

Long-term renal graft function and survival in patients with high-risk for cytomegalovirus infection receiving preemptive therapy

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ABSTRACT

Background. Preemptive therapy reduces the risk of cytomegalovirus disease in high-risk kidney transplant patients. The advantage of this strategy is that only a fraction of patients receive antiviral drugs for a limited time, which decreases costs and toxicity but requires frequent monitoring and may not prevent complications of asymptomatic cytomegalovirus replication. **Material and methods.** Long-term graft-function and patient survival of high-risk kidney transplant patients who received preemptive therapy guided by pp65 antigenemia was compared to those whose assay remained negative throughout the first post-transplant year. **Results.** Between August 1997 and March 2005, 24 of 272 patients were CMV D+/R-. Thirteen of the 24 (54.2%) developed a positive CMV assay during follow-up; the time between transplant and first positive antigenemia was 66.7 ± 58.3 days (range 29-251 days). Four patients developed symptoms associated with CMV, one of whom succumbed from complications of CMV pneumonitis. Overall, no significant differences were observed in SCr, eGFR, Δ SCr, and Δ eGFR during a 60-month follow-up between patients who developed CMV infection or disease and those who remained pp65 antigenemia-negative throughout the first 12 post-transplant months. Additionally, no deaths or graft loss occurred during the long-term follow up of this cohort. **Conclusions.** Our results suggest that in this high risk group of kidney transplant recipients, treating CMV replication using a preemptive strategy during the first post-transplant year is associated with a low rate of CMV complications and probably interferes with the alleged long-term negative indirect effects of CMV on kidney function and survival.

Sobrevida y función del injerto renal a largo plazo en pacientes con alto riesgo de infección por Citomegalovirus que reciben tratamiento anticipado

RESUMEN

Introducción. La terapia anticipada reduce el riesgo de enfermedad por citomegalovirus (CMV) en receptores de trasplante renal de alto riesgo. La ventaja de esta estrategia es que solamente una fracción de los pacientes recibirá fármacos antivirales por tiempo limitado, lo cual disminuye costos y toxicidades; sin embargo, se requiere monitorización frecuente y podrían no prevenirse las complicaciones de la replicación viral asintomática. **Material y métodos.** La función renal a largo plazo y la supervivencia de pacientes receptores de trasplante renal de alto riesgo para enfermedad por CMV que recibieron terapia antiviral anticipada guiada por antigenemia pp65, se comparó con la evolución de estos parámetros en pacientes que teniendo alto riesgo permanecieron con antigenemia pp65-negativos a lo largo del primer año post-trasplante. **Resultados.** De agosto de 1997 a marzo de 2005, 24 de 272 receptores de trasplante renal fueron CMV D+/R- pretrasplante (alto riesgo). Trece de los 24 (54.2%) desarrollaron positividad del ensayo para CMV durante el seguimiento; el tiempo transcurrido entre el trasplante y la primera antigenemia pp65 positiva fue de 66.7 ± 58.3 días (rango 29-251 días). Cuatro pacientes desarrollaron síntomas asociados a CMV, uno de los cuales falleció por complicaciones de neumonitis por CMV. No se observaron diferencias significativas en CrS, TFG, Δ CrS y Δ TFG durante los 60 meses de seguimiento promedio entre los pacientes que desarrollaron infección o enfermedad por CMV y aquellos que permanecieron antigenemia-negativos a lo largo de los primeros 12 meses

Key words. CMV Infection. Preemptive CMV therapy. Kidney transplantation. Graft function. Graft survival.

