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Cytomegalovirus (CMV) excretion as a factor in the severity of CMV disease in kidney and simultaneous kidney and pancreas transplantation

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Abstract. The aim of the study was to evaluate the virological parameters associated with the severity of cytomegalovirus (CMV) disease in renal and simultaneous renal and pancreatic transplantation. The association of the viral profile and the severity of the viral disease was analysed taking into account different confounding variables susceptible to linkage with the severity of the CMV infection and the viral parameters. All the patients transplanted between 1 January 1989 and 31 December 1990, a total of 242, were prospectively followed by viral cultures in blood and urine and by serological methods using the detection of CMV-specific IgM and the complement fixation (CF) test. The samples were taken systematically each week for the first month and then at day 90, 180 and every 6 months and also in cases of clinical manifestations related to viral disease. CMV infection was diagnosed virologically by the presence of viraemia, viruria, IgM, or a significant rise in CMV antibody titre in CF. CMV disease was classified as asymptomatic, mild (fever and/or leukopenia), moderate (fever, leukopenia and liver abnormalities), severe (CMV pneumopathy and/or gastrointestinal disease) or fatal. The incidence of CMV infection was 65% (157/242): 32% asymptomatic, 36% mild, 30% moderate and 2% severe. The presence of IgM was associated with the severity of CMV disease: 51.4% of moderate and severe CMV infections in the group with IgM versus only 16% in the group without IgM ($P < 0.0001$). The risk of having severe or moderate CMV disease was 3.28 times higher in patients with positive IgM. However the serological changes in CF were not significantly associated with the severity of the viral disease since 34.6% of the patients with CF changes had a severe form versus 20.8% in the group without CF modification. Viruria was significantly associated with moderate or severe infection: 43.6% of the patients with viruria had severe infection versus only 12.5% in the patients without viruria ($P < 0.0002$). The risk of having moderate or severe CMV disease was 3.48 times higher in the patients with viruria. Viraemia was also associated with more severe CMV infection: 48.6% of moderate or severe CMV infection in the group of patients with viraemia versus 19% in the group without viraemia ($P < 0.0001$). The risk of having severe or moderate CMV infection was 2.58 times higher in the patients with viraemia. Viraemia was not more associated with severe CMV infection than viruria. Using the maximum likelihood ratio method and the logistic regression model, CMV-specific IgM, viruria and viraemia were each shown to be associated with the severity of CMV disease and the addition of one parameter to the other(s), whatever the type (except the CF changes) and whatever the order of this addition, did not remove the link between the severity and IgM, viruria and viremia. The incidence of severe and moderate CMV disease increased with the number of positive viral parameters (PVP) from 2% of moderate and severe infections in the group with one PVP, to 28% in the group with two PVP, to 39% in the group with three PVP and 68% in the group with four PVP (trend, 35.95; $P < 0.0001$). Taking the absolute risk of the group of patients without IgM, viruria or viraemia as the basal level, the observed relative risk of severe CMV infection varied from 6.45 in the group with positive IgM without viruria or viraemia, to 10.74 in the group with positive IgM and viruria without viraemia and to 22.5 in the group with the three positive parameters IgM, viruria and viraemia. The different potential confounding factors (recipient and donor serology, renal or renal and pancreatic transplantation, DR compatibility, rejection before CMV infection) did not modify the link between the viral profile and the severity of CMV disease. This study suggests that the severity of CMV disease might be linked to the overspread of the virus as well as to the consequences of a CMV-specific humoral immune response.

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Cytomegalovirus (CMV) infection remains the most frequent viral infection in bone-marrow transplantation and in organ transplantation and is responsible for a high morbidity and mortality. Some patients will develop asymptomatic infection detected by systematic viral monitoring and others will develop clinical signs ranging from a self-limited syndrome to severe CMV disease with pneumonia or gastrointestinal disease. However, it is not known whether the viral profile is different in asymptomatic infection than in severe CMV disease, and whether the different clinical forms depend on the viral charge, on the intensity of the viral replication or on the immune status of the recipient.

