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# Cytomegalovirus infection and disease in renal transplant patients treated with cyclosporin

## A prospective study

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**Abstract.** In this prospective study, the incidence of cytomegalovirus (CMV) infection and CMV disease was determined in 175 renal transplant recipients on cyclosporin and low-dose prednisone. CMV infection occurred in 51.4% of the patients, CMV disease in 13.7%. The major manifestations of CMV disease were fever of unknown origin and leukopenia. In the group with CMV infection, there was an increased occurrence of rejection (60% in infected vs 27% in noninfected patients). In most patients (41/54), the rejection preceded the CMV infection. CMV infection did not lead to a decreased graft survival. There was no close time relationship between the onset of clinical symptoms of CMV disease and the laboratory confirmation of CMV infection. A subgroup of patients at risk for the development of severe CMV disease could not be identified.

**Key words:** Cytomegalovirus infection in kidney transplantation - Cytomegalovirus disease and rejection - Rejection and cytomegalovirus disease - Risk factors for cytomegalovirus disease.

Cytomegalovirus (CMV) infections frequently occur after renal transplantation, partly because of exposure to CMV and partly as a consequence of the immunosuppressive regimen [7, 16]. The use of more intensive immunosuppressive therapy for treatment of a rejection is often associated with a high infection rate [1, 16].

The clinical spectrum ranges from asymptomatic infection to a life-threatening disease with pneumonitis and death in up to 10% of all patients [11]. Leukopenia, thrombocytopenia, hepatitis, pneumonitis, gastrointestinal ulcerations, encephalitis, and chorioretinitis are among the possible manifestations. Since the introduction of cyclosporin, some authors have found a reduction in CMV infection rate [7] and in CMV disease [15]. This has been attributed to the different mechanism of action of cyclosporin compared with azathioprine or to the decreased use of additional immunosuppression in patients receiving cyclosporin [1]. Most studies, however, are retrospective or use azathioprine as the main immunosuppressive drug.

It has been clearly established that in seronegative recipients the virus is most often transmitted through the donor kidney [3]. CMV infection in seropositive recipients may be due to reactivation of a latent virus or to infection with a new strain of CMV [3, 6].

The aims of the present study were (1) to analyze the incidence of CMV infection and disease in our transplant patients, (2) to determine the best way to establish the diagnosis of CMV infection, and (3) to determine which patients are at risk for severe CMV disease. This latter aspect is important because DHPG (9-(13-dihydroxy-2-propoxy-methyl) guanine) has become available as a possible therapy for CMV infection [4].

### Patients and methods

Between 1 October 1985 and 30 September 1987, 197 patients received a renal transplant at our hospital. Twenty-two patients were not included in the study, either because they died early or because there was insufficient follow-up available on them. Thus, a total of 175 patients participated in the study. The immunosuppressive therapy consisted of cyclosporin and low-dose prednisone in all patients, whereas 11 patients were treated with a monoclonal antibody against the CD3 antigen (WT32) and azathioprine during the first 14 days after transplantation. Cyclosporin was started as an intravenous infusion 6 h after vascularization of the graft in a dose of 3 mg/kg for 3 days and was then administered orally, 12 mg/kg daily. The dose was decreased by 2 mg/kg every 14 days. Doses were adjusted according to cyclosporin trough levels in whole blood or when nephrotoxicity was suspected. Trough levels (normal range 400-800 ng/ml) were measured by radioimmunoassay (Sandoz). All patients also received 100 mg prednisone for 3 days after transplantation (in divided doses, twice daily) and 10 mg daily thereafter. After 3 months, cyclo-

