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Study on the viral load ant antigenemaia as predictive values of CMV infection relapse in renal transplantation

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SUMMARY

Cytomegalovirus (CMV) is a pathogen, commonly found in the donors and recipients of solid organ transplantation. CMV is one of the major causes of morbidity and mortality in these patients. Relapsing episodes of CMV infection occur in 23-33% of transplant patients which is likely a reflection of incomplete suppression of viral replication following antiviral treatment with intravenous ganciclovir. We have studied CMV DNA load and antigenemia as markers for relapse of CMV infection in 49 renal transplant patients out of 68 with CMV infection who received a course of intravenous ganciclovir among 300 transplants carried out between january of 2001 and june of 2005. Viral load and antigenemia were measured in blood samples obtained before, during and at the completion of treatment. We also studied different viral load as predictors of relapse CMV infection. Twelve (24.5%) of 49 recipients developed relapsing CMV infection. The relapsing group had higher viral loads after treatment than the no relapsing group. There was no difference in antigenemia level between both groups. The viral loads before and during the treatment, the age and sex of donors and recipients, inmunosupresión, percentage of seronegative recipients with seropositive donors, duration of the therapy and the percentage of patients with heavy inmunosupression were similar in the two groups, but the incidence of acute rejection was higher in the relapsing group. We also evaluated the range of viral load after treatment which is able to trigger the relapse of CMV infection. We conclude that CMV DNA load after treatment is a useful marker for individualizing antiviral treatment of CMV infection in renal transplant recipients. Acute rejection is a risk factor to the relapsing CMV infection.

Key words: ***Renal transplantation. CMV infection. Viral load. Relapse.***

ESTUDIO DE CARGA VIRAL Y ANGIGENEMIA COMO VALORES PREDICTIVOS DE RECIDIVA DE INFECCIÓN CMV EN EL TRASPLANTE RENAL

RESUMEN

El citomegalovirus (CMV) es un patógeno que se encuentra frecuentemente tanto en donantes como en receptores de trasplantes de órganos sólidos. La in-

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fección por CMV es una de las mayores causas de morbilidad y mortalidad en estos enfermos. Entre el 23% y 33% de los pacientes trasplantados presentan episodios de recidiva de infección por CMV, debido probablemente a una supresión incompleta de la replicación viral tras el tratamiento con ganciclovir intravenoso. Hemos evaluado la carga viral y la antigenemia como marcadores de recidiva de infección por CMV en 49 de los 68 receptores de trasplante renal que presentaron una infección por CMV y recibieron un curso de tratamiento con ganciclovir intravenoso de entre los 300 trasplantes realizados en el periodo comprendido entre enero de 2001 y junio de 2005. Se analizó la carga viral y la antigenemia en estos pacientes antes del tratamiento durante y al final del mismo. Además hemos estudiado el valor predictivo en la aparición de recidiva de infección de diferentes cargas virales a la finalización del tratamiento. Doce (24,5%) de los 49 pacientes desarrollaron recidiva de la infección CMV, presentando dicho grupo de pacientes una carga viral significativamente más alta después del tratamiento que el grupo de pacientes sin recidiva de la infección. No había diferencias entre el nivel de antigenemia entre ambos grupos en ninguno de los momentos estudiados, ni en la carga viral al inicio ni durante el tratamiento. No encontramos diferencias significativas entre la edad y el sexo del donante y del receptor, tipo de inmunosupresión basal, porcentaje de receptores seronegativos con donantes seropositivos, duración del tratamiento, porcentaje de pacientes que recibieron inmunosupresión de alto riesgo en los grupos estudiados, pero la incidencia de rechazo agudo fue significativamente superior en el grupo con recidiva. Hemos hallado diferentes puntos predictivos para el desarrollo de la recidiva. Concluimos que la carga viral al finalizar el tratamiento es un marcador útil para individualizar el tratamiento antiviral de la infección por CMV en los receptores del trasplante renal. La aparición de rechazo agudo es un factor de riesgo asociado a la recidiva de la infección.