In bone-marrow transplantation, viraemia is associated with the severity of CMV disease [4, 8] and would be predictive of CMV disease [5]. This marker could be used to determine the initiation of antiviral chemotherapy and to prevent the progression from mild infection to severe CMV disease. In organ transplantation, the association of the clinical signs of CMV disease with the recovery of CMV from sources other than blood, such as urine, remains to be determined. Moreover the use of serological methods such as detection of CMV-specific IgM antibodies by enzyme-linked immunosorbent assay (ELISA) and the significant rise in CMV antibodies using a complement fixation (CF) test need to be assessed in the context of the association with the severity of CMV disease. The aim of the study was to evaluate the virological parameters associated with the severity of CMV disease in renal transplantation and renal and pancreatic transplantation in order to understand the physiopathology of the viral infection better. The viral profile associated with serious disease was determined taking into account the different confounding factors susceptible to linkage with the severity of CMV disease and with the viral parameters. The factors studied were the presence of CMV-specific IgM, the significant increase in CMV antibodies in CF, viraemia and viruria.

Material and methods

Patients

All the patients attending our Transplantation Unit transplanted between 1 January 1989 and 31 December 1990 were included in the study. A total of 242 patients, 191 renal transplant and 51 renal and pancreatic transplant, were thus enrolled.

Virological diagnostic methods and viral monitoring

The serological methods used were the CF test and ELISA. The CF test was performed in microplates with two units of antigen and two units of complement. The detection of CMV-specific IgM was done with the cytomegalovirus IgM EIA (Wellcome) according to the manufacturer's instructions [6].

Cultures were prepared from blood and urine. Specimens were inoculated to human embryonic fibroblasts (MRC5) and cultures were maintained for at least four weeks to detect the CMV isolation because of the slow cytopathogenic effect. A rapid identification was performed by low speed centrifugation of the samples onto MRC5-grown cells followed by detection after 48 h of CMV immediate early antigen by the immunoperoxidase technique with monoclonal antibody E13 (Clonotec).

The viral monitoring was systematic and prospective. Viraemia, viruria and serology were performed weekly from the day of transplantation for the first month and then at day 90, day 180 and then every 6 months. Viral cultures and serology were also performed when clinical symptoms of viral infection occurred.

Definition of CMV infection

The definition of CMV infection was virological and was based on any number of the following signs: viraemia, viruria, seroconversion, the presence of CMV-specific IgM antibodies and significant four-fold rise in the anti-CMV antibody titres by CF.

Classification of CMV disease

Clinical CMV disease was classified in five grades as follows:

- asymptomatic;
- mild (fever and/or leukopenia);
- moderate (fever, leukopenia and liver abnormalities);
- severe (CMV pneumopathy or gastrointestinal disease);
- fatal.

Parameters studied

All the 157 patients included in the study group had at least one positive viral parameter since being infected. The parameters studied were CMV specific IgM antibodies, a significant rise in the CMV antibodies in CF, viraemia and viruria.

Confounding factors

The confounding factors were the factors susceptible, from the literature and from our study, to linkage with the severity of the CMV infection and the viral parameters. They were: recipient age (≤ 55 or > 55 years), donor age (≤ 55 or > 55 years), recipient CMV serology before transplantation, donor serology at the time of organ procurement, the type of transplantation (kidney alone or simultaneous kidney – pancreas transplantation), the degree of HLA A, B, DR compatibility, the degree of anti-HLA immunization before transplantation, the number of transplantations, the type of immunosuppressive therapy, the occurrence of rejection before CMV infection (from the day of the transplantation to 4 days before the diagnosis of CMV infection).

Statistical analysis

The association of severe and moderate CMV infection with viral parameters was compared by means of a two-by-two table for univariate analysis. The Mantel Haenszel test was used to take into account only one confounding variable. Logistic regression was used to take into account more than one confounding variable and to model the relation between the viral profile and the severity of CMV disease.

Results

The incidence of CMV infection was 63%, 157 patients having a CMV infection proved virologically. Of these infections, 32% were asymptomatic, 36% mild, 30% moderate and 2% severe. The presence of CMV IgM was associated with the severity of CMV disease, since 38 patients of the 74 with IgM (51.4%) developed a moderate

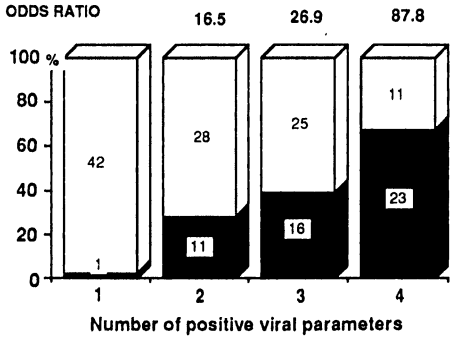

Fig. 1. Severity of CMV infection and the number of positive viral parameters. The incidence of severe and moderate CMV infection increased with the number of positive viral parameters (trend = 35.95; $P < 0.0001$). □, Asymptomatic/mild; ■, moderate/severe