INTRODUCTION

Cytomegalovirus (CMV) continues to be a frequent infectious cause of morbidity in solid organ transplant recipients.¹ Its clinical presentation varies from a viral syndrome presenting with fever and malaise (with or without leucopenia or thrombocytopenia) to a symptomatic tissue-invasive disease.² In addition to its direct morbid effects, CMV has a known immunosuppressive effect and is an independent risk factor for the development of other opportunistic infections and graft dysfunction.³

High-risk scenarios for post-transplant CMV disease are well recognized and include seronegative recipients who receive an organ from a seropositive donor and patients receiving anti-lymphocytic therapy regardless of their serologic status.^{2,4} In these circumstances, active symptomatic infections occur in 60% of kidney transplant recipients (KTR).⁵

The role that CMV infection plays in the development and progression of a graft's histological and functional deterioration is one of the most studied and debated aspects of CMV in KTR. Several clinical and experimental studies have provided information supporting that direct and indirect mechanisms associated with CMV are involved in its pathogenesis.⁶

Various preventive strategies have been designed and applied clinically in order to prevent or quickly treat CMV infection, due to its high morbidity and mortality. One of these strategies is to administer prophylactic antiviral therapy in "high-risk" patients (D+/R-), usually during the first three months after transplantation.⁷⁻⁹ Even though this therapy prevents CMV disease in a high percentage of patients, 14-18% will develop symptoms during the first post-transplant year, usually after prophylaxis has concluded.^{8,10} However, this strategy is costly and unnecessarily administered to approximately half of patients who even without prophylactic treatment will not develop symptoms or positive viral markers. This treatment strategy has also been associated to

post-trasplante. Adicionalmente, no ocurrieron otras defunciones o pérdidas de injerto al seguimiento a largo plazo de esta cohorte. **Conclusiones.** Los resultados obtenidos en este grupo de receptores de trasplante renal de alto riesgo, sugieren que tratar la replicación viral mediante una estrategia anticipada durante el primer año post-trasplante, se asocia con un índice bajo de complicaciones por CMV y probablemente interfiera con los efectos virales indirectos negativos a largo plazo en la función renal y supervivencia del paciente.

Palabras clave. Infección por CMV. Terapia anticipada CMV. Trasplante renal. Función del injerto. Sobrevida del injerto.

antiviral drug-induced toxicity, development of drug-resistant viruses, and the possibility of only delaying disease onset.¹¹⁻¹⁴ An alternative to universal prophylaxis is preemptive therapy, which consists of administering antiviral therapy before symptoms arise in patients at high risk of developing clinical disease as reflected by the presence of predictive viral markers.¹⁵⁻¹⁹ Its advantage is that only a fraction of patients receive these drugs during a limited time, thus decreasing costs and toxicity.

A recent study, comparing prophylactic versus preemptive treatment with valganciclovir as antiviral in KTR, points out that prophylaxis was superior in decreasing CMV infection (evidenced by viral replication) and possibly less effective in prevention of symptomatic disease that occurred after discontinuation of prophylaxis. No significant difference between treatment arms was found in the frequency of acute rejection episodes, graft failure, neutropenia, or bacterial infections.¹⁰

Given the possible risk imposed by CMV infection to graft and patient survival, the aim of this study was to analyze the long-term renal graft function/survival of KTR at high risk for CMV disease who were managed by a strategy of CMV preemptive therapy guided by detection of pp65 antigenemia during the first post-transplant year. Patients included were transplanted and followed at the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran. Sixteen patients were included in a previous publication and have a longer follow-up in the present study.²⁰

MATERIAL AND METHODS

This is a prospective cohort study.

Patients

All CMV seronegative KTR with a seropositive donor (R-/D+) since August 1997 were prospective-

ly included for follow-up with sequential pp65 antigenemia and administration of preemptive therapy. All were adult subjects, and the last patient included was transplanted on March 2005. Studied variables included the following: age at transplant, gender, donor source, histocompatibility, immunosuppressive scheme, induction with anti-IL-2r, delayed graft function, clinical or biopsy-proven acute rejection episodes and their relation in time to positive pp65 antigenemia. Renal function, as well as the occurrence of graft loss or patient death was recorded, as was the cause of such events.

CMV Follow-up

Antigenemia pp65 was performed in all patients every two weeks during the first four post-transplant months and monthly thereafter for one year. After November 2004, antigenemia pp65 was performed weekly during the first four months, every two weeks during the fifth and sixth months, and monthly thereafter for one year. All patients whose assay became positive and thus received preemptive therapy or treatment had an anti-CMV antibody determination at the end of the first post-transplant year.

