The current burden of cytomegalovirus infection in kidney transplant recipients receiving no pharmacological prophylaxis

O fardo atual da infecção por citomegalovírus em receptores de transplante renal que não recebem profilaxia farmacológica

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Abstract

Cytomegalovirus (CMV) infection in kidney transplantation has changed its clinical spectrum, mostly due to the current and more effective immunosuppression. In the absence of preventive strategies it is associated with significant morbi-mortality. **Objective:** This study evaluated the incidence of CMV events and its effect on outcomes of kidney transplantation in recipients without pharmacological prophylaxis or targeted preemptive treatment. **Results:** The study cohort comprised 802 recipients of kidney transplants between 04/30/2014 and 04/30/2015. The majority received induction with anti-thymocyte globulin (81.5%), tacrolimus and prednisone in combination with either mycophenolate (46.3%) or azathioprine (53.7%). The overall incidence of CMV events was 42% (58.6% infection and 41.4% disease). Patients with CMV showed higher incidence of first treated acute rejection (19 *vs.* 11%, p = 0,001) compared with those without CMV but no differences in graft loss, death or loss to follow-up. The incidence of delayed graft function was higher (56% *vs.* 37%, p = 0.000) and the eGFR at 1 (41 ± 21 *vs.* 54 ± 28 ml/min, p = 0.000) and 12 months (50 ± 19 *vs.* 61 ± 29 ml/min, p = 0.000) were lower in patients with CMV. Recipients age (OR = 1.03), negative CMV serology (OR = 5.21) and use of mycophenolate (OR = 1.67) were associated with increased risk of CMV. Changes in immunosuppression was more often in patients with CMV (63% *vs.* 31%, p = 0.000). **Conclusion:** the incidence of CMV events was high and associated with higher incidence of acute rejection and changes in immunosuppression. Besides traditional risk factors, renal function at 1 month was independently associated with CMV infection.

Keywords: cytomegalovirus; kidney transplantation; immunosuppression.

Resumo

A infecção por citomegalovírus (CMV) no transplante renal mudou seu espectro clínico, principalmente devido à atual e mais efetiva imunossupressão. Na ausência de estratégias preventivas, está associado a significativa morbimortalidade. **Objetivo:** este estudo avaliou a incidência de eventos de CMV e seu efeito nos desfechos do transplante renal em receptores sem profilaxia farmacológica ou tratamento preventivo direcionado. **Resultados:** A coorte do estudo envolveu 802 receptores de transplantes de rim entre 30/04/2014 e 30/04/2015. A maioria recebeu indução com globulina anti-timocitária (81,5%), tacrolimus e prednisona em combinação com micofenolato (46,3%) ou azatioprina (53,7%). A incidência global de eventos de CMV foi de 42% (58,6% de infecção e 41,4% de doença). Os pacientes com CMV apresentaram maior incidência de rejeição aguda do primeiro tratamento (19 *vs.* 11%, p = 0,001), em comparação com aqueles sem CMV, mas sem diferenças na perda de enxerto, morte ou perda de seguimento. A incidência de função retardada de enxerto foi maior (56% *vs.* 37%, p = 0,000) e a TFGe a 1 (41 ± 21 *vs.* 54 ± 28 ml/min, p = 0,000) e 12 meses (50 ± 19 *vs.* 61 ± 29 ml/min, p = 0.000) foram menores em pacientes com CMV. A idade dos receptores (OR = 1,03), a sorologia negativa para CMV (OR = 5,21) e o uso de micofenolato (OR = 1,67) foram associados ao aumento do risco de CMV. As alterações na imunossupressão foram mais frequentes em doentes com CMV (63% *vs.* 31%, p = 0,000). **Conclusão:** a incidência de eventos relacionados a CMV foi alta e associada a maior incidência de rejeição aguda e alterações na imunossupressão. Além dos fatores de risco tradicionais, a função renal com 1 mês foi associada de forma independente à infecção por CMV.

Palavras-chave: citomegalovírus; imunossupressão; transplante de rim.

Introduction

The current standard immunosuppressive regimen for kidney transplant recipients includes induction with basiliximab and maintenance therapy with tacrolimus (TAC), mycophenolate (MPS) and prednisone (PRED).[1] Induction therapy with rabbit anti-thymocyte globulin (r-ATG) has been used in an increasing proportion of patients with higher immunological risk and in recipients of kidney recovered from expanded criteria donos.[2] This strategy determines lower incidence of acute rejection and superior long term graft survival,[3,4] but are associated with higher incidence of cytomegalovirus (CMV)[5] and polyomavirus infection.[6,7]

Due to the increasing use of more effective immunosuppressive regimens, the spectrum of clinical presentations of CMV infection has progressively changed, with significant morbidity and mortality in the absence of preventive strategies. Management of CMV infection is then mandatory but associated with increased direct and indirect costs.[8]

Currently there are two alternatives for the prevention of CMV infection, universal pharmacological prophylaxis and preemptive treatment. Both strategies have proven efficacy and safety.[9] However, the access to pharmacological prophylaxis and to the laboratorial tests for monitoring viral replication, used for preemptive treatment, are limited and not reimbursed by our National Health System.

This study aims to evaluate the incidence of CMV infection and its effect on the major outcomes of kidney transplantation using a contemporary cohort of renal transplant recipients receiving no pharmacological prophylaxis.

Methods

Study design

This single center retrospective study included data extracted from an electronic database and adjudicated by review of medical records. The study was approved by the local Research Ethics Committee (CEP) at UNIFESP under registration C.A.A.E ID: 56366516.5.0000.5505.

