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Cytomegalovirus prevention strategies in seropositive kidney transplant recipients: an insight into current clinical practice

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Summary

There is notable heterogeneity in the implementation of cytomegalovirus (CMV) prevention practices among CMV-seropositive (R+) kidney transplant (KT) recipients. In this prospective observational study, we included 387 CMV R+ KT recipients from 25 Spanish centers. Prevention strategies (antiviral prophylaxis or preemptive therapy) were applied according to institutional protocols at each site. The impact on the 12-month incidence of CMV disease was assessed by Cox regression. Asymptomatic CMV infection, acute rejection, graft function, non-CMV infection, graft loss, and all-cause mortality were also analyzed (secondary outcomes). Models were adjusted for a propensity score (PS) analysis for receiving antiviral prophylaxis. Overall, 190 patients (49.1%) received preemptive therapy, 185 (47.8%) antiviral prophylaxis, and 12 (3.1%) no specific intervention. Twelve-month cumulative incidences of CMV disease and asymptomatic infection were 3.6% and 39.3%, respectively. Patients on prophylaxis had lower incidence of CMV disease [PS-adjusted HR (aHR): 0.10; 95% confidence interval (CI): 0.01–0.79] and asymptomatic infection (aHR: 0.46; 95% CI: 0.29–0.72) than those managed preemptively, with no significant differences according to the duration of prophylaxis. All cases of CMV disease in the prophylaxis group occurred after prophylaxis discontinuation. There were no differences in any of the

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The authors have declared no conflicts of interest.

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secondary outcomes. In conclusion, antiviral prophylaxis was associated with a lower occurrence of CMV disease in CMV R+ KT recipients, although such benefit should be balanced with the risk of late-onset disease.

Introduction

Despite notable advances in the last decades, human cytomegalovirus (CMV) remains as one of the major causes of infection-related morbidity in solid organ transplant (SOT) recipients [1]. There is growing evidence suggesting that CMV infection exerts a detrimental impact on patient and graft outcome through indirect effects [2]. The combination of donor (D) and recipient (R) CMV serostatus plays a definitive role in defining the risk of post-transplant CMV infection, with those recipients with no pretransplant immunity (D+/R−) suffering from the highest incidence [1]. Alternatively, recipients seropositive for CMV at the time of transplantation face the risk of either viral reactivation or superinfection, according to the donor CMV serostatus.

Although showing variation across socioeconomic and age groups, CMV seroprevalence in the Western population ranges between 50% and 85% [3–5]. Therefore, CMV-seropositive patients constitute by far the most common risk group among the SOT population. However, most of large randomized clinical trials (RCTs) have been focused on the highest risk category [6–8], whereas few studies have examined the optimal prevention approach in CMV-seropositive recipients [9–12]. Although there is some evidence of the superiority of antiviral prophylaxis, current guidelines contemplate both approaches as acceptable [13,14]. The recent update of the consensus guidelines from The Transplantation Society suggest that antiviral agents should be maintained for 3 months when the prophylaxis strategy is chosen, while put emphasis on the importance of stringent viremia monitoring to achieve an effective preemptive therapy [14]. Nevertheless, there remains considerable heterogeneity regarding management strategies in CMV-seropositive recipients [15] and the role of some relevant variables, such as the donor serostatus [16], in the clinical decision-making process is unclear.

Our study was aimed at describing the contemporary clinical practice patterns regarding the prevention of CMV disease in a multicenter cohort of CMV-seropositive kidney transplant (KT) recipients. We also compared the effect of the different strategies on the incidence of CMV disease in the daily practice. Finally, we analyzed whether the occurrence of asymptomatic CMV infection exerted some discernible impact on the graft or patient outcome.

Subjects and methods

Study population

We performed a prospective, observational, cohort study at 25 transplant centers in Spain (The OPERA Study). Eligibility criteria included patients aged 18 years or older that underwent single KT throughout a 6-month period at one of the participating institutions between March 2011 and December 2012 and were CMV-seropositive before transplantation. Recipients of a double or combined transplant were excluded. The Clinical Research Ethics Committees approved the study protocol, and written informed consent was obtained from each participant. The present study was performed in accordance with the ethical standards laid down in the Declarations of Helsinki and Istanbul. Funding source had no involvement in the study design and conduction, data analysis, or manuscript preparation.

Study design

Participants were enrolled at the time of transplantation and followed up for at least 12 months, unless death or graft loss occurred earlier. Scheduled follow-up visits were carried out at baseline, week 2, and months 1, 3, 6, 9, and 12. Further visits were additionally performed depending on local practices at each center. Pretransplant, perioperative, and post-transplant variables were prospectively recorded by means of a standardized data collection form (DCF) and entered into a dedicated database. Detailed information was specifically gathered on the CMV prevention strategies used and the occurrence of CMV infection and disease. The choice and implementation of the prevention strategy were not standardized, but rather based on local institutional protocols. The *primary outcome* was the 12-month incidence of CMV disease. *Secondary outcomes* included the 12-month incidence of asymptomatic CMV infection, the 6- and 12-month cumulative incidences of biopsy-proven acute rejection (BPAR), non-CMV infection, new-onset

diabetes after transplantation (NODAT), graft loss and all-cause mortality, and the evolution of graft function at months 3, 6, and 12 after transplantation.