Table 1. Test of significance of the gain when a viral parameter was added. The method used was the maximum likelihood ratio and the model used was logistic regression. The presence of IgM was significantly associated with the severity of CMV disease. The introduction of viruria to IgM in the model did not remove the link between the viral profile and the severity ($chi^2 = 7.8$; $P < 0.006$). The introduction of viraemia did not modify the association between the severity of the CMV disease and viruria and IgM ($chi^2 = 7.9$; $P < 0.006$). However, the addition of the CF changes did not add anything to the model ($chi^2 = 1.54$; $P < 0.22$). Thus, the variables IgM, viruria and viraemia were kept in the logistic regression model to model the risk of having a severe infection according to the viral parameters

Number of positive viral parameters	Parameter	Chi squared	*P* value
1	IgM	23.4	< 0.0001
2	IgM and viruria	7.8	$P < 0.006$
3	IgM, viruria and viremia	7.9	< 0.006
4	IgM, viruria, viremia and CF changes	1.54	< 0.22 NS

or severe CMV infection versus only 13 out of the 83 patients without IgM (16%). The odds ratio was 5.68 (95% confidence interval, 2.78–11.61) ($P < 0.0001$). The risk of having severe or moderate CMV disease was 3.28 times higher in patients with positive IgM than in patients free of CMV-specific IgM antibodies. However, the serological changes in CF were not significantly associated with the severity of the viral disease, since 34.6% of the patients with CF changes (46/133) had a severe or moderate form versus 20.8% (5/24) in the group without CF modification (odds ratio = 2.01; 95% confidence interval, 0.72–5.64) ($P = 0.3$). Viruria was significantly associated with moderate or severe infection since 44 of the 101 patients (43.6%) with viruria had a severe infection versus 7 of the 56 patients (12%) without only viruria. The odds ratio was 5.40 (95% confidence interval, 2.36–12.4) ($P < 0.0002$). The risk of having moderate or severe CMV disease was 3.48 times higher in patients with viruria than in patients without viruria.

Viraemia was also associated with more severe CMV infection: 48.6% of the patients (35/72) with viraemia developed moderate or severe CMV infection versus 19% (16/85) in the group of patients without viraemia ($P < 0.0001$). The odds ratio was 4.08 (2.04–8.16) and the risk of having a severe or moderate CMV infection was 2.58 times higher in patients with viraemia than in patients free of viraemia. Viraemia was not more associated with a severe CMV infection than viruria since the absolute risk of severe CMV disease was 29% in cases of viruria and 26% in cases of viraemia in the patients having only one positive viral culture, either viraemia or viruria (chi squared = 0.01).

The incidence of severe and moderate CMV disease in increased with the number of positive viral parameters from 2% in the group with one positive parameter, to 28% in the group with two positive parameters, to 39% in the group with three positive parameters and 68% in the group with four positive parameters (trend = 35.95; $P < 0.0001$) (Fig. 1). Specific anti-CMV IgM, viruria and viraemia were each associated with the severity of CMV disease and the addition of one parameter to the other(s) whatever the type (except the CF changes) and whatever the order of this addition, did not remove the link between the severity and IgM, viruria and viraemia, using the method of maximum likelihood ratio and the logistic regression model (Table 1).

The observed absolute risk of severe or moderate CMV infection was 3.1% in patients without IgM, viraemia or viruria, 20% in patients with IgM only, without viraemia or viruria, 33.3% in patients with IgM, with viruria and without viraemia, and 68% in patients with the three parameters positive. Taking the absolute risk of the group of patients without IgM, viruria or viraemia as the basal level, the observed relative risk of severe CMV infection according to the number and the type of viral parameters is shown Fig. 2. The analysis of the different potential confounding factors (recipient serology, donor serology, renal or renal and pancreatic transplantation, DR compatibility, rejection before CMV infection) did not show any modification of the link between the viral profile and the severity of CMV disease (Table 2).