Population

For this analysis, all patients who underwent transplantation between 04/30/2014 and 04/30/2015 were initially selected. Patients undergoing simultaneous pancreas-kidney transplants, receiving kidney transplant from HLA identical living donors, and receiving initial immunosuppressive therapy including cyclosporine or everolimus were excluded from the analysis.

Objective

The aim of this study was to determine the incidence and risk factors for CMV infection or disease and its influence on clinical outcomes during the first year after kidney transplantation.

Immunosuppression

Induction therapy with basiliximab was used in pediatric recipients while r-ATG (single 3 mg/kg dose within 24 hours of graft revascularization) was the choice for the majority of adult recipients. All patients received tacrolimus with doses adjusted to maintain blood concentrations between 5-15 ng/mL, combined with MPS (1440 mg/day) or azathioprine (AZA, 2 mg/kg/day). All patients received an initial dose of 0.5 mg/kg/day of prednisone which was progressively reduced to 5 mg/day within 30-45 days after transplantation.

Management of CMV infection/disease

None of the patients received pharmacological prophylaxis for CMV infection. Instead, preemptive treatment was performed in patients deemed as high risk for developing CMV infection: (1) seronegative CMV kidney transplant recipients from seropositive CMV donors (D+/R-); (2) use of r-ATG for induction (3) use of MPS for maintenance therapy; (4) after treatment of acute rejection episodes. The preemptive treatment consisted of monitoring of viral replication every other week from week three until the end of the third month, using the pp65 CMV antigenemia test.

CMV infection was defined as the presence of more than 5 infected cells with pp65 CMV antigen in a total of 200,000 peripheral blood neutrophils in the absence of symptoms or signs of CMV infection. For D+/R- patients the diagnosis of CMV infection was done with any number of infected cells. CMV disease was defined as any number of cells infected with pp65 antigen associated plus characteristic symptoms or signs such as fever, asthenia, myalgia, leukopenia, thrombocytopenia and liver enzymes abnormalities.

All episodes of CMV infection or disease were treated with intravenous ganciclovir with dose adjustments according to renal function, as per package insert. Treatment was monitored weekly and extended for one more week after a negative pp65

CMV antigenemia test, with a minimum of 14 days of ganciclovir. Recurrence of CMV infection or disease was defined as a new episode diagnosed after a previous successful treatment confirmed by a negative pp65 CMV antigenemia test.

Definitions

The episodes of biopsy proven acute rejection (BPAR) were classified according to Banff 2009 criteria. Clinical rejections were defined as graft dysfunction without histological evidence of rejection and treated with methylprednisolone for at least three days. The composite endpoint of efficacy failure included the incidence of the first biopsy proven acute rejection, graft loss, death or loss to follow up. Delayed graft function was defined as the need of dialysis during the first week of transplant. The estimated glomerular filtration rate (eGFR) was calculated using the MDRD-4 equation. Permanent discontinuation of initial immunosuppressive treatment was defined as the withdrawal of one drug or conversion to another alternative drug during the first year of transplantation.

Statistical analysis

Continuous variables were summarized by mean and standard deviation and categorical variables as proportions. The differences between groups were identified using the Student t or Chi square test, respectively. The differences in cumulative survival obtained by the Kaplan-Meier curves were identified by Log Rank test. Logistic regression using one or more variables was used to identify risk factors associated with CMV infection (age and type of donor; age, race, cause of chronic kidney disease, degree of sensitization to HLA antigens [PRA], time on dialysis; pretransplant CMV serology, HLA mismatches, induction therapy, initial immunosuppression with MPS, delayed graft function and acute rejection) and with reduced renal function at 12 months of transplantation (age and type of donor, age, gender, time on dialysis, PRA, HLA mismatches, pretransplant CMV serology, cold ischemia time, delayed graft function, induction therapy, MPS use, acute rejection and recurrent CMV infection or disease).

Only variables with $p < 0.05$ on univariable analysis were used in the multivariable models. Best surrogate variable was selected among variables showing colinearity to include in multivariable models. All statistical analyzes were performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA) and differences were considered significant for values of $p < 0.05$.

Results

Population

From April 2014 to April 2015, 938 kidney transplants were performed. One-hundred-thirty-six patients were excluded: 22 simultaneous kidney-pancreas transplants, 37 HLA identical living donor transplants, and 69 patients who received everolimus and 8 who received cyclosporine in the initial immunosuppressive regimen (Figure 1).