The variables studied with respect to CMV follow-up included: time from kidney transplant to first positive pp65 antigenemia, recurrent positive pp65 antigenemia assays (a positive antigenemia after achieving conversion to a negative assay with preemptive therapy) and number of patients who developed CMV disease or syndrome and its form of presentation. The number of positive cells per assay and the maximum peak per patient and per event were reported in positive cases of pp65 antigenemia.

We defined CMV infection as a positive antigenemia in the absence of clinical symptoms or typical laboratory results (leucopenia or abnormal liver function); CMV syndrome in cases where positive pp65 antigenemia with or without leucopenia was associated with viral symptoms: fever > 48 hours and/or malaise, myalgia, arthralgia, asthenia, when other accompanying infectious diseases were ruled out; and CMV disease in cases with CMV syndrome plus (specific) organ involvement (enteritis, pneumonitis, esophagitis, etc.).

CMV pp65 antigenemia assay

The test was performed as previously described by Van der Bij.¹⁶ Polymorphic leucocytes were se-

parated from 5 mL of anticoagulated blood using NH_4Cl 0.8% and were suspended in PBS at 1.5×10^6 mL. Afterwards, 100 μL were centrifuged in each slide and fixed with formaldehyde. The slides were covered and incubated with a monoclonal antibody against the CMV pp65 antigen (Biotest Diagnostics, Denville, NJ), washed, and then a second incubation with fluorescein-conjugated anti-IgG monoclonal antibodies was performed. When one or more cells were reported positive, the test was considered positive in our study.

Preemptive therapy

We administered a daily 5 mg/kg of body weight intravenous dose of ganciclovir for 15 days to patients who presented a positive pp65 antigenemia assay at any point during the first post-transplant year. Ganciclovir was administered at the ambulatory care ward. Repeat antigenemia assays were performed at the end of treatment and if positive, treatment was continued until the assay became negative. Patients with a positive antigenemia were physically examined and directly questioned. Tests such as complete blood cell count, liver function tests, and any other test considered necessary by the attending physician were performed in order to determine the presence of CMV syndrome or disease.

Patients who presented CMV-related symptoms during the first postrasplant year (associated with a positive pp65 antigenemia) were treated for two weeks with 5 mg/kg body weight of intravenous ganciclovir every 12 hours, adjusted to renal function. If antigenemia continued at the end of the two-week period, treatment was continued with 5 mg/kg BID until a negative result was obtained.

Finally, in cases that presented after November 2004, 900 mg/day of valganciclovir was administered as preemptive therapy in patients presenting positive antigenemia.

Antigenemia at the previously mentioned intervals was performed once preemptive therapy or treatment of CMV disease concluded. Antigenemia was performed every two weeks in patients who were receiving preemptive therapy or treatment for CMV disease on completion of the first post-transplant year, in order to identify a negative assay and conclude treatment.

Renal function

Serum creatinine (SCr) was determined at 3, 6, and 12 months and yearly thereafter until the last

visit according to the follow-up period in each patient. The MDRD formula was used to calculate glomerular filtration rate (eGFR).

Statistical analysis

Results are presented as mean \pm standard deviation for parametric variables and median with range for non-parametric variables. Nominal variables are shown as frequency and proportion. Statistical significance for proportions between CMV-positive and -negative patients was determined by chi-square test or Fisher's exact test when applicable. Student's t-test and Mann-Whitney U test were used to evaluate differences for parametric and non-parametric variables, respectively. Statistical significance was considered at $p < 0.05$.

RESULTS

Two hundred seventy-two patients received 274 kidney transplants between August 1997 and March 2005. Pre-transplant CMV serology was available for all recipients and donors as part of routine evaluation. Twenty-four patients (all first KTR) constituted the high-risk group for post-transplant CMV (D+R-), which equals 8.8% of KTR during this period. Seronegative status was confirmed in all 24 KTR the day before the transplant was performed. All 24 recipients –10 women and 14 men, age 30.1 ± 10.3 (19-63) years old– were first-time KTR, with 19 (79%) live donors and five (21%) deceased donor recipients. The immunosuppressive scheme used was diverse, yet usually included three drugs and was distributed as follows: cyclosporine-azathioprine-prednisone (15 patients), tacrolimus-azathioprine-prednisone (five patients), cyclosporine-mofetil mycophenolate-prednisone (one patient), tacrolimus-mofetil mycophenolate-prednisone (two patients), and azathioprine-prednisone (one patient). Eleven patients received induction with anti-IL-2r monoclonal antibody (humanized: daclizumab = 9 and chimeric: basiliximab = 2). In no case was anti-lymphocytic treatment used for induction.