Discussion

The presence of CMV-specific IgM antibodies was associated with the severity of CMV disease whatever the donor and recipient serology and whatever the other confound-

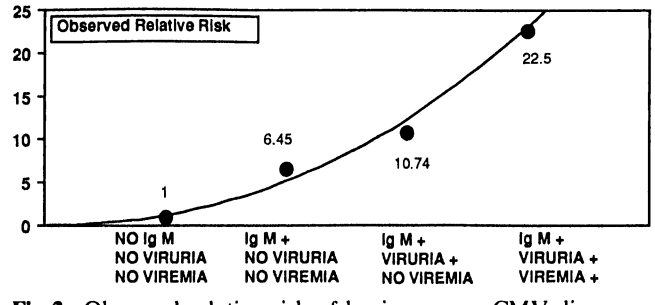

Fig. 2. Observed relative risk of having severe CMV disease and viral parameters in the 157 infected patients. Taking the absolute risk of the group of patients without IgM, viruria or viraemia as the basal level, the observed relative risk of severe CMV infection varied from 6.45 in the group with positive IgM only, without viruria or viraemia, to 10.74 in the group with positive IgM and viruria, without viraemia, and to 22.5 in the group with the three parameters positive

Table 2. Analysis of the confounding factors in the model of the association of the severity of CMV disease with the viral parameters. The method used was the maximum likelihood ratio and the model used was logistic regression. The recipient and donor serology are known from the literature to be linked with the incidence and the severity of CMV infection. In this model, no modification was observed in the link between the severity of CMV disease and the viral parameters (IgM, viruria, viraemia) after taking into account the recipient and donor serology ($P < 0.0001$). CMV infection was more severe in kidney and pancreas transplantation. After taking into account the type of transplantation, the association between the severity of CMV disease and the viral parameters was not modified ($P < 0.0001$). The degree of DR compatibility from 0 to 2 was associated with the severity of CMV disease, a good compatibility protected from serious CMV infection. After taking into account this parameter, the association remained significant ($P < 0.0001$). The occurrence of rejection before CMV infection increased the risk of severe CMV infection. However, this factor was not a confounding factor. Thus, the different potential confounding factors tested did not modify the link between the viral profile and the severity of CMV disease

Confounding variable	Chi^2	*P* value
None	39.06	< 0.0001
Recipient serology	33.63	< 0.0001
Donor serology	32.51	< 0.0001
Type of transplantation (kidney or kdiney – pancreas)	33.26	< 0.0001
DR compatibility	37.01	< 0.0001
Rejection before CMV infection	38.63	< 0.0001

ing factors. The detection of IgM has previously been found to be associated with a poor prognosis in renal transplantation [7], mainly because it was associated with primary infections. IgM could be the witness of severe infection, but could also play an active role in the aggravation of CMV disease by the formation of immune complexes. The presence of IgM immune complexes (IgM – CIC) using a C1q solid-phase assay has been detected in kidney graft recipients during the second month in infected patients only and as early as the urinary excretion [2]. The dissociation of IgM immune complexes and the characterization of the antibody specificity allowed the CMV specificity of the IgM-CIC to be demonstrated, including IgM antibodies and a 45–47 kDa viral polypeptide [3]. This anti-p 45–47 IgM detected by immunoblotting was always present before the anti-CMV IgM was detected with ELISA and was present in primary infections as well as in recurrent infections [1]. This 45–47 kDa polypeptide could be the main viral target for the CMV-specific IgM antibody response in the early phase of CMV infection, since anti-p 45–47 IgM was detected as early as or before urinary viral excretion. However, the physiopathological meaning of the production of CMV-specific IgM, not only during primary infections but also during recurrent CMV infection, remains to be elucidated. Complement fixation changes were not associated with severe CMV disease and the significant increase in the titres could reflect the recovery of a normal immune status, when the immunosuppressive therapy is decreased, or be the consequence of an increased immune reactivity concomitant with a mild CMV infection.

Viruria and viraemia were associated with the severity of CMV infection in renal transplantation and this result would be important to confirm in bone-marrow transplantation with the same methodology, since viruria is easier to determine than viraemia, especially in aplastic patients. IgM, viraemia and viruria were each associated with severe or moderate CMV infection. The addition of one factor to the other(s) not only did not remove the link with the severity of CMV infection, but increased the severity of the disease. The striking increased risk of severe or moderate CMV infections with the number of positive viral parameters suggests that the severity of CMV disease might partly be due to the overspread of the virus as well as to the consequences of a CMV-specific IgM immune response.

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