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HCMV infection in renal transplant recipients: a retrospective cohort study

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SUMMARY

Human Cytomegalovirus (HCMV) represents the most common viral complication affecting solid organ transplant recipients (SOTRs) and its management is still debated. This study analyzes the association between HCMV infection and renal transplant recipients' outcomes. From January 2008 through December 2009, 97 consecutive renal transplant recipients were retrospectively studied. HCMV disease prevention was pursued by pre-emptive therapy, reserving long-term prophylaxis for high-risk patients. A total of 32/97 patients (32.9%) developed HCMV positivity in blood for a cumulative estimated proportion at 3 months post-transplantation of 0.21. HCMV disease developed in 7 patients (7.2%), while 25 patients had asymptomatic infection (25.7%). No patient died from HCMV. HCMV disease, older graft age and post-transplant renal dysfunction were independent predictors of rejection while HCMV infection without disease was associated with a higher number of other complications. The use of basiliximab was independently associated with a reduced hazard of HCMV infection/disease. In renal transplant recipients HCMV infection still represents a major issue influencing the outcome, not only because of the potential to develop the disease and its link to graft rejection, but also in terms of higher number of complications. The choice of different immunosuppressive strategies might be associated with HCMV replication.

KEY WORDS: HCMV, Renal transplant, Prophylaxis, Pre-emptive therapy, Rejection.

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INTRODUCTION

Despite recent improvements in transplantation practices, *Human Cytomegalovirus* (HCMV) infection still represents the most common viral complication affecting solid organ transplant recipients (SOTRs), resulting not only in significant morbidity but also in mortality, and influencing many short and long-term indirect effects that collectively contribute to reduced allograft and patient survival (Fishman *et al.,* 2007). Therefore, HCMV disease prevention remains one of the principal goals of SOTRs management and several approaches are used. Some guidelines recommend specific pre-emptive anti-HCMV therapy in asymptomatic viremic patients (Gerna *et al.,* 2007; Baldanti *et al.,* 2008), whereas others combine this strategy with a pharmacological prophylaxis for patients belonging to high-risk groups, such as HCMV seronegative recipients from an HCMV seropositive donor (KDIGO Transplant Work Group, 2009; Kotton *et al.,* 2010). Other approaches range from universal, delayed or prolonged pharmacological prophylaxis for established durations (San Juan *et al.,* 2009; Kotton *et al.,* 2010; Leone *et al.,* 2010; Blumberg *et al.,* 2010), to extremely selective pre-emptive treatment strategies, still under investigation, evaluating HCMV viral load and specific immune response (Gerna *et al.,* 2006; Gerna *et al.,* 2011). The former is based on the rationale that HCMV replication itself, even at low levels

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and without symptoms, leads directly or indirectly to several complications (not only to organ damage) and therefore it is more useful and practical to use extensive prophylaxis; the latter relies on the fact that complications and organ damage should be linked to HCMV disease rather than to HCMV replication per se and therefore it should be more effective to employ pharmacological resources to individuals really needing them. Whatever the strategy might be, constant correct virological monitoring is essential to guide a correct therapeutic strategy. Risk factors predisposing to HCMV diseases, including the different immunosuppressive strategies employed, are still under investigation. The aim of the present study was to describe virological, clinical and therapeutic management characteristics of HCMV infection as well as their association with patients' outcomes in a complete single site cohort of renal transplant recipients.

PATIENTS AND METHODS

Study population and design

This is a retrospective, observational analysis including all renal transplants performed from January 2008, through December 2009 at the Transplantation Surgery Department of University Hospital of Siena, Italy. At this Department all transplant recipients' data are prospectively collected in an electronic medical record that includes characteristics of the transplant itself, laboratory tests, follow-up outpatient visits and any hospitalization following transplantation in which an HCMV infection might have been documented. Moreover, all virological tests are recorded in a database at the Microbiology and Virology Department of the hospital. For each patient, the following variables were extracted from these databases and analyzed: age, gender, baseline renal diseases leading to end-stage renal disease, length and modality of pre-transplant dialysis, co-morbidities, type of transplantation, donor's characteristics, post-operative clinical progress, delayed graft function, immunosuppressive therapies employed, virological follow-up, post-transplant complications and their management, occurrence of rejection, graft function measured by plasma creatinine, plasma creatinine, laboratory abnormalities and their management. HCMV infection or disease development, its management and outcomes.