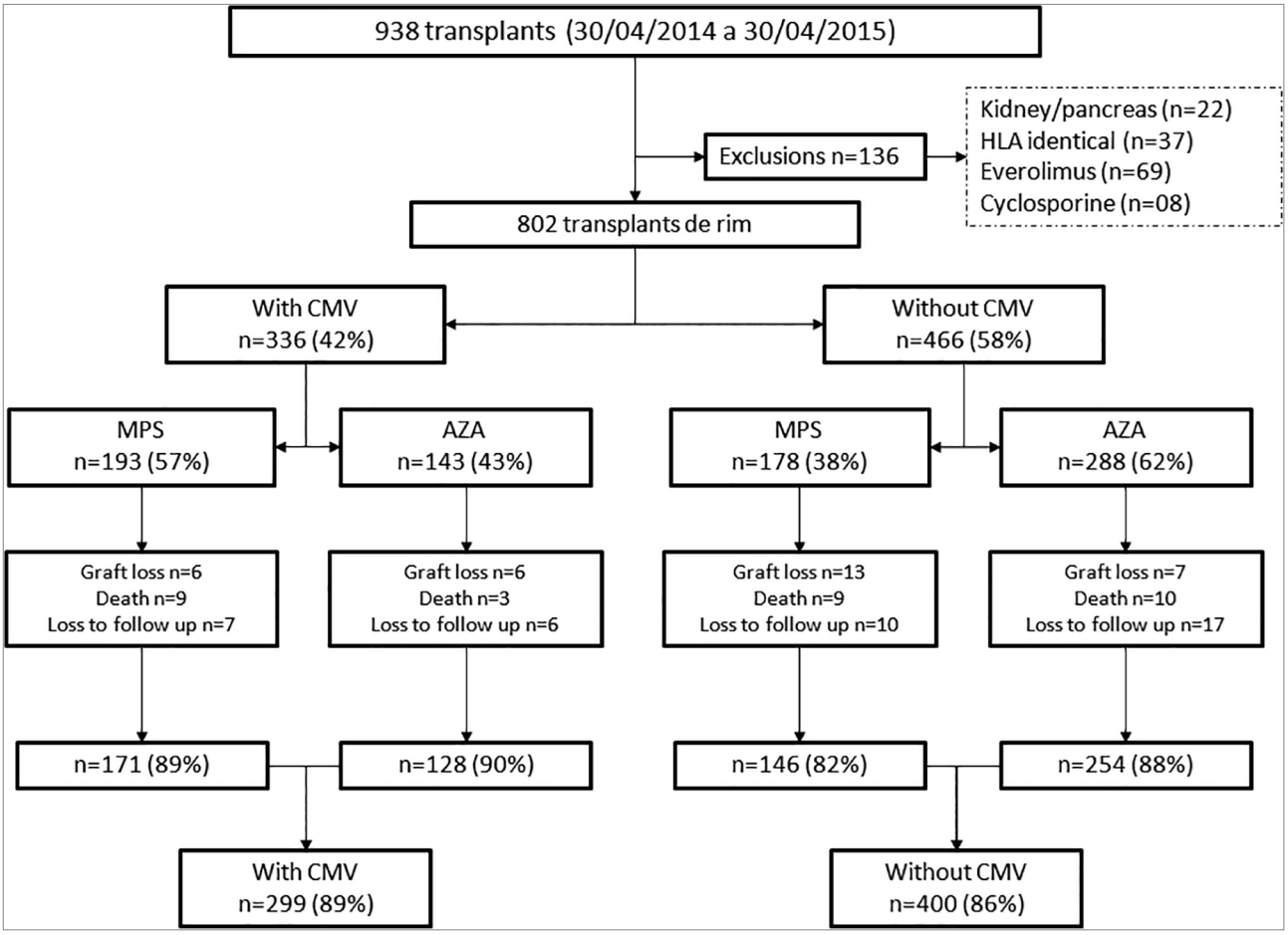
The study cohort consisted of 802 recipients; most were adults, males and with a higher prevalence of white and mixed ethnicities. The proportion of patients with chronic kidney disease secondary to *diabetes mellitus* was 14.9% and the average time on dialysis was 46.1 months. Patients showed low degree of sensitization against HLA antigens. Only 6.2% were CMV D+/R- with additional 2.1% of CMV R- who received kidneys from donors with unknown CMV serostatus. Mean donor age was 44.9 years and 20.9% of the patients received kidneys from living donors. Induction therapy with r-ATG was used in 81.5% and all patients received initial immunosuppression regimens consisted of TAC and PRED, in combination with either MPS (46.3%) or AZA (53.7%).

CMV Infection or disease

The global incidence of CMV infection or disease was 42% (336/802). Patients with CMV infection were older and were on dialysis for a longer period. This group had a higher proportion of patients with *diabetes mellitus*, with CMV D+ or unknown/R- combinations and higher mean class II PRA. Patients with CMV infection received grafts from older donors and with a higher proportion of expanded criteria donors but with lower HLA mismatches. Finally, of the patients receiving no induction or basiliximab, only 23.6% developed CMV infection (Table 1).

CMV infection (58.6%) was more frequent than CMV disease (41.4%). Because of the pretransplant serostatus prevalence, the majority of the episodes (66.1%) occurred among CMV D+/R+. Of the 336 first episodes of CMV infection/disease, 47 (14%) were preceded by an episode of acute rejection. Of 65 patients with first treated acute rejection episodes, 19 (29%) occurred after the first episode of CMV infection/disease.

Figure 1. disposition of patients evaluated and enrolled in the study cohort.



The incidence of recurrence of CMV infection or disease was 36%, ranging between 1 and 5 episodes per patient, and yielding a total of 514 episodes of CMV infection or disease diagnosed in 336 patients. Importantly, 46% of the patients with CMV infection/disease required transient or permanent changes in MPS or AZA dosages (Table 2).

Of 139 patients with first CMV disease 87 (63%) occurred during the period of pp65 antigenemia monitoring every other week. Of them, 19 were D+/R-, 63 were receiving MPS and 5 occurred after treatment for acute cellular rejection. Only 2 patients (D+/R-) presented CMV disease after day 105, the end of pp65 antigenemia monitoring.

The majority of the episodes of CMV diseases were mild, with either leucopenia and/or gastrointestinal manifestations, predominantly diarrhea, with no involvement of lung, liver or pancreas. Prompt recover was observed after treatment with ganciclovir and MPS or AZA dose reduction or interruption. No episodes of invasive CMV disease were confirmed by histologic analysis and there were no deaths directly related to episodes of CMV infection or disease.