Palabras clave: ***Trasplante renal. Infección CMV. Recidiva.***

INTRODUCTION

Citomegalovirus (CMV) infection is the most frequent viral infection in renal transplant recipients.[1-3] Exposure to the virus increases with patient's age, and CMV is detected in more than two thirds of donors and recipients before performing renal transplantation.[1] CMV infection is associated with development of acute rejection,[4-5] which in turn is an important risk factor for further onset of chronic graft nephropathy.[6] In spite of having available an effective agent for treating the infection, such as intravenous ganciclovir, optimal duration of the therapy is unknown.[7] There is a high relapse rate after treatment withdrawal ranging 23%-33% of the cases according to different published series.[7-10] It is very likely that infection relapse is due to incomplete suppression of viral replication, which could be prevented prolonging treatment duration, although the relapse risk must be weighted against toxicity and cost that this represents. Thus, it is important to find a marker that screens those patients susceptible of presenting a relapse in order to prolong the therapy only in them.

Several markers have been proposed such as quantification of sub-populations of T lymphocytes,[10] although the development of new techniques in viral detection, such as quantification of the viral load[7,11,12] or antigenemia[13] opens up new perspectives in CMV viremia identification in those patients at risk of relapsing.

We have studied the progression of viral load and antigenemia during therapy with intravenous ganciclovir in CMV infection and we have evaluated its efficacy as predictors of infection relapse.

MATERIAL AND METHODS

A prospective study was done on 49 renal transplant recipients out of 68 patients that had CMV in-

fection in our hospital from January 2001 to June of 2005.

All the recipients of the study groups received triple immunosuppressant therapy with calcineurin inhibitors (6 tacrolimus, 43 cyclosporin A), mycofenolate, and methyl-prednisolone. Thymoglobulin administration was considered as high-risk immunosuppression, for both acute rejection prophylaxis and treatment. Mycofenolate was withdrawn in all patients developing the infection.

In all recipients and donors, their serological status for CMV before transplantation. Specific anti-CMV gammaglobulin was administered in sero-negative patients receiving a graft from a sero-positive donor since we considered that these patients had a special risk for developing the infection, being defined as high serological risk.

CMV antigenemia was determined in all recipients from the fourth week post-transplantation and weekly thereafter for the first 3 months and fortnightly from the 3^{d} to the 6th months.

CMV infection was defined as the presence of antigenemia 10 cells/200,000 leucocytes, that could be associated or not to a viral syndrome or invasive disease. In case of infection, ganciclovir therapy, 5 mg/kg/day, was started with dose adjustment according to renal function, and maintained for at least tow weeks with resolution of any symptom or sign of infection.

Total blood antigenemia and plasma viral load were determined in patients with CMV infection before starting the treatment, at weeks 1 and 2, and on ending the therapy after antigenemia became negative.

A relapse was defined as antigenemia recurrence higher than 10 cells/200,000, 14 after having stopped the therapy and during the follow-up period.

The COBAS Amplicor CMV Monitor Test (Roche Diagnostics, Branchburg, NJ) was used for analytical determination of viral load, expressing the results as logarithmic units. The detection limit of the technique is 2.60 logarithmic units and the linear range goes up to 5.0 logarithmic units. The pp65 antigen was used to detect antigenemia by means of the Monofluokit Pasteur reactive, the result being expressed as the number of marked cells per 200,000 leucocytes (Figure 1).

All laboratory determinations were done by microbiologists unaware of the patients' clinical condition.

Viral load and antigenemia at the beginning of therapy, at weeks 1 and 2, and at the end of treatment, gender, age (years) of donors and recipients, baseline immunosuppression, percentage of high serological risk receptors, the incidence of acute rejection, the percentage of patients having received high-risk immunosuppression, and treatment duration (days) in the group of recipients having had a relapse and in those without it were compared.

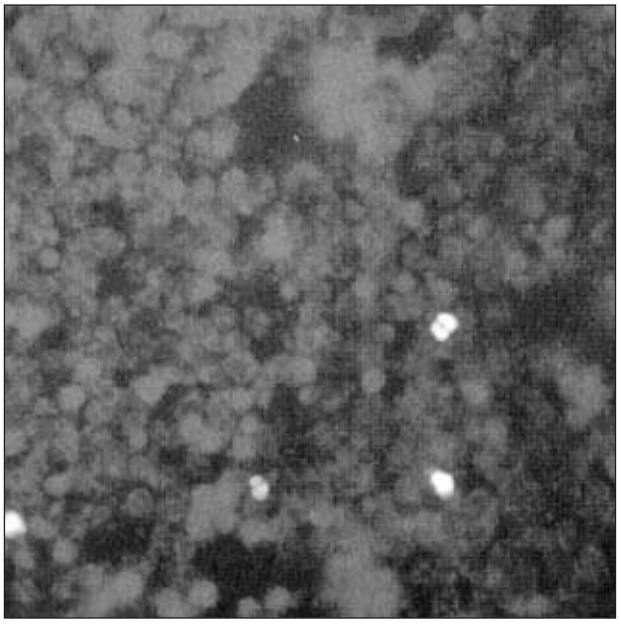

Fig. 1.—

Minimum follow-up time was 12 months after renal transplantation and 3 months after each episode of CMV infection.