Diagnosis and virological monitoring of HCMV infection

HCMV-virological monitoring followed a shared protocol, according to the most recent available guidelines (Baldanti *et al.,* 2008; Consensus SIV-AMCLI, 2008; KDIGO Transplant Work Group, 2009). During post-transplant follow-up the following HCMV assays were performed at the Microbiology and Virology Department of the hospital:

1) HCMV pp65 antigenemia;
2) HCMV DNAemia on whole blood by real-time PCR in blood (Abbott CMV PCR Kit);
3) HCMV DNA quantitative detection from biopsies (intestinal, lung and other tissues) and biological fluids (bronchoalveolar lavage, liquor *et al.)* by Real time-PCR (RT-PCR).

Besides these tests, additional HCMV assays performed by the patients in other structures and included in the clinical records were also considered.

Definition of HCMV infection

HCMV infection was defined as the detection of HCMV in blood or body tissues in the absence of clinical manifestations or organ function abnormalities (Consensus SIV-AMCLI, 2008).

Definition of HCMV disease

Patients were considered to be affected by systemic HCMV disease if HCMV viral syndrome (body temperature of >38°C plus the presence of HCMV infection with no other apparent underlying cause) and one of the following findings were present: leukocyte count <4,000 cells/mm^3; atypical lymphocytes of ≥3%; platelet count <100,000 mm^3 (San Juan *et al.,* 2008). End-organ HCMV disease was defined by symptoms and signs of organ involvement associated with either immunohistochemical or virological detection of HCMV in biopsy tissues or local secretions (independently of virus detection in blood), absence of other possible causes of organ disease and clinical and virological response to anti-HCMV therapy (Consensus SIV-AMCLI, 2008). Probable end-organ disease was considered for patients without histopatological evidence of HCMV, with a compatible clinical presentation, evidence of HCMV

antigenemia or DNAemia, and clinical and/or virological response to specific treatment with ganciclovir or valganciclovir (San Juan, 2008).

Definition of graft rejection and other complications

Graft rejection was defined according to Banff diagnostic categories for renal allograft biopsies. Any other disorder occurring after transplantation was grouped into major categories of complications: infectious (other than HCMV itself, as urinary tract infections, sepsis, devices infections, upper and lower respiratory tract infections, herpes zoster etc.), renal (acute creatinine increase requiring intervention other than rejection), surgical (bleeding, explant, lymphocele, stones etc.), cardiovascular (heart failure, hypertension, heart attack, arrhythmias, venous thrombosis etc.), neurological (cerebral stroke, persistent headache, neuralgia, tremor etc.), endocrine-metabolic (diabetes, parathyroids dysfunction etc.), gastrointestinal (esophagitis, gastritis, diarrhea, diverticulitis, constipation etc.), respiratory complications (exacerbation of chronic obstructive bronchopathy, pulmonary edema etc.) and laboratory abnormalities requiring intervention (other than renal dysfunction, like agranulocytosis, thrombocytopenia, gammopathy etc.).

Therapeutic management of HCMV infection/disease

The latest available guidelines and clinical judgment guided the therapeutic choices (prophylaxis, pre-emptive therapy, symptomatic disease treatment) in this patient cohort (Consensus SIV-AMCLI, 2008; KDIGO Transplant Work Group, 2009). A HCMV antigenemia >100 pp65 positive/2x10^5 leukocytes or a DNAemia >100,000 copies/ml were used as criteria for pre-emptive therapy initiation, due to their strong association with the risk of developing HCMV disease (Gerna *et al.,* 2007).

Statistical analysis

Standard methods were used to describe patients and viral characteristics. Separate survival analyses with the Kaplan-Meier method were employed to estimate the time from renal transplantation to specific outcomes such as HCMV infection, HCMV disease, transplant rejection, transplant loss, death. For patients not reaching a specific outcome, follow-up was right-censored at the last HCMV measurement (only for the HCMV-specific outcomes), the last clinical observation, death or December 31st 2010, whichever came first. Univariable and multivariable Cox regression models were employed to analyze the association of variables with the above-mentioned outcomes.

RESULTS

Patients characteristics at renal transplant

In this study, 97 renal transplant recipients were enrolled: 35 females, 62 males; their median age was 55 years (IQR:49-60).

Glomerulonephritis was the most common cause leading to end-stage renal disease (20.6%), followed by polycystic kidney disease (17.5%) and chronic renal failure of unknown origin (14.4%). The median duration of the underlying disease was 10 years (IQR: 6-21), and pre-transplant dialysis lasted a median of 30 months (IQR:18-57).

