15]. In fact, a cut-off point of 900 copies/mL had 100% sensitivity and 82.5% specificity for the diagnosis of active and symptomatic CMV infections [22].

As described in the literature [12, 14], most of the CMV infections observed in the standard-group occurred during the protocol period. On the other hand, in the high-risk group, these infections occurred with almost the same incidence during and after the protocol period, in line with the observation that universal prophylaxis could delay the appearance of CMV infection [13, 14, 16]. Although these two groups had different risk factors for CMV in-

fection, when patients randomized into either prophylaxis or pre-emptive therapy without significant differences in terms of their CMV serostatus or immunosuppression, late CMV viremia occurred more frequently in prophylaxis group [14].

We had 25 late CMV infections, mostly in the high-risk group and rarely in the standard-risk group. The incidence of late CMV infection in D+/R– patients reported previously is variable [8, 13, 17, 18]. In a randomized controlled trial, the incidence of CMV disease (CMV syndrome or tissue invasive disease) in D+/R– patients during a 200-day period of prophylaxis was 7.1%; one year post-transplantation, it was 16.1% [13]. The same cohort was followed for two years and the incidence of CMV disease came up to 21.3% [17]. However, the incidence of CMV viremia (viral load >600 copies/mL) was 18.7% at six months and 37.4% one year post-transplantation [13]. Jamal, *et al*, also reported a 30% cumulative incidence of CMV infection in D+/R– patients after one year after cessation of a 3–6 month prophylaxis course [10]. Other authors reported an incidence of 37% primary late-onset CMV infection (positive viremia with symptoms) in D+/R– patients after six months of valganciclovir prophylaxis [8]. We reported 34.7% incidence of positive CMV antigenemia in D+/R– patients that could overestimate the CMV infection rates reported in previous studies. Regarding to patients treated with lymphocyte-depleting agents, we only identified one small study reporting the frequency of late CMV viremia in patients receiving a 100-day prophylaxis course in which 98% were treated with lymphocyte-depleting agents. The frequency of late CMV viremia was 22% (11 of 49 patients). The higher incidence observed could be attributed to the shorter prophylaxis period and different screening methods used. However, most studies [8, 10, 13, 17-19] did not consider screening during the period of prophylaxis and only screened patients with symptoms. In our study, the high-risk group was submitted to a more aggressive surveillance, including valganciclovir prophylaxis and screening for CMV antigenemia during the protocol period. This allowed switching for valganciclovir therapeutic dose in antigenemia positive cases. However, this measure did not seem to have contributed to a lower incidence of late CMV disease because those continued to be primary CMV infections. This result suggested that late CMV infection in high-risk patients did not appear to be related to undiagnosed low-grade CMV infection during the protocol period since its identification by antigenemia and treatment did not seem to reduce the incidence of the infection. On the other hand, positive viral load seemed to have a prognostic value. In a recent study, CMV testing was performed monthly throughout the first year post-transplantation. R+ patients received valganciclovir prophylaxis for 100 days; D+/R– received the prophylaxis for 200 days. In 30.6% of patients, the viral load was detectable at least once during the follow-up; a viral load >656 copies/mL was significantly associated with higher mortality [23]. In another study, asymptomatic CMV viremia was associated with chronic graft disfunction [24].

The low incidence of late CMV infection observed in the standard-risk group is also reported in two others studies. In one, the authors did not observe primary late-onset CMV viremia after 100 days of pre-emptive therapy, but recurrence occurred in 4% (1 of 13 patients) of D+/R+ but in none of nine D-/R+ patients [14]. In another study, asymptomatic CMV infection rate was 48% after six months; it only increased to 49% after 12 months [25].

Screening after prophylaxis period has been recommended. But only few reports address this issue [7]. Many of those were done after 100 days of prophylaxis in high-risk patients (D+/R–) and only one reported results in lower-risk patients (R+) [2]. Blanco, *et al*, concluded that the performance of a CMV viremia monitoring every 15 days during and after three months of valganciclovir prophylaxis did not appear to be useful in R+ patients with less conclusive data on D+/R– patients [20]. The authors described that 18 (32%) of 56 R+ patients had positive CMV viremia (all bellow the established cut-off value of 10,000 copies/mL) and only two developed CMV disease. In D+/R– patients 13 (43%) of 30 had CMV

viremia, of whom seven developed late-onset CMV disease [20]. Our screening also had limited results, as just allowed for the diagnosis of 56% of those with late CMV antigenemia. Regarding D+/R– solid organ transplant recipients, Lisboa, *et al*, showed that 8-weekly screening for CMV viremia after 3–6 months of valganciclovir prophylaxis, did not appear to be of value because of rapid viral doubling time, and that 55.2% of CMV disease occurred after the surveillance period [9]. We observed similar results probably because our median time to late CMV infection after the protocol was similar to their screening period (55 days ≈ 8 weeks). In a cohort of D+/R– kidney transplant, the median time to late CMV infection after six months of prophylaxis was also similar—67 days [8]. A longer screening period could increase the ability to identify positive CMV antigenemia patients. However, the specificity of those results, the frequency of screening and the cost-effectiveness of that measure need to be addressed in future studies.

When considering all patients, D+/R– was almost significantly associated with CMV infection in multivariate analysis. D+/R– has been identified as a risk factor for late CMV disease in a cohort of kidney transplant recipients (D+/R– and R+) receiving the same protocol (a 3–6-month prophylaxis course) [10]. In our study the patients received different protocols according to their CMV infection risks. In the high-risk group, D+/R– was associated with late CMV antigenemia independently of induction therapy and prophylaxis duration. This result was expected since the incidence of late CMV antigenemia was higher in D+/R– compared with that in R+ patients treated with lymphocyte-depleting agent. Furthermore, our high surveillance protocol, D+/R– continued to be an important factor to be considered; a previous report did not find other risk factors for late CMV infection in this group of patients [8]. In the standard-risk group, age appeared to be an independent risk factor associated with late CMV infection. However, this group only had five cases of late CMV infection and thus recommendations on higher surveillance on younger patients is limited and further studies are needed. Estimated GFR <45 mL/min at prophylaxis cessation and delayed graft-function have been identified as risk factors for late CMV infection [10, 21].

This study had few limitations to address. As this was a retrospective analysis, it was not possible to identify all patients with CMV disease from patients' records. Because the cut-off values used to start treatment after a positive CMV antigenemia varied according to the caring physician, we used a broader definition of CMV infection, including all patients with positive CMV antigenemia. This might overestimated our results. The screening surveillance of CMV replication was done using CMV pp65 antigenemia, which is a semi-quantitative test with limitations. Samples need to be processed within 6–8 hours and assay performance diminishes with less than 1000 neutrophils/μL. The recommendations of the Spanish Societies of Transplantation and Infectious Diseases propose real-time QNAT methods for monitoring CMV infection [7]. Additionally, we did not have a control group without screening during administration of the prophylaxis to compare the effect of this measure on the incidence of late CMV infection. The screening after prophylaxis/treatment had an important limitation since it was done in an irregular period. Finally, our sample only developed 25 late CMV infections that could limit the study power of our research.

In conclusion, we found that the incidence of CMV infection in our cohort was similar to what had been reported in the literature. Antigenemia surveillance during prophylaxis in high-risk patients did not appear to reduce the incidence of late CMV infections when comparing to literature reports. Screening in the two visits after prophylaxis or treatment turned out to be of limited value in the diagnosis of late CMV infections. D+/R– serostatus was identified as a risk factor for late CMV infection in our high-risk group. Lower age appeared to be related to late CMV infection in standard-risk patients.

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