Statistical analysis

The studied variables are viral load and antigenemia at baseline, the first and second weeks, and at the end of treatment, as well as gender and age (years) of donors and recipients, the percentage of recipients with high serological risk, treatment duration counted in days, the incidence of acute rejection, and the use of high-risk immunosuppression.

For qualitative variables, absolute and relative frequencies (%) were used. For quantitative variables, mean and standard deviation were used for variable with a parametric distribution, and median with 25th and 75th percentiles for non-parametric variables. The Kolmogorov-Smirnov test was used to differentiate between both types.

A multivariate analysis was done using those variables found to be significant in the bivariate analysis ($p \leq 0.05$).

To predict the behavior of plasma CMV viral load, we performed an analysis using the receiver-operator curves (ROC). With this type of analysis, each point of the studied variable is represented, and the

maximally possible sensitivity and specificity as area under the curve. The area under the curve as well as the sensitivity and specificity were calculated for the plasma viral load to detect CMV nucleic acids. With these data it is possible to obtain the positive and negative predictive values for the test at a certain cut-off point of viral load.

The statistical significance level used in hypothesis testing has been $p \leq 0.05$. The result analysis was done with the statistical software package SPSS PC version 10.0.

RESULTS

Between January of 2001 and June of 2005, 300 renal transplants were done at the General University Hospital of Alicante. Of them, 68 patients, corresponding to 22.7%, were diagnosed with CMV infection.

In our study, 49 of these 68 patients with CMV infection were selected. The reason for exclusion of the remaining 19 patients was the inability to obtain some of the samples to determine viral load or early withdrawal from the study due to loss to follow-up or death.

Twelve (24.5%) out of the 49 renal transplant recipients developed one episode of infection relapse versus 37 (75.5%) that did not.

Relapse episodes were detected between days 14 and 50 after therapy discontinuation.

We did not observe statistically significant differences between the relapsing group and the non-relapsing group with regards to recipient's and donor's age and gender, baseline immunosuppression, percentage of recipients with high serological risk, days of antiviral therapy during the first episode, type of immunosuppressant agent used, or the percentage of patients with high-risk immunosuppression (Table 1).

There is, however, a significant association between the development of acute rejection and the group of relapsing patients (Table 1).

There were no significant differences with regards to antigenemia between both groups throughout all the study periods (Table 2), or with regards to CMV viral load at baselines or at the first treatment week (Table 3). CMV viral load determined at the end of antiviral therapy was significantly higher ($p < 0.05$) in the relapsing group of patients (Table 3).

Since the variables «acute rejection» and «viral lad» at the end of therapy were significantly associated with relapsing episodes, they were selected to be included into the multivariate analysis. Both variables sustained statistical significance ($p \leq 0.05$), independently relating with relapse development.

Figure 2 shows the ROC curve for plasma CMV viral load in relation to development of a second episode of CMV infection. The area under the curve is statistically significant ($p < 0.05$).

Several cut-off points of plasma viral load may be drawn from the ROC curve with different sensitivity and specificity values. This type of test has to be as sensitive as possible to detect the highest number of patients that may relapse although this may imply, within certain limits, treating a moderate number of patients that may not need it. With this in mind, the highest sensitivity that may be obtained is 75% an, within this range, the highest specificity that can be obtained is 76.5%, representing a cut-off point of 3.81 logarithmic units, corresponding to 6457 copies/mL. Looking at other cut-off points with higher specificity and still having good sensitivity we found the value of 3.95 logarithmic units (8913 copies/mL) yielding a 66.7% sensitivity and 91.2% specificity (Figure 2).