Transplant characteristics and immunosuppressive treatments. All the transplants performed were simple (no combined kidney-pancreas transplant), including 95 from deceased donors and 2 from living donors. There were 18 double kidney transplants 17 of which were subsequent to a first transplantation followed by graft loss. The median graft donor age was 63 years (IQR 53-70).

Initial standard immunosuppression was usually performed with a triple-drug regimen including cyclosporine (CyA, n=49, 50.5%) or tacrolimus (Tac, n=48, 49.4%) combined with mycophenolate mofetil (MMF, n=53, 54.6%) and steroids (n=96, 98.9%).

In the majority of patients with stable graft function, steroids were usually slowly tapered until discontinuation within 6 months after transplantation. Basiliximab was used as induction therapy in 67 patients (69%). The remaining 30 patients did not undergo an induction therapy. Apart from basiliximab and lower mean initial exposure to steroids, the immunosuppressive regimen of this group of patients did not generally differ from that used in basiliximab-exposed patients. Biopsy-proven acute rejections of grade I-II were treated with high dose intravenous corticosteroids combined with rituximab in selected cases.

Clinical events and complications

For each patient there was a minimum follow-up of 3 months, with the exception of 5 cases lost due to early complications, resulting in a median follow-up time of 666 days. Seven patients (7.2%) died (incidence 3.8/100 PYFU; 2-year estimated proportion dying 0.063). Causes of death were pneumonia (two cases), liver failure (two cases), acute respiratory failure, graft bleeding and unknown due to unavailable record (each one case). Four patients were explanted (1 due to BK-related nephropathy, 1 with a septicemia, 1 due to grade III acute rejection and 1 due to unknown reasons), while 40/97 had a favorable outcome with regular graft function. During the study follow-up, 33/97 patients (34%) showed at least one rejection episode. Of these, 5 presented 2 episodes and 1 patient 3 episodes. The overall incidence of rejection was 24.0/100 PYFU with estimated 1-year and 2-year proportions showing rejection of 0.28 and 0.36, respectively. Sixteen (34%) lost graft function, including 9 cases undergoing explants (incidence of graft loss 9.6/100 PYFU; 12 and 24 months estimated proportion with graft loss of 0.13 and 0.16, respectively).

Excluding rejection and surgical complications (including graft explants), 88/96 patients (90.7%, data unavailable for one patient) presented at least one post-transplant complication requiring intervention, accounting for a total of 200 different episodes; the most frequent were infectious diseases (85 episodes, other than HCMV), followed by cardiovascular disorders (34 episodes), laboratory abnormalities (22 episodes, excluding renal dysfunction), endocrine-metabolic affections (18 episodes) and renal dysfunction other than rejection (17 episodes). Other different types of complications were represented by a lower number of cases (n=24).

HCMV infection/disease and its management

A serological positivity for IgG anti-HCMV was documented in 91/97 (93.8%) transplant recipients and 86/97 (88.6%) donors at the time of transplantation. Patients were stratified into 4 groups on the basis of the recipient/donor (R/D) HCMV serostatus: 80/97 couples presented an R+/D+ profile (82.5%), 10/97 showed an R+/D- mismatch (10.3%), 6 an R-/D+ mismatch (6.2%) and 1 couple (1%) showed a serological R-/D- profile. The high-risk group (6 seronegative recipients with seropositive donors, R-/D+) underwent 3 months of antiviral prophylaxis with oral valganciclovir (900 mg once daily or dose adjusted according to renal function).

Patients were monitored for HCMV DNAemia and, in a smaller number of patients, HCMV antigenemia at pre-established intervals or in case of suspected HCMV disease. During the study period, 32/97 patients (32.9%) developed a virological HCMV positivity in their blood. These patients (21 males and 11 females) had a median age of 58 years (IQR 52-60). A cumulative estimated proportion of 0.21 patients developed a virological positivity within the first 3 months after transplantation (median latency: 86 days). Among these positive patients, 30 had episodes of reactivation, while 2 cases had a primary HCMV infection (2%). HCMV disease developed in 7 patients (7.2%), while 25 patients had virological positivity alone (25.7%). Table 1 summarizes the analysis of the predictors of HCMV infection. The use of the IL-2 receptor antagonist basiliximab as induction immunosuppressive therapy was associated with a reduced risk of HCMV infection, independently from donor age, duration of the renal disease, number of post-transplantation dialysis sessions and HCMV serostatus of donor and recipient.