Definitions

Antiviral prophylaxis was defined as the administration of a course of ganciclovir (GCV) or valganciclovir (VGCV) within the first 3 weeks after transplantation, irrespective of its planned duration. A patient was assumed to be managed by *preemptive therapy* when the following conditions were met: explicit statement by the local researcher in the DCF, absence of antiviral prophylaxis, and presence of ≥3 monitoring points for CMV viremia or antigenemia during the first 6 months after transplantation. *Asymptomatic CMV infection* was defined by the laboratory confirmation of CMV replication [a single positive result in either the pp65 antigenemia assay or a polymerase chain reaction (PCR)-based quantitative nucleic acid amplification test (qNAT)] in the absence of symptoms. In view of the heterogeneity across participating centers in their technical procedures, for the purposes of the present study we considered as significant any antigenemia value and/or any CMV DNAemia level irrespective of the threshold established in each institution for initiating preemptive therapy. Episodes of CMV infection separated by both a minimum 2-week interval and at least one negative specimen were considered to be different episodes. *CMV disease* included both viral syndrome and end-organ disease. Viral syndrome was defined by the demonstration of CMV infection plus fever plus at least one of the following: leukopenia, atypical lymphocytosis, thrombocytopenia, or elevation of ALT or AST, as detailed elsewhere [17]. End-organ disease comprised both probable and definitive cases. The definitive diagnosis of gastrointestinal disease required the detection of CMV in tissue by culture, immunohistochemical analysis or *in situ* hybridization, whereas the definitive diagnosis of retinitis was established on the basis of the presence of typical retinal lesions confirmed by an expert ophthalmologist [17]. The graft function was assessed by estimated glomerular filtration rate using the abbreviated Modification of Diet in Renal Disease (MDRD-4) equation [18]. *Leukopenia* was defined as a total white blood cell (WBC) count $<4.00 \times 10^3$ cells/mm^3, with severe leukopenia (grade 3–4) defined by a WBC count $<2.00 \times 10^3$ cells/mm^3. *Delayed graft function* denoted the need for dialysis within the first week after transplantation. *Acute graft rejection* was suspected in case of an elevation of the serum creatinine and diagnosed by histological examination [19]. *Graft loss* was defined as permanent return to dialysis, allograft nephrectomy, or retransplantation.

Statistical analysis

Quantitative data were shown as the mean ± standard deviation (SD) or the median with interquartile range (IQR). Qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the χ^2 test, whereas Student's *t*-test or Mann–Whitney *U*-test were applied for continuous variables, as appropriate. Survival probabilities were estimated by the Kaplan–Meier method with CMV disease and infection as events, and differences between strategy groups (antiviral prophylaxis and preemptive therapy) were compared by the log-rank test. Univariate and multivariate (backward conditional selection) Cox regression models were used to evaluate the association between the prevention strategy and both outcomes, with results expressed as hazard ratios (HRs). Patients not receiving any specific preventive intervention were excluded from these analyses. To partially overcome the limitation posed by the nonrandomized design of our study, we calculated the propensity to receive antiviral prophylaxis (versus preemptive therapy) given the patient's baseline and transplant-related factors. The propensity score (PS) was estimated using a backward stepwise logistic regression model including variables with *P*-values < 0.1 in the univariate analysis, and the fit of the resulting model was assessed by means of the area under the receiving operator characteristics curve (auROC). The estimated PS was then used as a covariate in a multivariate analysis to adjust for potential confounding by factors associated with type of prevention approach [20]. Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). In addition, we performed various sensitivity analyses restricted to those patients with an appropriate monitoring schedule for CMV infection (arbitrarily set at ≥4 monitoring points throughout the first 6 months after transplantation). All the significance tests were two-tailed. Statistical analysis was performed using SPSS v. 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Study population and follow-up

Overall a total of 403 patients were included, with a median number of patients per center of 13 (IQR: 6–24) and a median recruitment period of 5.5 months (IQR: 3.3–6.8). Relevant data were not available in 16 of them (4.0%). Therefore, the study population comprised 387 patients, whose clinical characteristics are shown in Table 1. Data on immunosuppression and non-CMV-related events are detailed in Table 2. The median follow-up period for the entire cohort was 365 days (IQR: 355–373). Thirteen patients (3.4%) experienced graft loss after a median inter-

Table 1. Demographics and clinical characteristics of the study cohort (n = 387).

Variable	
Recipient age in years, mean ± SD	53.4 ± 12.4
Recipient gender (male), *n* (%)	224 (57.9)
Race, *n* (%)	
Caucasian	358 (92.5)
Black	24 (6.2)
Asian	5 (1.3)
Body mass index at transplantation (kg/m^2), mean ± SD	26.1 ± 4.4
Etiology of underlying ESRD, *n* (%)	
Glomerulonephritis	107 (27.6)
Diabetic nephropathy	38 (9.8)
Nephroangiosclerosis	38 (9.8)
Polycystic renal disease	60 (15.5)
Chronic interstitial nephropathy	37 (9.6)
Congenital nephropathy	7 (1.8)
Systemic vasculitis	7 (1.8)
Unknown	73 (18.9)
Others	20 (5.2)
Pretransplant renal replacement therapy, *n* (%)	338 (87.3)
Type of therapy, *n* (%)*	
Hemodialysis	242 (80.9)
Continuous ambulatory peritoneal dialysis	57 (19.1)
Dialysis vintage in years, median (IQR)	2.3 (1–3.7)
Previous kidney transplantation, *n* (%)	27 (7.0)
≥2 previous transplants	11 (2.8)
Donor age in years, mean ± SD	55.2 ± 14.5
Donor gender (male), *n* (%)	223 (57.6)
Type of donor, *n* (%)	
DBD donor	308 (79.6)
CDC donor	26 (6.7)
Living donor	53 (13.7)
Donor cause of death, *n* (%)†	
Cerebrovascular accident	207 (65.5)
Head trauma	47 (14.9)
Anoxia	34 (10.8)
Other	28 (8.9)
Baseline immunological risk, *n* (%)‡	
Peak PRA > 10%	35 (10.9)
Peak PRA > 50%	18 (5.6)
HLA mismatches in number, median (IQR)	4 (2–5)
Donor and recipient CMV serostatus, *n* (%)	
D+/R+	305 (78.8)
D−/R+	57 (14.7)
R+ (donor serostatus unknown)	25 (6.5)
Recipient serostatus (other than CMV), *n* (%)	
Hepatitis C virus positive	14 (3.6)
Hepatitis B virus (HBsAg) positive	4 (1.0)
Human immunodeficiency virus positive	4 (1.0)
Cold ischemia time in h, mean ± SD§	13.3 ± 7.3