Table 4 summarizes the cut-off points obtained at 3.81 and 3.95 logarithmic units, as well as other va-

Table I. Patients' demographic and clinical variables

	Relapse YES N = 12 (24.5%)	Relapse NO N = 37 (75.5%)	p
Recipient's age (mean)	51.1 ± 13.7	51.0 ± 12.0	NS
Donor's age (mean)	50.8 ± 15.8	41.4 ± 15.9	NS
Recipient's gender (males)	5 (41.7%)	24 (64.9%)	NS
Donor's gender (males)	8 (66.7%)	27 (73.0%)	NS
Serological risk	4 (33.3%)	6 (16.2%)	NS
Antiviral therapy (days)	17.5 ± 4.9	17.3 ± 4.2	NS
Inmunosuppressant			
• Tacrolimus	3 (25%)	3 (8.1%)	
• Cyclosporin A	9 (75%)	34 (91.9%)	
High-risk immunosuppression	3 (25.0%)	4 (10.8%)	NS
Acute rejection	6 (50.0%)	4 (10.8%)	0.008*

NS: Not significant.

Table II. CMV Antigenemia at baseline and during therapy

	Relapse YES Median (P25%-P75%)	Relapse NO Median (P25%-P75%)	p
Baseline	19.5 (15.3-40.0)	30,0 (11.3-72.3)	NS
First week	8.0 (0.5-544.5)	2,0 (0.0-20.0)	NS
Second week	0.0 (0.0-9.0)	0,0 (0.0-0.0)	NS
End of therapy	0.0 (0-1.5)	0,0 (0.0-0.0)	NS

NS: No significant.

Table III. CMV plasma viral load at baseline and during therapy

	Relapse YES Mean (± SD)	Relapse NO Media (± SD)	p
Baseline	4.44 (± 0.74)	4.39 (± 0.65)	NS
First week	3.96 (± 0.53)	4.17 (± 0.81)	NS
Second week	3.89 (± 0.78)	3.64 (± 0.79)	NS
End of therapy	4.01 (± 0.93)	3.29 (± 0.64)	0.005*

NS: Not significant.

lues of viral load and antigenemia related or not with detectability, at the end of therapy, and that would be associated to development of relapses. Above a viral load value of 3.81 logarithmic units at the end of treatment, there is a likelihood higher than 5 of having a relapse.

Finally, Table 5 shows the multivariate analysis of the variables associated with the onset of a second episode of CMV infection ($p \leq 0.05$). In this Table we have replaced the quantitative variable "final viral load" by the dichotomous variable "final viral load ≥ 3.81 logarithmic units (LUs) versus < 3.1 LUs.

DISCUSSION

The relapse percentage described in different series varies according to the consideration of infection or disease, as well as the solid organ transplanted in the population studied. The relapse incidence in our study (24.48%) is similar to that reported in other series in the literature,[7-10] although slightly lower to that expected, although we should point out that all of our patients are renal transplant recipients, which brings to the series greater homogeneity than others.[7,8]

We would like to stress that, when considering a cut-off point in the number of copies/mL to consider whether or not continuing with ganciclovir therapy, other parameters should considered such as the patient's clinical condition and the assessment of possible benefits and risks related with this therapy. This is due to the high variability in the sensitivity and specificity of the test depending on the result of the viral load.

In the study performed by Sia *et al.*[7], 24 patients with solid organ transplantation and CMV infection received a course of intravenous ganciclovir therapy for 14 days having a 33% relapse rate (8 out of 24 patients) versus 24.48% in our series. These authors evidenced a statistically significant higher viral load before treatment and at the end of therapy in the re-

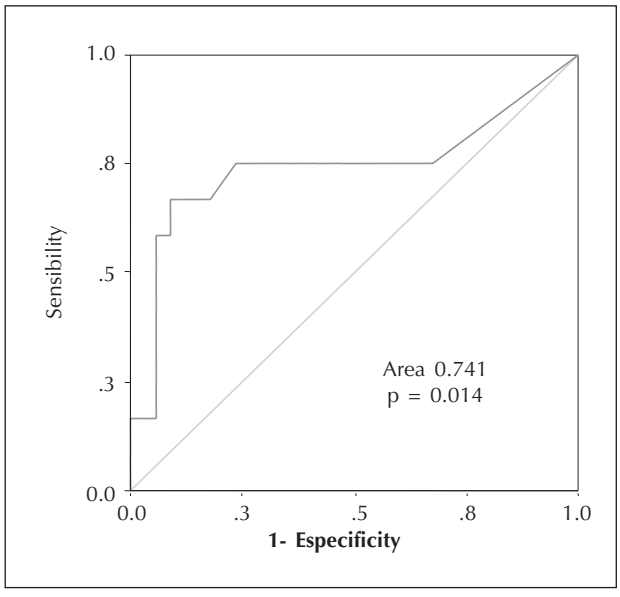

Fig. 2.—ROC curve.