No significant differences in age, gender, graft origin, histocompatibility, delayed graft function, or acute rejection episodes, were found between patients who developed antigenemia throughout the first post-transplant year and those that were negative.

Patients who developed pp65 antigenemia

Thirteen of the 24 (54.2%) high-risk KTR presented a positive pp65 antigenemia at some point throughout the first year of follow-up. It is worth mentioning that 70.8% of the 24 patients complied with the programmed laboratory visits for assay processing during the first year, and therefore, the average follow-up was 10.1 ± 3.2 months with a median of 12 months. The time between kidney transplant and the first positive antigenemia was 66.7 ± 58.3 days (range 29-251 days). Figure 1 shows the pp65 antigenemia kinetics for each of the 13 patients and depicts the number of positive cells per assay. It is interesting to note that 9/13 (69.2%) patients had one or more recurrences after completion of initial preemptive treatment and having a negative assay. The average number of positive assay recurrences in the 13 patients was 2.54 ± 1.8 with a median of 2.

Four of the 24 (16.7%) patients developed symptoms associated with CMV. A viral syndrome with leucopenia developed in two cases and CMV disease in another two cases (one with biochemical evidence of CMV hepatitis and the other with pneumonitis with a fatal outcome). Symptom presentation coincided with the first positive pp65 antigenemia in three of these four cases, depicted in figure 1. The median time to symptoms in these patients was 44.5 (range 33-48) days from kidney transplant. The median maximum number of positive cells per

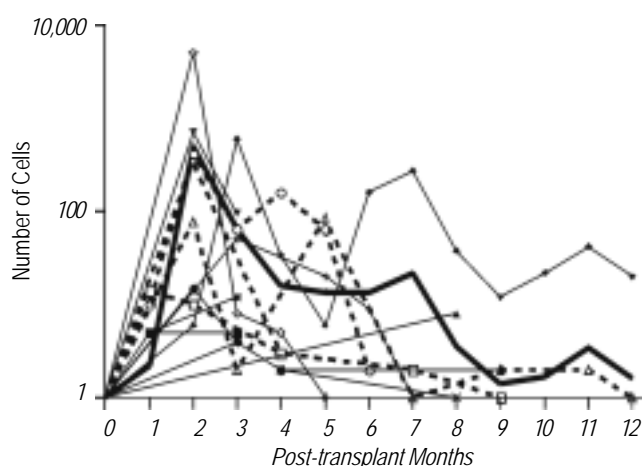


Figure 1. Evolution of the CMV-pp65 antigenemia assay for positive patients through the first post-transplant year. The number of cells detected is presented in a log scale. The dotted line represents those patients who developed CMV-syndrome or disease. The wider line is the mean of number of cells for all positive patients.

pp65 antigenemia assay was not statistically different between KTR who developed symptoms and those who remained asymptomatic throughout the first post-transplant year, being 203 (range 14-527) and 15 (range 4-5,000) cells, respectively ($p = 0.71$).

Patients whose assay remained negative did not develop symptoms or laboratory results suggestive of CMV syndrome or disease during the first post-transplant year. However, one patient whose viral assay was negative throughout the first 12 months presented viral syndrome and pancytopenia 24 months after transplant concurrent with a positive pp65 CMV antigenemia assay that was treated successfully with two weeks of IV ganciclovir.