CMV, cytomegalovirus; D, donor; DBD, donation after brain death; DCD, donation after circulatory death; ESRD, end-stage renal disease; HLA, human leukocyte antigen; IQR, interquartile range; PRA, panel reactive antibody; R, recipient; SD, standard deviation.
*Data available for 299 patients.
†Data available for 316 patients.
‡Data available for 319 patients.
§Data available for 346 patients.

Table 2. Immunosuppressive regimens and post-transplant adverse events.

Variable	
Induction therapy, *n* (%)	
Basiliximab	164 (42.2)
Antithymocyte globulin	93 (24.0)
Antilymphocyte globulin	3 (0.8)
None	127 (32.8)
Immunosuppressive regimen at day 14, *n* (%)	
Steroids	372 (96.1)
Tacrolimus	353 (91.2)
Cyclosporine	8 (2.1)
Mycophenolate mofetil/mycophenolic acid	339 (87.6)
mTOR inhibitors	11 (2.8)
Azathioprine	1 (0.3)
Use of mTOR inhibitors, *n* (%)*	
At month 6	11 (3.0)
At month 12	13 (3.6)
Delayed graft function, *n* (%)†	85 (25.1)
Non-CMV post-transplant infection, *n* (%)‡	109 (28.2)
Episodes per patient (mean ± SD)	1.5 ± 0.9
Cumulative incidence of BPAR, *n* (%)	
At month 1	35 (9.0)
At month 6	44 (11.4)
At month 12	47 (12.1)
NODAT, *n* (%)§	13 (4.9)
Graft loss, *n* (%)	13 (3.4)
All-cause mortality, *n* (%)	7 (1.8)

BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; mTOR, mammalian target of rapamycin; NODAT, new-onset diabetes after transplantation; SD, standard deviation.
*Percentages calculated over the total number of patients alive and with functioning graft at each time point.
†Data available in 339 patients.
‡The most common sites of non-CMV infection were lower urinary tract (48 episodes), bloodstream (19 episodes), and intra-abdominal (18 episodes). The most frequently isolated agents were *Enterobacteriaceae* (66 episodes) and *Pseudomonas aeruginosa* (22 episodes).
§Data available in 264 patients.

val of 71 days (IQR: 13.5–215.5). Causes of graft loss included graft thrombosis (4 patients), perioperative complications (3 patients), chronic allograft rejection (2 patients), and recurrent glomerular disease, BK virus-associated nephropathy, and microangiopathic thrombosis (one patient each). The death-censored 1-year graft survival was 96%. Seven patients died after a median interval from transplantation of 84 days (IQR: 15–267), accounting for an all-cause mortality rate of 1.8%. The causes of death

were infection and cardiovascular disease (4 and 3 patients, respectively).

CMV prevention strategies

One hundred and ninety patients (49.1%) were managed by preemptive therapy, 185 patients (47.8%) were given antiviral prophylaxis, and 12 patients (3.1%) did not receive any specific intervention. The prevention approach largely varied across the 25 participating centers: Most of them applied one or other strategy according to the patient's profile, whereas five centers (20%) only used antiviral prophylaxis, and one center (4%) only used preemptive therapy. When compared those managed preemptively, the patients who received antiviral prophylaxis had longer dialysis vintage (3.2 ± 2.4 vs. 2.4 ± 1.9 years, respectively; $P = 0.001$) and cold ischemia time (14.4 ± 6.8 vs. 12.5 ± 7.4 h; $P = 0.015$) and were more likely to have a peak PRA >10% (18.5% vs. 4.5%; $P < 0.0001$) and to have received a graft from a donor after circulatory death (10.3% vs. 2.6%; $P = 0.003$) and T-cell depleting agents as induction therapy (43.2% vs. 7.4%; $P < 0.0001$). Multivariate analysis identified dialysis vintage, cold ischemia time, and induction with T-cell depleting agents as independent factors predicting the receipt of antiviral prophylaxis (Table 3). The PS was constructed from these three variables (auROC 0.738, $P = 0.029$).