Compared to AZA, patients receiving MPS had a higher incidence (33% *vs.* 52%) and a shorter interval between transplant and the first CMV infection/disease episode, respectively. The incidence of recurrence of CMV infection/disease was higher in patients using MPS (41.4%) compared with AZA (28.7%). The mean duration of treatment with ganciclovir was not different between patients who received MPS or AZA.

The incidence of the first episode of CMV after treatment of acute rejection was higher in patients on AZA (23.8%) compared with MPS (6.7%). Moreover, the incidence of acute rejection after the first episode of CMV infection or disease was higher in treated MPS (7.2%) compared with AZA (3.5%). Changes in the initial immunosuppressive regimen after CMV infection/disease episodes were higher in patients who received MPS (52%) compared with AZA (38%) (Table 2).

Recipients age (OR = 1.03 per year), CMV serological combination D+/R- (OR=5.21), and use of MPS (OR = 1.67) were associated with increased risk of CMV infection/disease. On the other hand, number

Table 1 Demographic characteristics of study population

	Total N = 802	With CMV N = 336	Without CMV N = 466	*p*
Recipient age, years (mean ± SD)	40.2 ± 14.7	48.6 ± 13.4	41.1 ± 14.8	0.000
Recipient gender, N (%)				
Male	497 (62.0)	198 (39.8)	299 (60.2)	0.132
Female	305 (38.0)	138 (41.2)	167 (54.8)	
Recipient race, N (%)				
White	321 (40.0)	135 (42.1)	186 (57.9)	0.011
Black	105 (13.1)	36 (34.3)	69 (65.7)	
Mixed	354 (44.5)	149 (42.1)	205 (57.9)	
Other	22 (2.7)	16 (72.7)	6 (27.3)	
Cause of renal chronic disease, N (%)				
Glomerulonephritis	182 (22.7)	64 (35.2)	118 (64.8)	0.000
Hypertension	86 (10.7)	47 (54.7)	39 (45.3)	
Diabetes Mellitus	119 (14.9)	64 (53.8)	55 (46.2)	
Polycystic kidney disease	57 (7.1)	31 (54.4)	26 (45.6)	
Unknown	284 (35.4)	104 (36.6)	180 (66.4)	
Other	74 (9.2)	26 (35.1)	48 (64.9)	
Time on dialysis, months (mean ± SD)	46.1 ± 45.7	51.6 ± 47.6	42.2 ± 44.0	0.004
Previous treatment, N (%)				
Hemodialysis	684 (85.2)	284 (41.5)	400 (58.5)	0.784
Peritoneal dialysis	43 (5.4)	21 (48.8)	22 (51.2)	
Conservative	36 (4.5)	14 (38.9)	22 (61.1)	
Hemodialysis/Peritoneal dialysis	39 (4.9)	17 (43.6)	22 (56.4)	
Panel of reactive antibodies (mean ± SD)				
Class I	9.1 ± 22.0	10.9 ± 24.0	7.8 ± 20.3	0.064
Class II	4.7 ± 16.5	6.0 ± 18.2	3.8 ± 15.1	0.050
HLA mismatch (mean ± SD)	2.5 ± 1.3	2.3 ± 1.2	2.6 ± 1.3	0.001
Pre-transplant CMV IgG, N (%)				
Donor +/Recipient +	573 (71.5)	222 (38.7)	351 (61.3)	0.001
Donor +/Recipient -	50 (6.2)	33 (66)	17 (34)	
Donor -/Recipient +	34 (4.2)	13 (38.2)	21 (61.8)	
Donor unk/Recipient +	126 (15.7)	55 (43.7)	71 (56.3)	
Donor -/Recipient -	2 (0.3)	1 (50)	1 (50)	
Donor(unk/Recipient -	17 (2.1)	12 (70.6)	5 (29.4)	
Donor age, years (mean ± SD)	44.4 ± 15.3	47.3 ± 14.8	42.3 ± 15.4	0.000
Donor, N (%)				
Living donor	168 (20.9)	41 (24.4)	127 (75.6)	0.000
Standard criteria deceased donor	451 (56.3)	177 (39.2)	274 (60.8)	
Expanded criteria deceased donor	183 (22.8)	118 (64.5)	65 (35.5)	
Cold ischemia time (mean ± SD)	24.5 ± 6.7	24.9 ± 6.7	24.4 ± 6.8	0.417
Induction therapy, N (%)				
No induction	97 (12.1)	26 (26.8)	71 (73.2)	0.000
Thymoglobulin	654 (81.5)	301 (46)	353 (54)	
Basiliximab	51 (6.4)	9 (17.6)	42 (82.4)	
Initial immunosuppression, N (%)				
TAC+MPS+Pred	371 (46.3)	193 (52)	178 (48)	0.000
TAC+AZA+Pred	431 (53.7)	143 (33.2)	288 (66.8)	

SD: standard deviation; CMV: cytomegalovirus; HLA: human leucocyte antigen; (unk): unknown; TAC: tacrolimus; MPS: sodium mycophenolate; AZA: azathioprine; Pred: prednisone.

of HLA mismatches (OR = 0.88 per mismatch) and kidney function at 1 month (OR = 0.98 per ml/min/1.73 m^2) were associated with reduced risk of CMV infection/disease (Table 3).