In order to identify the effect that an immunosuppressive scheme and induction therapy with anti-IL-2r had on the result of the pp65 antigenemia assay, we analyzed the number of positive assays in reference to the immunosuppressive scheme prescribed. Table 1 shows the number of patients per immunosuppressive scheme according to the pp65 antigenemia assay result. Most patients²⁰ received combination schemes with tacrolimus-azathioprine-prednisone and cyclosporine-azathioprine-prednisone. Comparison between these two groups showed that the scheme with tacrolimus had a greater number of patients with positive antigenemia during the first post-transplant year ($p = 0.04$). Of note, when all patients who received tacrolimus as part of the scheme were grouped and compared to those who received cyclosporine, only a trend was observed ($p = 0.09$). On the other hand, independent analysis of anti-IL-2r monoclonal antibodies on antigenemia development was not statistically significant ($p = 0.20$).

Given that various patients had more than one antigenemia event during the first post-transplant year, the effect of immunosuppression on the num-

ber of events was evaluated. Even though tacrolimus was associated with a greater number of events, when compared to cyclosporine no difference was found ($p = 0.10$).

Eight acute rejection events occurred in eight patients during the first post-transplant year; four of them biopsy-confirmed and four based on increases in SCr without other explanation (such as urinary obstruction, urinary tract infection, etc.). All events were managed successfully with methylprednisolone pulse therapy (10 mg/Kg body weight). Five of the eight acute rejection events occurred in the positive antigenemia group. Noteworthy is the fact that in four of these events, rejection had preceded by 5, 16, 17, and 33 days the first positive antigenemia.

It is worth mentioning that at the end of the first year of follow-up, all serologic assays of patients who developed pp65 antigenemia showed seroconversion. In contrast, for those 11 patients who remained pp65 antigenemia negative at follow-up, CMV serology was available in 6 of them at the end of the first post-transplant year and persisted negative.

Preemptive therapy

Nine of the 13 patients who presented a positive pp65 antigenemia during follow-up and never developed symptoms received a total of 13 preemptive treatments. As interruption of preemptive therapy was based on a negative pp65 antigenemia, two patients from this group received IV ganciclovir in a continuous fashion during 405 and 147 days, respectively. The remaining 11 treatments –six with IV ganciclovir and five with valganciclovir– had a duration of 14 days each and between 14 and 150 days (mean 63 days) of ganciclovir and valganciclovir, respectively. Seven treatments were given to four patients who presented CMV syndrome or disease, all with IV ganciclovir. Six of these treatments were administered during 15 to 56 (mean 28) days. None had symptom recurrence.

One patient developed pneumonia after three months of treatment with IV ganciclovir for a repeated positive antigenemia; however, for reasons not clear, the dose of ganciclovir was not increased by the treating physician as it should have, until a full viral syndrome developed. In spite of transient clinical improvement with the larger dose of ganciclovir, CMV pneumonia developed in the following days and was treated with ganciclovir, hyperimmune globulin, and foscarnet. No other microorganis-

Table 1. Immunosuppressive therapy according to the status of antigenemia throughout the first year.

Immunosuppression	CMV pp65 Ag + pts N	CMV pp65 Ag – pts N
Tacro-Aza-Pdn*	5	0
CsA-Aza-Pdn*	6	9
Tacro-MMF-Pdn	1	1
CsA-MMF-Pdn	1	0
Aza-Pdn	0	1
Total	13	11

* $p = 0.04$ between both groups. **Tacro:** Tacrolimus. **CsA:** Cyclosporine, **Aza:** Azathioprine. **MMF:** Mycophenolate mofetil. **Pdn:** Prednisone.

Table 2. Comparison between serum creatinine mean values at 3, 6, and 12 post-transplant months for the CMV pp65 Ag + and - group.

Post-Transplant time	CMV pp65 Ag + pts Mean \pm SD	CMV pp65 Ag - pts Mean \pm SD	p
3 months	1.25 \pm 0.27	1.41 \pm 0.26	0.18
6 months	1.28 \pm 0.26	1.37 \pm 0.25	0.39
12 months	1.36 \pm 0.24	1.43 \pm 0.28	0.54
Δ SCr (3 vs. 12 mo)	0.13 \pm 0.28	0.02 \pm 0.39	0.45

SD: Standard deviation.