Antiviral prophylaxis was initiated at a median of 3 days (IQR: 1–7) after transplantation and mostly consisted of VGCV (76.2% of patients). Median duration was 92 days (IQR: 78–105), with 52 and 20 patients (28.1% and 10.9% of those in the prophylaxis group) receiving prophylaxis for >100 and >150 days, respectively. Dosage information was available for 172 patients (92.9%). All patients on GCV prophylaxis received 5 mg/kg/day or renal function-adjusted equivalent dose. Most of those on VGCV were given 900 mg/day or renal function-adjusted equivalent (87.9%), with the remaining receiving low-dose VGCV prophylaxis (i.e., 450 mg/day). Forty-five patients (24.3%) required dose adjustment of GCV or VGCV at some point during the course of prophylaxis. Forty-one (21.6%) of 190 patients managed by preemptive approach actually received at any point antiviral therapy for asymptomatic CMV infection. Such treatment was administered at a median of 59 days (IQR: 45.5–83.5) after transplantation.

Regarding the monitoring for CMV infection, 378 patients (97.7%) had at least one monitoring point throughout the post-transplant period. The median number of monitoring points per patient was 7 (IQR: 5–8), with no differences between those managed by preemptive therapy and those under prophylaxis [7 (IQR: 5–9) vs. 7 (IQR: 5–8); $P = 0.570$]. Most of patients in the latter group (93.0%) were screened at least once for CMV infection while receiving antiviral prophylaxis [median of 3 monitoring points (IQR: 2–6)]. Two hundred and seventy-nine patients (72.1%) had ≥4 monitoring points during the first 6 months. The method for the monitoring of CMV replication varied across participating centers: Nine of them (36.0%) only relied on qNAT and two (8.0%) only used pp65 antigenemia assay, with the remaining using both methods in variable combinations. Nine different commercial PCR-based assays were used for qNAT, the most common being the Cepheid Smart® CMV real-time PCR kit and the COBAS® AmpliPrep/COBAS® TaqMan CMV kit (three centers each).

Incidence of CMV infection and disease

A total of 152 patients had at least one documented episode of asymptomatic CMV infection, accounting for a cumulative incidence of 39.3% (95% CI: 34.4–44.1) in the entire cohort. There were 187 separate episodes of asymptomatic CMV infection (31 and 4 patients experienced a second and a third episode, respectively). Fourteen patients had 15 episodes of probable or definitive CMV disease [cumulative incidence of 3.6% (95% CI: 2.2–5.9)]. Six episodes were

Table 3. Baseline and transplant-related factors associated with the administration of antiviral prophylaxis (versus preemptive therapy) as CMV prevention strategy.

	Univariate analysis			Multivariate analysis*		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Dialysis vintage, years†	1.19	1.07–1.34	0.002	1.19	1.02–1.39	0.026
Peak PRA > 10%	4.84	2.09–11.17	0.000	–	–	–
DCD donor	4.23	1.55–11.59	0.003	–	–	–
Cold ischemia time, h†	1.04	1.01–1.07	0.016	1.06	1.01–1.11	0.028
Induction therapy with T-cell depleting agents‡	9.58	5.17–17.75	0.000	21.20	9.10–49.39	0.000

CI, confidence interval; CMV, cytomegalovirus; DCD, donation after circulatory death; OR, odds ratio; PRA, panel reactive antibody.
*Hosmer–Lemeshow test: $P = 0.548$.
†Per unitary increment.
‡Antithymocyte or antilymphocyte globulin.

classified as viral syndrome and the remaining nine as end-organ disease [probable or definitive colitis (2 and 6 cases, respectively) and definitive retinitis (one case)]. The median interval between transplantation and the diagnosis of the first episode was 54 days (IQR: 28–104.3). All episodes of CMV disease were treated [median duration of 38 days (IQR: 22–56)], with no cases of directly attributable graft loss or death.

Impact of CMV prevention strategies

The CMV infection-free survival rates at 6 and 12 months in patients under antiviral prophylaxis were significantly higher as compared to those managed preemptively (69% and 56% vs. 52% and 51%, respectively; $P < 0.0001$; Fig. 1a). This effect persisted after adjusting for potential clinical confounders and the PS to receive antiviral prophylaxis in Cox regression models (Table 4). These results remained unchanged in sensitivity analyses restricted to those patients with ≥4 monitoring points throughout the first 6 months, or to those not receiving a T-cell depleting agent as induction therapy (data not shown). Within the group receiving prophylaxis, there were no significant differences in the CMV infection-free survival rates according to the duration of prophylaxis (≤100 days vs. >100 days; $P = 0.153$). Nevertheless, in the sensitivity analysis restricted to patients with ≥4 monitoring points throughout the first 6 months, we found a clear trend toward higher 6- and 12-month CMV infection-free survival rates among those receiving >100 days of prophylaxis (79% and 74% vs. 63% and 57%, respectively; $P = 0.055$; Fig. 1b).

The 6-month CMV disease-free survival rate was higher in those patients under antiviral prophylaxis as compared to those managed by preemptive therapy (99% vs. 93%, respectively; $P = 0.007$; Fig. 2). There were two episodes of CMV disease in the group of antiviral prophylaxis; in both cases, the diagnosis was established after prophylaxis cessation (64 and 104 days, respectively). We found no differences according to the duration of prophylaxis (≤100 vs. >100 days; $P = 0.500$). The protective effect of antiviral