Of the 7 patients who developed HCMV disease, 5 subjects had symptoms and signs suggesting a systemic viral syndrome and the remaining 2 showed probable end-organ diseases (one with gastrointestinal and one with pulmonary localization). One of these patients, belonging to the high-risk group, developed a primary infection showing a late-onset, systemic HCMV disease 45 days after prophylaxis interruption. All the episodes of HCMV disease were managed with intravenous ganciclovir (5 mg/kg twice daily or adjusted for renal function according to drug label) and/or oral valganciclovir (900 mg twice daily or adjusted for creatinine levels) with a median treatment duration of 26 days, and in some cases with reduction of immunosuppressive therapy. The outcome was favorable in all patients. Viral load decreased below the standard clinical cut-offs after a median of 19 days. No patient showed disease relapse. Only one transplant recipient had a subsequent virological relapse, without disease, requiring pre-emptive therapy, whereas all the other patients maintained viral replication levels below cut-offs.

TABLE 1 - *Predictors of HCMV infection/disease (n=32 episodes in 97 patients analyzed). Univariable and multivariable Cox regression.*

	Univariable analysis			*Multivariable analysis*		
	RH	*95% CI*	*p*	*RH*	*95% CI*	*P*
Renal disease duration (per 1 year more)	1.03	0.99-1.06	0.104	1.03	1.00-1.07	0.079
Number of post-transplantation dyalisis sessions (per 1 more)	1.06	1.00-1.13	0.051	1.06	0.98-1.14	0.136
Use of basiliximab	0.52	0.26-1.04	0.063	0.40	0.18-0.86	0.019
Donor age (per 1 year more)	1.02	0.99-1.04	0.129	1.01	0.99-1.04	0.305
HCMV serostatus			0.266			0.321
R+/D- (ref)	1.00			1.00		
R+/D+	4.99	0.68-36.69	0.114	3.41	0.43-26.70	0.243
R-/D+	3.55	0.32-39.20	0.301	1.24	0.07-21.34	0.881

Other tested variables without significant associations at univariable analysis: recipients age, gender, other type and number of immunosuppressive drugs used, type of transplantation (number of kidneys, re-transplantation), high risk category for CMV disease, comorbid conditions, delayed graft function. RH= relative hazard; CI=confidence interval; R/D = recipient/donor.

TABLE 2 - *Complications after transplantation in patients with/ without HCMV infection/disease (n= 200 episodes* in 96 patients**).*

		HCMV infection and disease	*Mean (episodes)*	*SD*	*95% CI*		*p value*▪	*HCMV infection without disease)*	*Mean (episodes*	*SD*	*95% CI*		*p value*▪
Total complication episodes*	Neg	65	1.7231	1.586				65	1.7231	1.586			
(n=200)	Pos	31	2.8387	2.001	-1.86	-0,36	0.004	24	3.000	2.146	-2.10	-0.44	0.003
Infectious complications°	Neg	65	0.6615	0.7960				65	0.6615	0.796			
(n=85)	Pos	31	1.3548	0.9503	-1.06	-3.26	0.000	24	1.4583	0.9770	-1.19	-0.39	0.000
Laboratory abnormalities episodes□	Neg	65	0.1538	0.3636				65	0.1538	0.3636			
(n=22)	Pos	31	0.3871	0.6672	-0.44	-0.02	0.029	24	0.4583	0.7210	-0.53	-0.07	0.010
Renal complications***	Neg	65	0.1231	0.3311				65	0.1231	0.3310			
(n=17)	Pos	31	0.2903	0.5287	-0.34	-0.01	0.061	24	0.3333	0.5646	-0.40	-0.17	0.033

*Including infectious, renal, cardiovascular, neurological, endocrine-metabolic, gastrointestinal, respiratory complications and laboratory abnormalities requiring intervention occurred after transplantation. **Data unavailable for one patient. °Other than HCMV itself. □Laboratory abnormalities requiring intervention (other than acute creatinine level increase). ▪According to T-test for independent samples. ***Acute creatinine level increase requiring intervention (other than rejection). SD= standard deviation; CI= confidence interval. Other tested complication categories without significant association with HCMV infection: respiratory, gastrointestinal, cardiovascular, neurological, endocrine-metabolic.