Clinical outcomes of efficacy and safety

The group with CMV showed a higher incidence of first treated acute rejection (19 *vs.* 11%) and first treated biopsy confirmed acute rejection (10% *vs.*

Table 2 Characteristics of CMV infection or disease episodes

Variable	Total N = 802	MPS N = 371	AZA N = 431
Incidence of first CMV event, N (%)	336 (42.0)	193 (52.0)[1]	143 (33.0)
Infection	197 (58.6)	121 (62.7)	76 (53.2)
Disease	139 (41.4)	72 (37.3)	67 (46.8)
Time to first CMV event, days (mean ± SD)	47.4 ± 30.1	41.1 ± 18.2[1]	55.8 ± 39.6
Duration of treatment, days (mean ± SD)	20.1 ± 9.1	20.3 ± 9.8	19.8 ± 7.8
Incidence of CMV events according to pre-transplant CMV serology, N (%)			
Donor +/Recipient +	222 (66.1)	125 (64.8)	97 (67.8)
Donor +/Recipient -	33 (9.8)	17 (8.8)	16 (11.2)
Donor -/Recipient +	13 (3.9)	7 (3.6)	6 (4.2)
Donor unk/ Recipient +	55 (16.4)	35 (18.1)	20 (14.0)
Donor -/Recipient -	1 (0.3)	1 (0.6)	0 (0)
Donor unk/ Recipient -	12 (3.5)	8 (4.1)	4 (2.8)
Incidence of recurrent CMV events, N (%)	121 (36.0)	80 (41.4)[2]	41 (28.7)
Total number of recurrent CMV events	178	110	68
Infection	120 (67.4)	73 (66.4)	47 (69.1)
Disease	58 (32.6)	37 (33.6)	21 (30.9)
Patients with 1 recurrence	83 (68.6)	59 (73.8)	24 (58.5)
Patients with 2 recurrences	24 (19.8)	13 (16.2)	11 (26.8)
Patients with 3 recurrences	10 (8.3)	7 (8.8)	3 (7.3)
Patients with 4 recurrences	3 (2.5)	1 (1.2)	2 (5.0)
Patients with 5 recurrences	1 (0.8)	0 (0)	1 (2.4)
Total of CMV events	514	303	211
Incidence of treated acute rejection before the first CMV event, N (%)	47 (14.0)	13 (6.7)[1]	34 (23.8)
Patients with 1 episode	40 (85.1)	12 (92.3)[a]	28 (82.4)
Patients with 2 episodes	7 (14.9)	1 (7.7)	6 (17.6)[b]
Total of treated acute rejection before the first CMV event, N	54	14	40
Incidence of treated acute rejection after CMV event, N (%)	19 (5.7)	14 (7.2)	5 (3.5)
Patients with 1 episode	17 (89.5)	12 (85.7)	5 (100)
Patients with 2 episodes	2 (10.5)	2 (14.3)	0 (0)
Total of treated AR after CMV event	21	16	5
Changes in MPS/AZA dosing after CMV events, N (%)	155 (46)	101 (52)[3]	54 (38)
Temporary interruption	63 (41)	30 (30)	33 (61)
Dose reduction	69 (44)	55 (54)	14 (26)
Permanent discontinuation	18 (12)	12 (12)	6 (11)
Conversion to other drug	5 (3)	4 (4)	1 (2)

SD: standard deviation; CMV: cytomegalovirus; unk: unknown; MPS: sodium mycophenolate; AZA: azathioprine.

[1] $p = 0.000$, [2] $p = 0.016$, [3] $p = 0.008$ MPS *versus* AZA.

[a] 2 patients presented 1 episode of acute rejection before the first CMV event and 1 episode of acute rejection after CMV event;

[b] 1 patient present 2 episodes of acute rejection before the first CMV event and 1 episode of acute rejection after CMV event.

6%) compared with the group without CMV. These differences were consistently when this analysis was limited to patients receiving either MPS or AZA (Table 4). There were no differences in the incidence of the composite endpoint of efficacy failure, graft loss, death or loss to follow-up. However, a higher percentage of deaths due to infection were observed in the group with CMV (Table 5).

Renal Function

The incidence of delayed graft function was higher in patients with CMV compared with those without CMV, even considering recipients of kidneys from standard donor (60% *vs.* 49%) or expanded criteria donors (70% *vs.* 55%) separately (Table 6). The eGFR at 1 and 12 months were also on average 10 mL/min lower in the group with CMV. This difference was

Table 3 Multiple variables analysis to risk of CMV infection or disease

Variable	Univariable analysis		Multivariable analysis	
	Odds Ratio (95% CI)	*p*	Odds Ratio (95% CI)	*p*
Recipient age, years	1.04 (1.03-1.05)	< 0.001	1.03(1.02-1.04)	0.000
Time on dialysis, months	1.00(1.00-1.01)	0.005	1.00(0.99-1.00)	0.703
PRA class I	1.01(1.00-1.01)	0.051		
PRA class II	1.01(0.99-1.02)	0.067		
HLA mismatches	0.83(0.74-0.93)	0.001	0.88(0.77-0.99)	0.035
CMV serology, Donor (+)/Recipient (-)	2.88(1.57-5.26)	0.001	5.21(2.55-10.66)	0.000
Recipient, Caucasian race	1.01(0.76-1.35)	0.940		
Chronic kidney disease due to *diabetes mellitus*	1.76(1.19-2.60)	0.005	1.26(0.81-1.94)	0.304
Donor type				
Living donor (ref.)				
Standard criteria deceased donor	2.00 (1.34-2.99)	0.001		
Expanded criteria deceased donor	5.6(3.53-8.95)	< 0.001		
Donor age, years	1.02(1.01-1.03)	< 0.001	1.00(0.99-1.01)	0.867
Delayed graft function, yes	2.45(1.84-3.23)	< 0.001		
induction therapy, r-ATG	2.75(1.83-4.14)	< 0.001	1.57(0.99-2.47)	0.053
immunosuppressive therapy, MPS	2.18(1.64-2.91)	0.000	1.67(1.22-2.29)	0.001
Acute rejection, yes	1.04(0.69-1.56)	0.849		
eGFR at 1 month	0.98(0.97-0.98)	< 0.001	0.98(0.98-0.99)	0.000

CI: confidence interval; MPS: sodium mycophenolate; r-ATG: rabbit anti-thymocyte globulin; eGRF: estimated glomerular filtration rate (MDRD4).

not observed in the subgroup of recipients of kidneys from expanded criteria deceased donors (Table 6). While donor age was associated with increased risk (OR = 1.04 per year), use of induction therapy with r-ATG (OR = 0.57) and eGFR at 1 month (OR = 0.93 per ml/min/1.73 m^2) were associated with reduced risk for reduced eGFR at 12 months (Table 7).