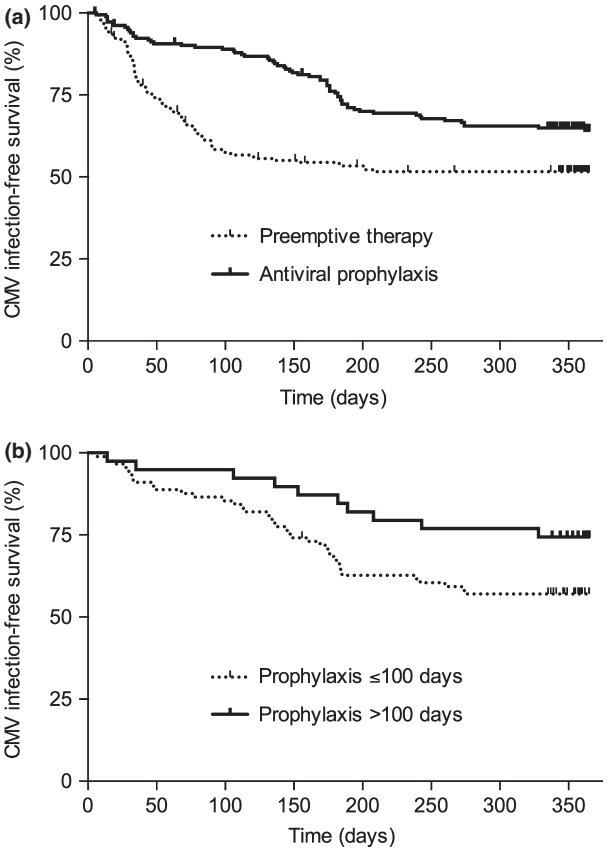

Figure 1 Kaplan–Meier cytomegalovirus (CMV) infection-free survival curves with follow-up truncated at 1 year: (a) according to the CMV prevention strategy (log-rank test $P < 0.0001$) and (b) according to the duration of antiviral prophylaxis in the subgroup of patients with ≥4 monitoring points during the first 6 months after transplantation (log-rank test $P = 0.055$).

Table 4. Risk factors for the occurrence of CMV infection (follow-up truncated at 1 year).

	Univariate analysis			Multivariate analysis			PS-adjusted model*		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Recipient age, years†	1.02	1.01–1.04	0.001	1.01	1.00–1.03	0.027	–	–	–
Donor age, years†	1.02	1.01–1.03	0.001	–	–	–	1.02	1.00–1.04	0.021
CMV serostatus D+/R+ (vs. D−/R+)	2.83	1.53–5.22	0.001	2.42	1.30–4.45	0.005	1.99	1.03–3.85	0.042
Graft function (eGFR) at month 1, ml/min†	0.98	0.97–0.99	0.002	0.99	0.98–0.99	0.028	0.99	0.98–0.99	0.016
Antiviral prophylaxis (vs. preemptive therapy)	0.61	0.44–0.83	0.002	0.62	0.44–0.86	0.004	0.46	0.29–0.72	0.021

CI, confidence interval; CMV, cytomegalovirus; D, donor; eGFR, estimated glomerular filtration rate; HR, hazard ratio; PS, propensity score; R, recipient.

*The propensity score accounted for the probability of receiving antiviral prophylaxis as CMV prevention strategy according to patient's baseline and transplant-related factors.

†Per unitary increment.

Table 5. Risk factors for the occurrence of CMV disease (follow-up truncated at 1 year).

	Univariate analysis			Multivariate analysis			PS-adjusted model*		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Dialysis vintage, years†	0.57	0.35–0.93	0.024	–	–	–	–	–	–
Diabetic nephropathy as underlying ESRD	3.79	1.19–12.09	0.024	4.06	1.24–13.32	0.021	–	–	–
Antiviral prophylaxis (vs. preemptive therapy)	0.17	0.04–0.74	0.019	0.19	0.04–0.91	0.037	0.10	0.01–0.79	0.030

CI, confidence interval; CMV, cytomegalovirus; ESRD, end-stage renal disease; HR, hazard ratio.
*The propensity score accounted for the probability of receiving antiviral prophylaxis as CMV prevention strategy according to patient's baseline and transplant-related factors.
†Per unitary increment.

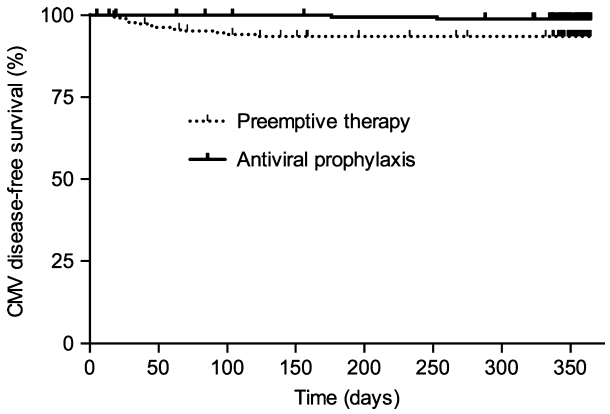

Figure 2 Kaplan–Meier cytomegalovirus (CMV) disease-free survival curves (with follow-up truncated at 1 year) according to the CMV prevention strategy (log-rank test $P = 0.007$).

prophylaxis was confirmed by the Cox regression model (Table 5). These later results were confirmed in a sensitivity analysis excluding those patients treated with T-cell depleting agents.

BPAR and CMV infection and disease

The specific impact of the occurrence of BPAR on the subsequent risk of CMV infection is detailed in Table 6. We only found a nonsignificant trend suggesting a lower CMV infection-free survival beyond day 30 after transplantation in patients receiving antiviral prophylaxis and diagnosed with BPAR within the first month (HR: 1.88; 95% CI: 0.92–3.81; $P = 0.081$), with no differences in the remaining groups or time points. There were no differences in the CMV disease-free survival beyond day 30 in the overall cohort according to the diagnosis of BPAR in the first month either (data not shown), whereas the low number of events prevented us from analyzing this association at other time points.