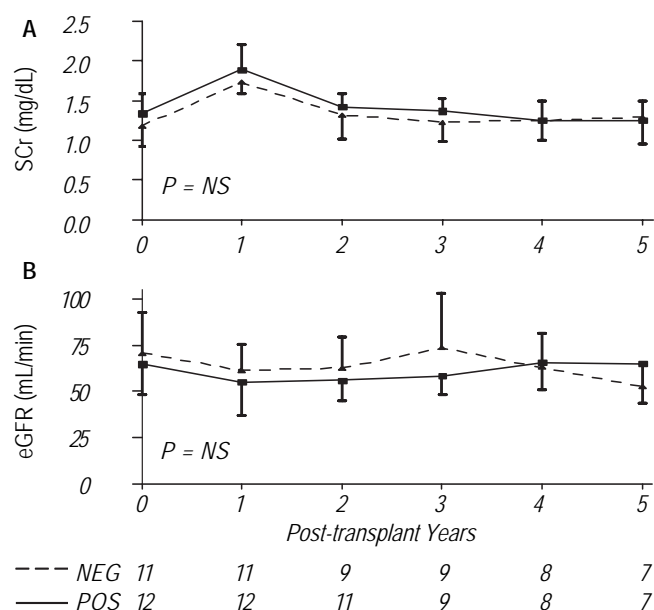


Figure 2. Post-transplant evolution of the SCr (A) and of the eGFR (B) according to the status of antigenemia through the first year. Values are presented as the mean and standard deviation.

ms were found in the lung biopsy and antigenemia became negative. However, a fatal outcome occurred after a prolonged course with mechanical ventilation and development of a “rigid lung”.

Renal function

Follow-up time was 54.2 (range 6.1-106.2) months for patients who developed CMV infection or disease and 100.8 (range 18.2-110.6) months for those who remained negative throughout the first 12 post-transplant months, $p = 0.04$. Up to this date, no additional mortality or graft loss has occurred. Even though there is a statistical difference in follow-up time between groups, comparisons of SCr and eGFR were performed at the specified intervals as described in *Material and methods*. Table 2 shows SCr values at 3, 6, and 12 months post-transplant in patients who

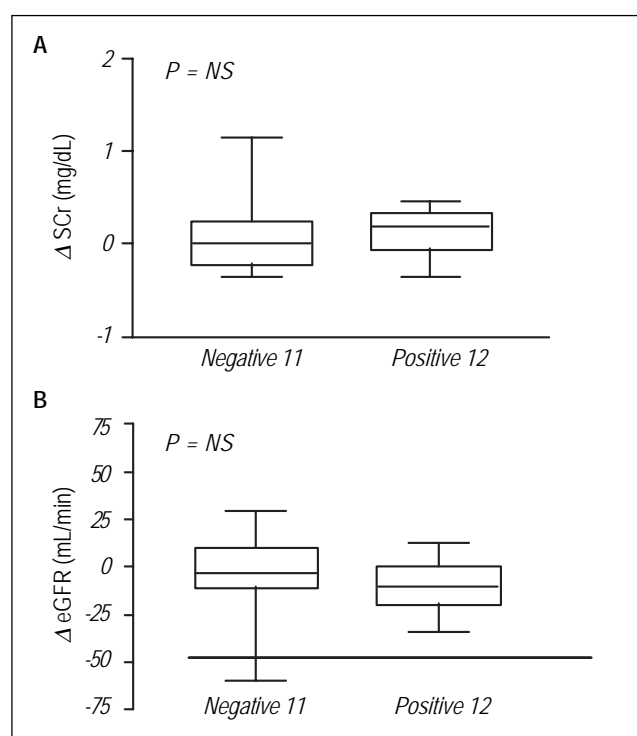


Figure 3. Differences in SCr (A) and eGFR (B) between the baseline values and the last follow-up values according to the status of antigenemia through the first year.

presented antigenemia versus those who did not. The Δ SCr (3 months versus 12 months) was not significantly different between groups (Table 2). Figure 2 shows the evolution of SCr (A) and eGFR (B) at 60 months post-transplant. Both variables show no difference between CMV pp65 antigenemia-positive and -negative groups. Additionally, SCr and Δ SCr were not different between patients who developed acute rejection and those who did not.