Table IV. Plasma viral load and Antigenemia at the end of therapy related with relapse occurrence

	Relapse Number/Total (%)	Relapse Prevalence ratio (IC 95%)	p
PVL			
• ≥ 3.81	9/17 (52.9)	5.1 (1.6 – 16.3)	0.004*
• < 3.81	3/29 (10.3)	1	
PVL			
• ≥ 3.95	8/11 (72.7)	6.4 (2.4 – 17.1)	0.000*
• < 3.95	4/35 (11.4)	1	
PVL			
• ≥ 2.60	9/32 (28.1)	1.3 (0.4 – 4.1)	NS
• < 2.60	3/14 (21.4)	1	
Antigenemia			
• ≥ 1	3/6 (50.0)	2.4 (0.9 – 6.4)	NS
• < 1	9/43 (20.9)	1	

NS: Not significant.

Table V. Multivariate analysis of variables associated with relapse occurrence. Expression of the recommended cut-off point for viral load

	Prevalence ratio	(IC 95%)	p
PVL ≥ 3,81	21.3	2.4 – 193.2	0.007*
Acute rejection	19.1	1.9 – 196.1	0.013*

lapsing group as compared with the non-relapsing group. In our series, viral load at the beginning of therapy also was higher in the relapsing group, although not reaching statistical significance, but viral load at the end of treatment did reach significance.

Humar *et al*.[8] have observed that infection relapse is associated with low decrease in viral load after the onset of ganciclovir therapy. Thus relapsing patients would take longer to clear their viral load and the initial response would be slower than in the non-relapsing group. Surprisingly, these authors observed that the non-relapsing group f patients had a viral load before treatment higher than the patients relapsing. These findings do not agree with ours, since viral load in our relapsing patients was higher, although not statistically significant, than that of non-relapsing patients. By contrast, they conclude that the presence of the virus at the end of treatment conditions the infection relapse, a conclusion that may superimpose to ours since we found an association between certain residual viral load and the presence of relapses in our patients. All this might indicate therapy extension while detecting certain level of viral load.

The data from our study show that the fact of antigenemia becoming negative with treatment administration occurs before viral load occurs. This means that, in some cases, we are leaving without treatment patients that are still infected, and thus very likely to have a relapse. With the data obtained and by using the ROC curve for selecting a cut-off point it may be suggested maintaining intravenous ganciclovir therapy until achieving a viral load of 3.95 Log_{10}/mL (≈ 7943 copies/ml) would decrease the relapse rate to approximately 12% at the cost of treating a high number of patients with already resolved CMV infection. By increasing the viral load threshold the number of cases with excessive treatment would be reduced but at the cost of increasing the relapse rate. Thus, this is a dynamic decision making that would have to be adapted to the particular setting of the Transplantation Unit and the patient. These data are corroborated by Roberts who detects a threshold of symptomatic relapse with viral loads higher than 1,000/100,000 and proposes, as in our case, an individualized decision based on serial viral load determinations at the end of therapy.[12]

The development of new drugs, such as oral ganciclovir and valganciclovir, forces reconsidering the prophylaxis. Both have shown their efficacy and safety for prevention of CMV infection in solid organ recipients,[17] although the poor bioavailability of oral ganciclovir of about 6%[18] versus valganciclovir would favor the use of the latter since its bioavailability is 10 times higher and it may be administered once a day, a regimen that is associated with higher treatment adherence according to a large number of studies.[19-20]

The financial burden and the medical consequences of maintaining a prophylactic strategy after renal transplant versus a surveillance and treatment strategy are known.[21] A study on the economics making cleat with is the health care cost of treating for more days than really needed, by prolonging the treatment, versus the cost that would suppose treating the relapses and their medical impact with shorter treatments would be of great importance.

In our experience, the only clinical risk factor associated with CMV infection relapse was the incidence of acute rejection, as a reflection of the patient's exposure to more profound immunosuppression. These findings are corroborated by Humar[22] who observed that both corticosteroid-sensible and corticosteroid-resistant acute rejection was the main risk factor for relapse development, although other authors do not support this experience.[8,18,12] In our case, the use of thymoglobulin was not a risk factor associated with infection occurrence.

We may conclude that viral load at the end of the therapy is a good viral marker to establish duration of ganciclovir therapy during CMV infection in order to prevent relapses, whereas antigenemia has null predictive value. On the other hand, he occurrence of acute rejection is a risk factor associated with CMV infection relapse.

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