Indirect effects on graft and recipient

There were no significant differences in the occurrence of BPAR, non-CMV infection, graft function, NODAT, graft loss, or all-cause mortality at month 12 according to the CMV prevention strategy used (secondary study outcomes). Patients receiving antiviral prophylaxis had a higher cumulative incidence of leukopenia at month 3 (22.3% vs. 11.1%; $P = 0.004$), although most cases were mild to moderate (Tables 7 and 8). However, the type of prevention strategy was no longer associated with the occurrence of leukopenia in a logistic regression model adjusted for the use of T-cell depleting agents as induction therapy (Table 9).

We also assessed whether the early development of asymptomatic CMV infection (<3 months) had any impact on outcome, irrespective of the prevention strategy group. Graft function in patients with early-onset CMV infection was worse compared to those without at months 6 (47.7 ± 16.2 vs. 51.9 ± 20.1 ml/min; $P = 0.065$), 9

Table 6. Impact of the occurrence of BPAR at different post-transplant time points on the subsequent CMV infection-free survival beyond those points stratified according to the CMV prevention strategy (univariate analysis).

	Overall cohort			Antiviral prophylaxis			Preemptive therapy		
Diagnosis of BPAR	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Within the first 30 days	1.15	0.65–2.03	0.640	1.88	0.92–3.81	0.081	0.61	0.22–1.68	0.343
Within the first 90 days	0.96	0.47–2.00	0.924	1.52	0.72–3.22	0.272	0.04	0.00–9.34	0.251
Within the first 180 days	1.62	0.76–3.47	0.211	1.56	0.65–3.77	0.323	1.45	0.33–6.50	0.624

BPAR, biopsy-proven acute rejection; CI, confidence interval; CMV, cytomegalovirus; HR, hazard ratio.

Table 7. Impact on graft and recipient outcome of the CMV prevention strategy.

	Antiviral prophylaxis (n = 185)	Preemptive therapy (n = 190)	P-value
Cumulative incidence of BPAR, *n* (%)			
At month 6	21 (11.4)	22 (11.6)	0.945
At month 12	22 (11.9)	24 (12.6)	0.827
Cumulative incidence of non-CMV infection, *n* (%)			
At month 3	46 (24.9)	44 (23.2)	0.699
At month 6	54 (29.2)	50 (26.3)	0.534
Graft function (eGFR), ml/min (mean ± SD)			
At month 3	49.4 ± 19.3	50.9 ± 17.7	0.438
At month 6	50.1 ± 19.7	51.3 ± 18.7	0.565
At month 12	53.4 ± 19.9	54.3 ± 18.7	0.667
Hemoglobin, g/dl (mean ± SD)			
At month 3	12.4 ± 1.6	12.5 ± 1.7	0.551
At month 6	12.6 ± 1.7	12.9 ± 1.7	0.052
At month 12	13.0 ± 1.7	13.4 ± 1.9	0.070
Total WBC count, $\times 10^3/mm^3$ (mean ± SD)			
At month 3	6.1 ± 2.4	6.9 ± 2.5	0.001
At month 6	6.4 ± 3.3	6.9 ± 4.7	0.183
At month 12	6.9 ± 2.2	7.1 ± 2.4	0.278
Leukopenia within the first 3 months, *n* (%)	41 (22.3)	21 (11.1)	0.004
Severe leukopenia (grade 3–4)	3 (1.6)	2 (1.1)	0.485
Graft loss, *n* (%)	6 (3.2)	5 (2.6)	0.726
All-cause mortality, *n* (%)	4 (2.2)	2 (1.1)	0.330

BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; SD, standard deviation; WBC, white cell blood count.

(50.0 ± 17.2 vs. 55.3 ± 19.8 ml/min; P = 0.023), and 12 (50.6 ± 18.8 vs. 55.1 ± 19.4 ml/min; P = 0.053; Fig. 3). These finding remained in sensitivity analyses restricted to patients with ≥4 monitoring points during the first 6 months or stratified by type of CMV prevention strategy. There were no differences in the cumulative incidences at month 12 for BPAR, non-CMV infection, NODAT, graft loss, or all-cause mortality (data not shown).

Discussion

A number of findings should be highlighted from the present study: (i) The 12-month cumulative incidence of CMV disease in a large contemporary cohort of CMV-seropositive KT recipients was below 4%; (ii) it is likely that such a low figure might be due, at least partially, to the widespread use of antiviral prophylaxis; (iii) as compared to the preemptive approach, prophylaxis significantly reduced the risk of both CMV disease and infection; (iv) there was no apparent benefit from using prophylaxis in terms of post-transplant adverse events theoretically attributable to the indirect effects exerted by CMV; and (v) a worse graft function was observed at the end of follow-up in patients experiencing early-onset CMV asymptomatic infection.

Recent studies have reported incidence rates of CMV disease among CMV-seropositive recipients similar to that we have observed [21–25]. Manuel *et al.* [25] observed an incidence as low as 4.2% in CMV-seropositive SOT recipients in the Swiss Transplant Cohort Study. Although differences between studies in disease definition should be taken into account, these figures seem to be lower than those reported over the previous decade, which often exceeded 10% [26–28]. It is unclear whether this trend is reflecting improvements in immunosuppression and follow-up protocols or secular changes in CMV prevention practices.