Independent of follow-up time we analyzed delta SCr and delta eGFR (Figure 3) considering initial levels (three months post-transplant) and those at last visit and found no difference between the CMV pp65 antigenemia-positive group and the group

who continued as negative throughout the first year of follow-up.

DISCUSSION

In the present study, the frequency of patients at high risk for CMV is low (8.8%) and similar to what was found previously in our setting, (data not published). Our center performs an average of 35 transplants per year, further clarifying the low number of patients included.

The Institute where this prospective study was performed is a tertiary care center that provides national medical attention. The decision to administer preemptive therapy in KTR at high risk for CMV as determined by pp65 antigenemia was made in 1997 based on the availability of expertise and infrastructure to perform this assay, and to lack of financial support to administer ganciclovir (oral or IV) prophylaxis to all patients. The Institute covered the cost of the assay. At the time the study was initiated, qualitative/quantitative PCR to determine CMV infection was not available at our center.

The incidence of CMV replication in 54.2% of the study population is similar to that of other reports and identical to a recent study comparing prophylaxis vs. preemptive therapy with valganciclovir. The latter study found a viral replication incidence (evaluated with CMV DNA) of 54% in a high-risk subgroup randomized to preemptive therapy and interestingly found the frequency of symptomatic CMV to be quite low.¹⁰ The prevalence of symptomatic CMV in our study was 16.4%; all cases occurred in patients with a positive viral assay; all cases coincided with the first positive result in CMV pp65 antigenemia; and the four cases occurred in the first 50 post-transplant days. As 21 of the 24 patients had a follow-up period in which the pp65 antigenemia assay was repeated every two weeks during the first four months and given that the patients that developed CMV symptoms also belong to this follow-up period, it can be assumed that a weekly evaluation during the first four months could identify active infections susceptible to preemptive treatment at an earlier stage. As previously reported, a positive assay precedes clinical symptoms by approximately seven days.¹⁶

It is noteworthy that recurrence of a positive CMV pp65 antigenemia assay occurred in a high percentage (69%) of patients receiving preemptive therapy, however in the majority of cases the antigenemia became negative with the administration of a full dose treatment. In only one case viremia

was not controlled in spite of continued ganciclovir administration, and this case developed CMV pneumonia as described. It is likely that resistance to ganciclovir developed during continued inadvertent administration of low dose ganciclovir instead of a full dose as indicated in the protocol. It is clear that when using the preemptive strategy, development of ganciclovir resistance is a risk in particular if subtherapeutic doses are used in spite of evidence of persistent viral replication. Additionally, this case underscores the need for closer supervision in the follow-up of these patients. Alternatively, this fatal event supports the concept of administering prophylactic valganciclovir for three months in R-/D+ patients and to continue frequent viral monitoring after completion of prophylaxis in order to allow opportune treatment of those at risk for disease development.¹⁰

Since preemptive therapy commenced upon the first positive antigenemia and given the fact that the median number of positive cells per assay was 15 (in the majority of patients), we could hypothesize that initial viral exposure was limited, thus possibly interfering with a "protective" immune response. Regardless of a protective endogenous immune response against CMV, optimal control mechanisms of viral replication can be chronically altered in KTR who receive maintenance immunosuppressive therapy. Consequently, these patients can present occasionally prolonged CMV antigenemia, yet disease instigation in these circumstances is very rare, suggesting that active infection is at least partially controlled.²¹ Interestingly, following the same line of thought, all patients developing antigenemia during the first year of follow-up presented seroconversion at the end of this period. Therefore, assay-confirmed viral replication (in an isolated event or after recurrence) allowed an immune response that conferred endogenous protection. This effect, proposed by several authors, is one of the most beneficial aspects of preemptive therapy: it allows early abortion of infection, limiting morbidity and guaranteeing viral exposure for the immune response.¹⁴