Tolerability

The incidence of changes in the initial MPS/AZA dosing regimens at 12 months of transplantation was higher in patients with CMV compared with those without CMV (63% *vs.* 31%). Dose reduction was more frequent in patients receiving MPS while drug discontinuation was more frequent among patients receiving AZA (Table 8).

Discussion

This analysis shows the negative influence of CMV infection on kidney transplant outcomes in patients receiving effective immunosuppressive regimens but no CMV pharmacological prophylaxis. Patients who developed CMV infection had a higher incidence of acute rejection episodes, required more changes in the immunosuppressive regimen and showed inferior renal function at 12 months after transplantation.

Considering the immunosuppressive regimens used, including a single dose of 3 mg/kg r-ATG in 82% of patients, and the use of preemptive treatment only in patients deemed at high risk for CMV infection,[10] the overall incidence of CMV infection/disease was 42% with almost 60% of them diagnosed by viral replication monitoring. In another study in kidney transplant recipients receiving induction with basiliximab, tacrolimus, mycophenolate and prednisone, the incidence of CMV infection/disease was 38% using a higher cutoff of 10 pp65 CMV positive cells to trigger treatment.[11]

In a comparable population, but without the use of preemptive therapy, the incidence of CMV disease was 17%. Interestingly, in this study 54% of patients had transient and self-limited CMV viremia[12] and there were no cases of invasive CMV disease.

While in this cohort 20% of patients developed CMV disease during pp65 monitoring every other week, only 12% developed CMV disease during weekly pp65 monitoring in our previous study,[11] suggesting that the frequency of testing may account, at least in part, for the higher incidence of CMV disease.

On the other hand, this cohort had higher risk for CMV disease, including a high use of r-ATG (82%). Also, in another cohort study we observed that patients developed CMV disease with lower level of viremia compared to those treated for CMV infection, suggesting that other factors such as the individual strength of immune response towards the virus, either cellular or the antibody titer, may be involved.[10]

Table 4 Incidence and characteristics of the acute rejection episodes

Parameters, N (%)	With CMV			Without CMV		
	Total N = 336	MPS N = 193	AZA N = 143	Total N = 466	MPS N = 178	AZA N = 288
First acute rejection episode, N(%)	65 (19)[a]	25 (13)[b]	40 (28)[c]	50 (11)	12 (7)	38 (13)
Biopsy proven acute rejection	34(10)[d]	11(6)[e]	23(16)	27(6)	7(4)	20(7)
IA	13(38)	3(27)	10(44)	18(67)	6(86)	12(60)
IB	11(32)	5(45)	6(26)	4(15)	0	4(20)
IIA	9(27)	3(27)	6(26)	5(19)	1(14)	4(20)
IIB	0	0	0	0	0	0
III	1(3)	0	1(4)		0	0
Borderline changes	21(6)	8(4)	13(9)	18(4)	4(2)	14(5)
Clinical acute rejection	10(3)	6(3)	4(3)	5(1)	1(1)	4(1)
Time to first acute rejection episode, days (mean ± SD)	49,0 ± 50,1	68,7 ± 78,7	42,8 ± 52,1	80,5 ± 129,1	123,1 ± 170,0	52,7 ± 91,8
Total number of treated acute rejection	75(22)	30(16)	45(33)	55(12)	13(7)	42(15)
Biopsy proven rejection	36(48)	11(6)	25(18)	30(55)	7(4)	23(8)
IA	13(36)	3(27)	10(40)	21(70)	6(86)	15(65)
IB	13(36)	5(45)	8(32)	4(13)	0	4(17)
IIA	9(25)	3(27)	6(24)	5(17)	1(14)	4(17)
IIB	0	0	0	0	0	0
III	1(3)	0	1(4)	0	0	0
Antibody mediated rejection	0	0	0	1(2)	1(1)	0
Borderline changes	28(37)	12(6)	16(11)	19(35)	4(2)	15(5)
Clinical acute rejection	11(15)	7(4)	4(3)	5(9)	1(1)	4(1)
Treatment						
Methylprednisolone	55(73)	24(80)	31(69)	46(84)	9(69)	37(88)
Antithymocyte globulin/ Methylprednisolone	20(27)	6(20)	14(31)e	8(14)	3(23)	5(12)
Immunoglobulin/ plasmapheresis	0	0	0	1(2)	1(8)	0
Episodes of acute rejection per patient						
Patients with 1 episode	52(16)	20(10)	32(22)	41(9)	9(5)	32(11)
Patients with 2 episodes	10(3)	5(3)	5(4)	5(1)	2(1)	3(1)
Patients with 3 episodes	1(0,3)	0	1(1)	0	0	0
Patients with 4 episodes	0	0	0	1(0,2)	0	1(0,3)

[a] $p = 0.001$, [b] $p = 0.046$, [c] $p = 0.000$, [d] $p = 0.023$, [e] $p = 0.030$ with versus without CMV.