Nearly half of the patients in our study received antiviral prophylaxis as compared to <15% of those enrolled in a previous Spanish cohort [29]. Not surprisingly, induction therapy with T-cell depleting agents was identified as the strongest clinical factor influencing the choice of prevention strategy among participating clinicians. Nevertheless, almost 40% of patients receiving basiliximab or no induction therapy – and, therefore, at no additional risk for CMV infection – were still managed with prophylaxis. A recent international survey revealed that antiviral prophylaxis is used for CMV-seropositive patients by about 60% of respondents [15]. Our findings point to an increasing trend in the use of such an approach in our setting, despite the fact that the latest Spanish consensus guidelines give no

Table 8. Detailed clinical characteristics and evolution of five patients who had developed grade 3–4 leukopenia (total WBC count <2.00 × 10^3 cells/mm^3) at month 3 after transplantation.

Patient number	Gender/age (years)	Induction/maintenance regimen	Administration of VGCV (strategy)	VGCV dosing and duration	eGFR at month 1 (ml/min)	WBC nadir (×10^3/mm^3)	Infectious complications	Management	Outcome
1	F/68	Antithymocyte globulin/Tac, MMF, S	Yes (prophylaxis)	450 mg/48 h for 105 days	8.4	1.96 (day 90)	Lower urinary tract infection (day 21)	VGVC discontinuation	Complete resolution
2	F/66	None/Tac, MPA, S	Yes (prophylaxis)	450 mg/48 h for 7 days	9.0	1.57 (day 31)	Acute pyelonephritis (day 122)	VGVC discontinuation	Complete resolution
3	F/50	None/Tac, MMF, S	Yes (prophylaxis)	450 mg/day for 88 days	34.5	1.42 (day 16)	None	MMF discontinuation, G-CSF	Complete resolution
4	F/53	Basiliximab/Tac, MMF, S	Yes (preemptive therapy)	450 mg/day for 12 days	33.4	1.60 (day 15)	Asymptomatic CMV infection (day 21)	MMF discontinuation	Complete resolution
5	M/53	Basiliximab/Tac, MMF, S	No	–	28.6	1.10 (day 15)	Asymptomatic CMV infection (day 35)	MMF discontinuation	Complete resolution

CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; F, female; G-CSF, granulocyte colony-stimulating factor; M, male; MMF, mycophenolate mofetil; MPA, mycophenolic acid, S, steroids; Tac, tacrolimus; WBC, white cell blood count; VGCV, valganciclovir.

clear preference to this strategy over the preemptive therapy for this risk category [13]. Interestingly, VGCV prophylaxis was administered in most patients at standard doses (900 mg/day or renal function-adjusted equivalent), with only a minority receiving low-dose regimens (450 mg/day). Although some studies suggest that both strategies might be similarly effective [30], opposing results have been reported [31] and current guidelines state that there is insufficient evidence to support the routine use of low-dose VGCV prophylaxis [14].

Laboratory methods for CMV infection monitoring widely vary across participating centers. Some institutions still relied exclusively on the pp65 antigenemia assay, even though there is general consensus favouring PCR-based molecular techniques on grounds of its superior sensitivity and reproducibility [13,14,32]. We also found considerable intercenter heterogeneity in the CMV PCR testing, thus hampering a direct comparison of DNAemia levels [33–35]. A surprising finding was the high frequency of monitoring for CMV infection in patients receiving prophylaxis. VGCV exhibits an oral bioavailability about 60% [36], and GCV resistance is uncommon among KT recipients [37,38]. Therefore, the risk of breakthrough CMV infection is assumed to not be significant enough to justify routine monitoring during the prophylaxis period [13,14]. Notwithstanding this, the visual inspection of the CMV infection-free survival curves depicted in Fig. 1 suggests the occurrence of some degree of breakthrough viremia that would reflect a suboptimal real-life implementation of the prophylaxis strategy (i.e., VGCV underdosing in patients with renal function impairment or lack of long-term adherence). The 12-month incidences of CMV infection in patients receiving ≤100 or >100 days of VGCV in our cohort (38.2% and 26.9%) were actually lower than those reported in the IMPACT Study (37.4% and 50.9%, respectively) [7], although differences in the stringency of viremia monitoring and in the risk profile of analyzed populations might partially account for this discrepancy.

The optimal approach to prevent CMV disease in the intermediate-risk group of CMV-seropositive KT recipients remains open to discussion [9–12]. Two RCTs have specifically compared the role of antiviral prophylaxis with VGCV for 100 days versus preemptive therapy [9,12]. Khoury *et al.* [9] found that prophylaxis reduced the occurrence of CMV DNAemia during the period in which VGCV was administered, although increased the risk of delayed-onset infection. The VIPP Study showed that the 12-month incidence of asymptomatic DNAemia was significantly lower in recipients under prophylaxis regardless of donor serostatus. The overall incidence of CMV disease was also lower in this arm, with the difference being only significant in D+/R+ patients [12]. A recent meta-analysis also favored prophy-

Table 9. Risk factors for the development of leukopenia (total WBC count <4.0 × 10^3/mm^3) within the first 3 months after transplantation.

	Univariate analysis			Multivariate analysis*		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Delayed graft function	2.05	1.13–3.72	0.017	1.89	0.99–3.63	0.055
CMV serostatus D+/R+ (vs. D−/R+)	1.96	0.99–3.89	0.050	–	–	–
Induction therapy with T-cell depleting agents	3.09	1.76–5.44	0.000	3.06	1.64–5.72	0.000
Antiviral prophylaxis (vs. preemptive therapy)	2.31	1.30–4.09	0.004	–	–	–

CI, confidence interval; CMV, cytomegalovirus; D, donor; OR, odds ratio; R, recipient; WBC, white blood cell.
*Hosmer–Lemeshow test: $P = 0.984$.