CMV infection has been suggested as a predisposing factor for acute and chronic graft rejection. Potential mechanisms include: increased expression of HLA class I and class II molecules and nuclear factor κ B with subsequent stimulation of genes that play an important role in the inflammatory response;²² increased expression of adhesion molecules on allograft endothelium and on their leukocyte ligands as well as increased liberation of

proinflammatory cytokines;²³ endothelial proliferation secondary to direct cytopathic effect or anti-endothelial cell antibodies²⁴ whose persistence can contribute to chronic graft dysfunction through vascular damage; and cross-reactivity between homologous sequences of CMV antigens and HLA class I heavy weight chains with subsequent increase in cytotoxic T cells directed against the graft.²⁵ However, a cause-effect relationship is difficult to establish in a clinical scenario as acute rejection frequently precedes CMV infection. The chronological relationship between the rejection event and the first positive antigenemia in four out of five patients suggests that TNF and other proinflammatory cytokines acted as promoters of graft CMV reactivation.²⁶ This chronological relationship also suggests that the high steroid dose used to treat the rejection episodes might have influenced donor-derived CMV reactivation/replication.

The indication for effective antiviral treatment concurrent with steroids in these high-risk patients is clear, even when the viral assay continues to be negative. The indication for antiviral treatment is indisputable when anti-lymphocytic therapy is employed for acute rejection events.²⁷

Currently, one of the issues pending elucidation is CMV's participation in chronic kidney graft damage through its direct or indirect effects. One of the main objectives of this study was to evaluate long-term renal function in patients developing pp65 antigenemia during the first year of post-transplant follow-up and to compare them with those whose assay continued to be negative. As shown in the graphs, renal function parameters were not different between groups. As renal biopsies were not taken at similar post-transplant intervals, evaluation of histological changes relevant to chronic rejection or chronic allograft nephropathy was not possible. However, our results suggest that preemptive therapy or treatment of symptomatic cases limited in a timely fashion the inflammatory process that would have favored uncontrolled viral replication and long-lasting viral exposure in asymptomatic infection. These results are concordant with data obtained from a retrospective analysis of KTR (not limited to high-risk groups) who received preemptive therapy on evidence of viremia. It did not show a difference in five-year patient and graft survival between patients who presented viremia during the first post-transplant trimester (and thus received preemptive therapy) and those who continued with a negative assay.²⁸ Another recent prospective study that did not administer pro-

phylactic nor preemptive treatment and rather only treated when symptoms appeared, pointed out that neither asymptomatic infection nor CMV disease showed decreased death-censored graft survival (death with a functional graft not considered graft loss). However, both asymptomatic infection and CMV disease were proven to be independent risk factors for mortality beyond 100 days post-transplant with a risk factor of 2.9; and resulted in a significant decrease in graft survival uncensored for death (death with a functional graft considered graft loss).²⁹ Few published studies similar to this one have investigated the impact of CMV as an independent risk factor integrating death-censored graft loss into the analysis. In this respect, another group reported that CMV disease was a significant risk factor for death-censored graft loss only in the presence of acute rejection. For example, recipients with CMV disease without acute rejection did not show an increased incidence of chronic rejection when compared to those without CMV disease and without acute rejection.³⁰ In summary, data proceeding from these studies emphasize the indication of prophylaxis or preemptive therapy to decrease the mortality index and pose questions about the isolated participation of CMV in chronic graft damage.

In the present study, a greater frequency of positive viral assays in patients whose scheme included tacrolimus probably translates into greater immunosuppression. Even though the mechanisms of action of cyclosporine and tacrolimus are similar, *in vitro* and *in vivo* studies have shown that tacrolimus is 10 to 100 times more effective than cyclosporine in immunosuppressive action.³¹ Regardless of tacrolimus' immunosuppressive potency clinically evidenced by a significant reduction in acute rejection events in KTR, comparative studies have not shown a difference between both drugs in incidence of CMV viremia or tissue-invasive disease.³²

Even with the current strategies for prevention of CMV disease, there is still a risk for delayed CMV disease for some patients who remain CMV seronegative—4% of the total transplants performed in our center during the study period—as illustrated by one of the patients in our cohort who developed a viral syndrome at 24 months of transplantation. It remains to be defined what is the best approach for this group of patients.

In conclusion, our results suggest that treating CMV replication in high-risk KTRs using a preemptive strategy during the first post transplant year

is associated with a low rate of CMV complications and will probably interfere with the indirect, long-term negative effects that CMV allegedly has on kidney function and survival.

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