TABLE 3 - *Predictors of graft rejection (n=33 episodes in 97 patients analyzed). Univariable and multivariable Cox regression.*

	Univariable analysis			*Multivariable analysis*		
	RH	*95% CI*	*P*	*RH*	*95% CI*	*P*
HCMV disease	3.00	1.23-7.34	0.016	2.63	1.07-6.48	0.035
HCMV infection	1.97	0.98-3.95	0.056	N.E.	N.E.	N.E.
Age of donor (per 1 year more)	1.04	1.01-1.07	0.005	1.03	1.00-1.06	0.043
Number of post-transplantation dyalisis sessions (per 1 more)	1.13	1.08-1.18	<0.001	1.11	1.06-1.17	<0.001

Other tested variables without significant associations at univariable analysis: recipients age, gender, renal disease duration, type and number of immunosuppressive drugs used, type of transplantation (number of kidneys, re-transplantation, high risk category for CMV disease, comorbid conditions, delayed graft function. RH= relative hazard; CI=confidence interval; N.E., not entered in the model.

Of the 25 patients who developed post-transplant HCMV infection without disease, 5 subjects (20%) were treated with pre-emptive therapy (valganciclovir p.o. 900 mg/bid or intravenous ganciclovir 5 mg/kg/bid) for 20 days, with an average time to plasma viral load decrease below standard cut-offs of 38 days. None of these 5 cases developed HCMV disease after a median follow-up after treatment discontinuation of 641 days. A high-risk patient (D+/R-) developed an asymptomatic HCMV replication 90 days after the discontinuation of valganciclovir treatment, whose levels remained spontaneously below high-risk cut-offs. In no case was antiviral treatment, either prophylactic or therapeutic, adjusted according to renal function, required interruption due to toxicity.

HCMV infection and associated complications

No patient died strictly from HCMV. HCMV infection, even without disease, was significantly associated with a greater number of different post-transplant complications, particularly other infectious events, acute renal dysfunction other than rejection and laboratory abnormalities requiring intervention (Table 2).

Results of univariable and multivariable analysis of predictors of transplant rejection are shown in Table 3. HCMV disease development, older graft age and post-transplant renal dysfunction requiring dialysis resulted independent predictors of rejection. On the contrary, the presence of HCMV infection without disease did not show any significant association with graft rejection. Patient deaths were significantly associated with post-transplant renal dysfunction as witnessed by a higher number of post-transplant dialysis sessions (RH per 1 more 1.13, 95%CI 1.05-1.20, p=0.001) while older patient age (RH per 1 year more 1.11, 95%CI 0.99-1.25, p=0.065) and longer duration of the renal disease (RH per 1 year more 1.05, 95%CI 0.99-1.11, p=0.09) showed only a trend. Multivariable analysis was not feasible due to the small number of events. The use of different immunosuppressive drugs was not associated with graft rejection. Among the 33 patients showing rejection, six developed HCMV viral replication and only one HCMV disease after the treatment of the rejection.

DISCUSSION

In this study, a complete cohort of patients receiving renal transplant over two years in a single unit at a University Hospital in central Italy was followed for an average time of 666 days. HCMV replication, sustained by post-transplant immunosuppression, was a common event involving one third of the analyzed population of renal transplants and the most common complication that affected patients during the first 3 months after transplantation.

Preventing HCMV disease with a pre-emptive therapy based on viral replication levels was the current procedure in SOTRs adopted at this unit,

according to the latest Guidelines while only high-risk groups (D+/R-) underwent prophylaxis (Consensus SIV-AMCLI, 2008; KDIGO Transplant Work Group, 2009: Andrew *et al.,* 2011).

With proper clinical and viral monitoring, only 5/25 patients showing HCMV infection received pharmacological therapy and none of them developed HCMV disease. Additionally, only 1/6 high-risk patients developed a late-onset disease after antiviral prophylaxis discontinuation (Paya *et al.,* 2004).

The incidence of HCMV disease in our population (7.2%), was lower than in other case studies, confirming the effectiveness of prophylaxis and pre-emptive therapy (Sagedal *et al.*, 2005; San Juan *et al.,* 2008).

From a clinical point of view, all the observed HCMV diseases (5 systemic diseases, 1 gastrointestinal and 1 pulmonary localization) had a good outcome and there were no deaths or relapses. Our experience highlighted the difficulty of making a definite diagnosis of end-organ HCMV disease, both for the possible dissociation with systemic high viral load and for the frequent lack of biological tissue samples (Gerna *et al.,* 2012).