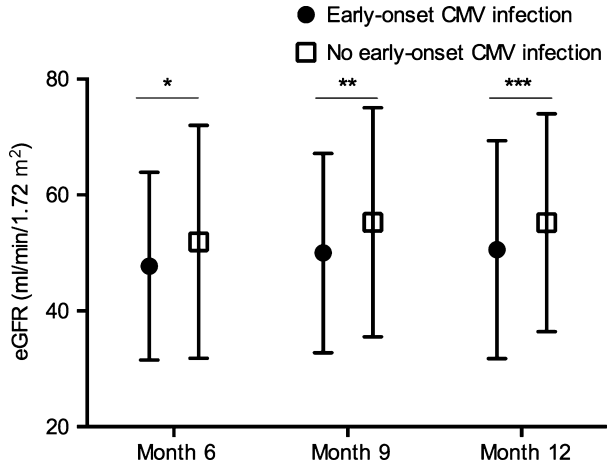

Figure 3 Evolution of graft function according to the occurrence of early-onset (<3 months) asymptomatic CMV infection (*$P = 0.065$; **$P = 0.023$; ***$P = 0.053$). CMV: cytomegalovirus; eGFR: estimated glomerular filtration rate.

laxis over preemptive therapy for the outcomes of asymptomatic viremia and end-organ disease [39].

Although limited by its observational nature, our experience supports the benefit from antiviral prophylaxis in decreasing the risk of CMV disease in CMV-seropositive recipients, even in those not receiving T-cell depleting antibodies. Of note, both episodes of CMV disease within the prophylaxis group occurred after prophylaxis cessation. The risk of late-onset CMV disease has been repeatedly cited as a potential drawback of the prophylactic strategy [1,13,14,16,39,40]. Extending the length of VGCV prophylaxis to 200 days has been demonstrated to reduce the occurrence of late-onset disease in D+/R− patients [7,41]. Despite the lack of analogous evidence for nonhigh risk groups, about 20% of patients under prophylactic approach received antiviral drugs for more than 100 days, thus suggesting that certain transplant centers are currently translating the results derived from the IMPACT Study into their daily practice involving CMV-seropositive patients.

In contrast to the benefit demonstrated by antiviral prophylaxis in terms of direct CMV effects, we have not been able to extend such an impact to its attributable indirect effects [2]. The investigators of the VIPP Study did not find significant differences at month 12 in graft function or in the incidence of BPAR or graft loss between treatment arms either [12]. On the opposite, other studies with longer follow-up periods have reported improved graft survival with prophylaxis [10,42]. Manuel *et al.* [25] observed that KT recipients managed by preemptive therapy had a higher incidence of graft loss or death. Interestingly, when early-onset asymptomatic CMV viremia was introduced in the multivariate model, the use of preventive therapy was no longer associated with outcome. This finding prompted us to also analyze the occurrence of asymptomatic CMV infection within the first 90 days despite of the prevention strategy used. In keeping with recent studies encompassing different CMV D/R constellations [43,44], we demonstrated a deleterious effect of this event on the renal allograft function in whose pathogenesis the pro-inflammatory role exerted by CMV could be hypothesized [2,45].

Our study comprised a large contemporary cohort of CMV-seropositive KT recipients prospectively followed up according to a standardized protocol. Notwithstanding these strengths, several limitations must be acknowledged. The incidence of CMV disease was too low to examine the potential impact of the duration of prophylaxis on this outcome. In addition, some degree of underdiagnosis may have occurred depending on the closeness of the clinical follow-up. As the allocation to the different prevention strategies was not random, patients prescribed antiviral prophylaxis had longer ischemia time and dialysis vintage and were more likely to have received induction with T-cell depleting agents. In an attempt to minimize confounding by indication bias, we performed an additional set of PS-bases analyses. However, it should be noted that PS only adjusts for known measured variables, so we are unable to exclude the potential effect of other confounders. Both the technical procedure (pp65 antigenemia or PCR assay) for and the intensity of CMV monitoring were heterogeneous across centers, as well as the threshold for initiating antiviral treatment in patients managed by

preemptive therapy, and we did not have detailed data on these points to assess the extent of the compliance with institutional protocols in individual patients, limitations common to similar multicenter studies [22,25,29]. The 12-month follow-up period might have been insufficient to reveal the impact of the prevention strategy on long-term outcomes. Finally, analyzing the cost-effectiveness of antiviral prophylaxis in intermediate-risk KT recipients was beyond the scope of this study.

In conclusion, the present multicenter study focused on a population of KT recipients – those at intermediate-risk serostatus for CMV infection – that have merited relatively little specific attention in the previous literature. The use of antiviral prophylaxis was associated with a lower risk of CMV disease, even though the overall incidence of this complication was low. Such benefit should be confirmed by large RCTs and cost-effectiveness analyses in order to clarify the optimal approach to prevent the CMV-associated direct effects and, eventually, its deleterious impact on the graft outcome.

Authorship

MFR, MA, JMC and JMA: designed research. MFR, DN, EGH, GCM, JMD, DH, GBB, FC, LJ, AFE, EG, FM, CGA, AM and ELH: performed research. MFR, MA, JMC and JMA: analyzed data. MFR and JMA: wrote the manuscript. MA, JMC and DN: revised the final draft of the manuscript.

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