(SD) standard deviation.

The incidence of CMV recurrence was 36% and comparable to other reports.[8] However is important to highlight the high number of patients with more than one episode of recurrence, which resulted in a high number of treated episodes, 514 in 330 patients. Patients receiving MPS showed a higher incidence and recurrence of CMV infection or disease, and underwent more changes in the initial immunosuppressive regimen during CMV treatment compared with those receiving AZA, confirming previous observations.

This study confirms that recipient age, D+/R- CMV serostatus, and use of MPS were independent risks factors associated with CMV infection/disease. The use of r-ATG was associated with an increased risk of CMV infection/disease, almost reaching statistical significance. Also, renal function at 1 month was associated with the incidence of CMV infection/disease, a finding observed in another recent cohort study.[13] In one interesting cohort study, patients with CMV infection showed more chronic allograft changes early on, even before developing CMV infection.[14]

Table 5 Efficacy and safety endpoints

Parameters	With CMV			Without CMV		
	Total (n = 336)	MPS (n = 193)	AZA (n = 143)	Total (n = 466)	MPS (n = 178)	AZA (n = 288)
Composite endpoint, N (%)	67 (20)	31 (16)	36 (26)	92 (20)	38 (21)	54 (19)
BPAR	34 (10)	11 (6)	23 (16)	27 (6)	7 (4)	20 (7)
Graft loss	8 (2)1	4 (2)	4 (3)	19 (4)3	12 (7)	7 (2)
Death	12 (4)2	9 (5)	3 (2)	19 (4)	9 (5)	10 (3)
Lost to follow up	13 (4)	7 (4)	6 (5)	27 (6)	10 (6)	17 (6)
Patient survival, (%)	96.1	95.3	97.2	95.9	94.9	96.5
Graft survival. (%)	92.9	92.2	93.7	91.6	87.6	94.1
Total of graft loss, N (%)	12 (4)	6 (3)	6 (4)	20 (4)	13 (7)	7 (2)
Time to graft loss, days (mean ± SD)	81.83 ± 30.0	65.67 ± 23.9	98.00 ± 28.0	75.90 ± 94.4	73.85 ± 97.3	79.71 ± 96.2
Cause to graft loss, N (%)						
Rejection	2 (17)	1 (17)	1 (17)	4 (20)	2 (15)	2 (29)
Technique	2 (17)	0	1 (17)	7 (35)	4 (31)	3 (42)
IF/TA	1 (8)	2 (33)	0	8 (40)	6 (46)	2 (29)
Glomerulonephritis	1 (8)	0	1 (17)	0	0 (0)	0
Other	6 (50)	3 (50)	3 (50)	1 (5)	1 (8)	0
Total of deaths. N (%)	13* (4)	9 (5)	4 (3)	19 (4)	9 (5)	10 (4)
Time to death, days (mean ± SD)	164.54 ± 88.70	149 ± 70.3	199.50 ± 126.3	97.16 ± 114.6	81.11 ± 93.8	111.60 ± 134.0
Cause to death, N (%)						
Infection	11 (84)	7 (78)	4 (100)	11 (58)	5 (56)	6 (60)
Stroke	0	0	0	3 (16)	0	3 (30)
Cardiovascular	1 (8)	1 (11)	0	3 (16)	2 (22)	1 (10)
Other	1 (8)	1 (11)	0	2 (10)	2 (22)	0

MPS: sodium mycophenolate; AZA: azathioprine; BCAR: Biopsy confirmed acute rejection; SD: standard deviation; IF/TA: interstitial fibrosis and tubular atrophy;

[1] 4 out of 12 patients that suffered graft loss had first BCAR; [2] 1 out of 13 patients that died had graft loss first; [3] 1 out of 20 patients that suffered graft loss had BCAR first;

* 1 patient suffered graft loss 174 days before death.

Table 6 Renal function

	With CMV	Without CMV	*p*
Total	336	466	
DGF, N (%)	189 (56)	171 (37)	0.000
eGFR at 1 month, ml/min, mean ± SD	41.4 ± 20.7	54.3 ± 28.1	0.000
eGFR at 12 months, ml/min, mean ± SD	50.0 ± 19.0	61.4 ± 29.4	0.000
Living donor	41 (12)	127 (27)	
eGFR at 1 month, ml/min, mean ± SD	52.0 ± 19.0	62.5 ± 21.8	0.006
eGFR at 12 months, ml/min, mean ± SD	50.7 ± 16.1	60.3 ± 18.9	0.004
Standard criteria deceased donor	177 (53)	274 (59)	
DGF, n (%)	116 (65)	142 (52)	0.004
eGFR at 1 month, ml/min, mean ± SD	43.4 ± 23.1	54.0 ± 31.0	0.000
eGFR at 12 months, ml/min, mean ± SD	54.4 ± 20.4	66.1 ± 34.5	0.000
Expanded criteria deceased donor	118 (35)	65 (14)	
DGF, n (%)	87 (74)	37 (57)	0.020
eGFR at 1 month, ml/min, mean ± SD	34.7 ± 14.4	38.8 ± 21.0	0.129
eGFR at 12 months, ml/min, mean ± SD	42.6 ± 15.2	44.0 ± 17.1	0.606

DGF: delay graft function; eGFR: estimated glomerular filtration rate by MDRD formula (Modification of Diet in Renal Disease Study equation).