In line with previous findings, in this study the onset of HCMV disease and graft age, but not HCMV infection per se, were found to be independent risk factors for graft rejection (San Juan *et al.,* 2008). Nevertheless, HCMV infection without disease, whether pharmacologically or spontaneously controlled, was associated with a greater number of other complications. The association of HCMV infection was particularly evident with other infectious complications, acute renal dysfunction and laboratory abnormalities requiring intervention.

While the efficacy of pre-emptive and prophylactic therapies in preventing HCMV disease is broadly accepted, some authors emphasize the role of continuous and subclinical viral replication in triggering rejection, chronic graft impairment, diabetes, atherosclerosis and leading to increased mortality, and thus suggest universal, prolonged prophylaxis administration to every solid organ transplantation recipient (Legendre *et al.,* 2008). In particular, the emerging hypothesis is that viral replication, even without disease development, might trigger different complication events (not necessarily related to graft damage) due to immune activation and inflammation, although this requires further investigation (Varani *et al.,* 2009; Van de Berg *et al.,* 2010; Aiello *et al.,* 2012). The pathogenetic links between HCMV replication and graft rejection still need to be clarified. HCMV may produce direct or immune-mediated renal injury and thus contribute to rejection or graft dysfunction. Conversely, rejection and, overall, its treatment could represent a risk factor for HCMV reactivation, as documented in 34% of patients who developed rejection (Varga *et al.,* 2008).

The reason active HCMV replication occurred only in a few transplanted patients remains unclear, considering that more than 90% of them had serological HCMV positivity. Several studies have already outlined the typical major risk factors for HCMV disease, such as HCMV D+/R- mismatch and type of immunosuppressive regimens (cyclosporine use, OKT3 or antithymocite globulin as induction treatment). Organs from elderly donors, kidney-pancreas transplantation and viral co-infections (such as EBV) represent additional risk factors. Moreover, an increasing number of studies underline how cellular immune response plays an important role against HCMV and virus-specific T-cell activation, both CD4 and CD8, seems to be protective towards viral replication and HCMV disease development (Zelini *et al.,* 2010; Gerna *et al.,* 2011). Analysing all the variables associated with post-transplant HCMV replication, we found a significant association between the use of basiliximab as induction immunosuppressive treatment and less HCMV infection/disease. Previous studies showed a more limited frequency of HCMV infection after basiliximab induction compared with anti-thymocyte antibodies (Sollinger *et al.,* 2001; Lebranchu *et al.,* 2002; Lawen *et al.,* 2003; Brennan *et al.,* 2006). This is a potentially interesting finding needing confirmation. A protective effect against HCMV disease was shown by sirolimus in other investigations (Luan *et al.,* 2010; San Juan *et al.,* 2009), but we could not confirm this observation in our series. Due to the retrospective nature of the study it was not possible to determine whether the use of basiliximab itself or the different power and combination of immunosuppressive drugs was protective against HCMV. It should be noted that the choice of drugs showed no significant difference in terms of risk of rejection. Besides pharmacological risk factors, other variables that

demonstrated an association trend with HCMV replication at univariable analysis (not confirmed in the adjusted analysis) were the duration of renal disease, donor age and HCMV positive serostatus in donors with a seropositive recipient. In this regard it is interesting to note that some authors have hypothesized how coinfection or superinfection by different HCMV genotypes in seropositive recipients could represent an additional risk factor for viral replication (Ishibashi *et al.,* 2011).

CONCLUSIONS

This study confirms that in renal transplant recipients HCMV infection still represents a clinically relevant issue influencing the outcome of SOTRs (Eid *et al.,* 2010). While HCMV disease is confirmed to be associated with graft rejection, it should be emphasized that HCMV infection without disease is associated with a higher number of other complication events. So, while HCMV disease prevention strategies and monitoring tools have shown a progressive improvement, the correct management of subclinical viral replication needs further clarification. The use of different immunosuppressive strategies, as suggested by our investigation, might be associated with a lower risk of HCMV infection/disease.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Author's specific contributions to the work: P.C., D.L.A, Z.G. collected the data, participated in research design, data analysis and writing of the manuscript, C.M., G.G., C.M.G., R.B., R.M.L., T.G. participated in study design, data analysis, and writing of the manuscript.

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