This is in agreement with our observation considering the higher prevalence of expanded criteria donors and the higher incidence of DGF in the group of patients who subsequently developed CMV infection/disease. How impaired renal function early increases the risk of CMV infection is unknown, but impaired immunological surveillance and altered pharmacokinetics and pharmacodynamics of the

Table 7 Risk factors associated with lower renal function (eGRF < 53 ml/min) 12 months after transplant

Variable	Univariable analysis		Multivariable analysis	
	Odds Ratio (95% CI)	*p*	Odds Ratio (95% CI)	*p*
Donor				
Age, years	1.06 (1.04-1.07)	< 0.001	1.04 (1.02-1.05)	0.000
Type				
Living donor (ref.)				
Standard criteria deceased donor	1.21 (0.83-1.78)	0.321		
Expanded criteria deceased donor	3.92 (2.42-6.38)	< 0.001		
Recipient				
Age, years	1.03 (1.02-1.04)	< 0.001	0.99 (0.98-0.97)	0.113
Male gender	0.87 (0.64-1.18)	0.359		
Time on dialysis, months	1.00 (1.00-1.01)	0.021	1.00 (0.99-1.00)	0.791
PRA class I	1.00 (0.99-1.01)	0.581		
PRA class II	1.00 (0.99-1.01)	0.237		
Transplant				
HLA mismatch [0- 6]	0.84 (0.74-0.95)	0.005	0.94 (0.81-1.09)	0.414
CMV serology, Donor (+)/Recipient (-)	1.07 (0.57-1.99)	0.831		
Delayed graft function	1.82 (1.35-2.46)	0.001		
Induction therapy, r-ATG	1.59 (1.07-2.36)	0.023	0.57 (0.33-0.98)	0.041
Immunosuppressive therapy, MPS	2.16 (1.60-2.93)	< 0.001	1.01 (0.67-1.53)	0.944
Acute rejection, yes	1.20 (0.78-1.86)	0.407		
CMV infection/disease, yes	1.99 (1.47-2.70)	< 0.001	1.14 (0.77-1.68)	0.518
Renal function at 1 month	0.93 (0.91-0.94)	< 0.001	0.93 (0.92-0.94)	0.000

CI: confidence interval; MPS: sodium mycophenolate.

Table 8 Changes in mycophenolate sodium or azathioprine dosing schedules 12 months after transplantation

Month 12	With CMV (n = 299)			Without CMV (n = 400)		
	Total (n = 299)	MPS (n = 171)	AZA (n = 128)	Total (n = 400)	MPS (n = 146)	AZA (n = 254)
No modification in the initial immunosuppression, n (%)	112 (37)	54 (32)	58 (45)	277 (69)	77 (53)	200 (78)
Modification in initial the immunosuppression, n (%)	187 (63)[a]	117 (68)[b]	70 (55)[c]	123 (31)	69 (47)	54 (22)
MPS/AZA dose reduction, n (%)	118 (63)	81 (69)	37 (53)	83 (67)	56 (81)	27 (50)
MPS/AZA permanent discontinuation, n (%)	69 (37)	36 (31)	33 (47)	40 (33)	13 (19)	27 (50)

[a] $p = 0.000$, [b] $p = 0.000$, [c] $p = 0.000$, with versus without CMV, respectively.

immunosuppressive drugs might be involved. On the other hand, the association between increasing number of HLA mismatches and reduced risk of CMV infection/disease is less clear and deserves further confirmation.

Patients with CMV infection/disease had higher incidence and more severe episodes of acute rejection, and required a higher number of treatments with r-ATG. As expected, no differences in the incidence of graft loss or death were observed between the two groups at the end of first year, although a higher proportion of deaths were due to infections in the group of patients who developed CMV infection/disease. It is recognized that CMV infection also induces a wide range of indirect effects, such as susceptibility to rejection and opportunistic infections.[15]

At 12 months, mean eGFR was lower in the CMV group compared with patients without CMV, suggesting a possible indirect effect. Nevertheless, among several risk factors associated with renal

function at the end of the first year, only recipient age (OR = 1.04 per year), r-ATG use (OR = 0.57) and eGFR at 1 month (OR = 0.93 per ml/min/1.73m^2) were independently associated with GFR at 12 months. eGFR at 1 month was used as a surrogate marker for donor type and DGF.

During the first year 46% of patients who developed CMV infection/disease required a temporary or permanent change in the immunosuppressive regimen was necessary, particularly due to hematologic adverse reactions such as leukopenia. At the end of first year, 63% of the patients with CMV had changes in the initial immunosuppressive regimen compared to only 31% in the group without CMV.

Patients who received MPS had a higher proportion of changes in immunosuppressive regimen compared with those receiving AZA. While the most common change in MPS was dose reduction, for AZA, permanent discontinuation occurred in nearly 50% of patients, either in the group with or without CMV. Changes in immunosuppressive regimens have been associated with increased incidence of acute rejection.[16] It is possible to speculate that these changes might be associated with subclinical acute rejections influencing renal function at 12 months.

This analysis has several limitations including the single center nature of the study, the demographic characteristics of the population, the immunosuppressive regimens used, and the lack of CMV prophylaxis even for the high risk CMV D+/R- group. On the other hand, the data well represent the national strategies of management of CMV infection, suggesting that more effective strategies to reduce this infection should be investigated and implemented. In the context of our public health system, the use of mTOR inhibitors may be one cost-effective strategy.[11]

In summary, in a contemporaneous cohort of kidney transplant recipients, the incidence and recurrence rate of CMV infection is high and is associated with higher incidence of acute rejection and need for immunosuppressive drug changes during the first year after transplantation. Besides traditional risk factors, renal function at 1 month was independently associated with CMV infection. Understanding this association is fundamental as we increase the use of kidneys recovered from expanded criteria donors.

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