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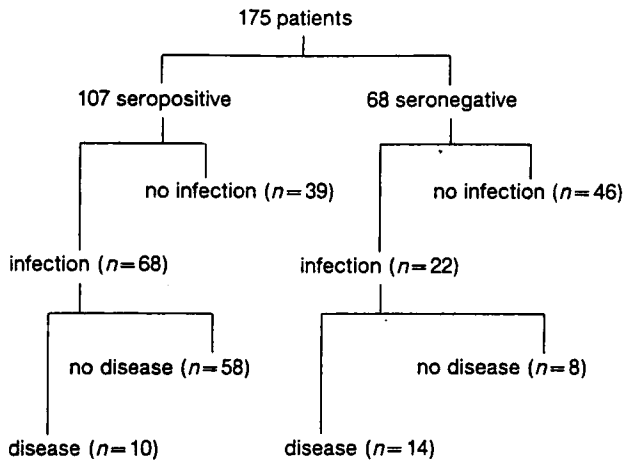


**Fig. 1.** Distribution of patients transplanted between 1 October 1985 and 30 September 1987 according to serological status before transplantation and to subsequent clinical course

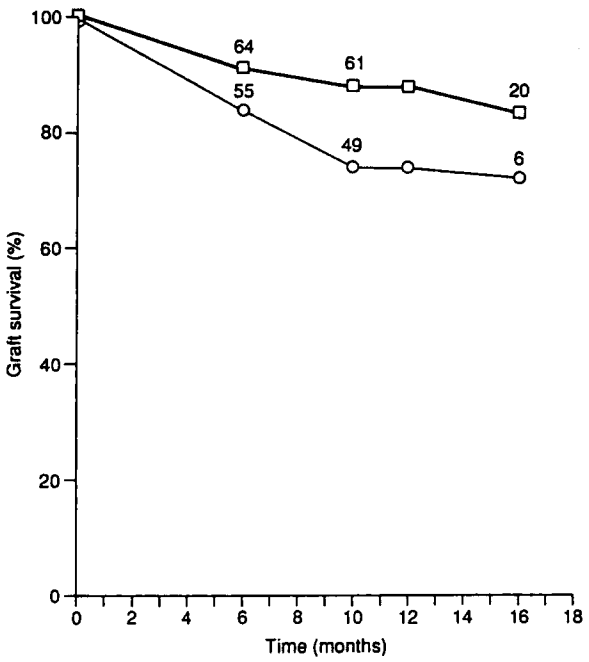


**Fig. 2.** Graft survival after CMV infection. Numbers denote patients at risk. O, CMV infection; □, no CMV infection; $P<0.05$

sporin therapy was replaced by azathioprine in a dose of 3 mg/kg once daily; the prednisone dose was increased to 25 mg daily. The prednisone dose was reduced by 5 mg every 14 days until a maintenance dose of 10 mg daily was reached [8]. First rejections were routinely treated with rabbit antithymocyte globulin (ATG), second rejections with high doses of oral prednisone.

There were 27 retransplantations and 16 living related donor transplantations. Fourteen patients were diabetics.

A serological diagnosis of CMV infection was made when there was a fourfold rise in IgG antibody titer or a seroconversion (IgM, IgG, IgA) of seronegative recipients. Virologically, CMV infection was detected by a positive reaction to CMV-induced early antigen (CMV-EA) after short-term culture or a positive virus culture in urine samples. Clinical diagnosis of CMV disease was made when there was fever of unknown origin (> 38.5° C) for more than 3 days together with serological or virological evidence of CMV infection. Patients were regularly tested for leukopenia ($<3.5\times10^9$/l), thrombocytopenia ($<100\times10^9$/l), and hepatitis. Every patient suspected of having CMV disease was examined for possible pneumonitis by chest X-ray. The IgM and IgA antibody titers were determined in an antibody capture ELISA and the IgG antibody titers in an indirect ELISA, as described elsewhere [12, 13]. CMV-EA was determined by means of indirect immunofluorescence with a monoclonal antibody after 1 and 6 days of urine culture [9].

During the first 3 months after transplantation, weekly determinations of IgM, IgG, IgA, and CMV-EA were performed, as well as conventional virus cultures (CVI). After 3 months, these tests were performed every 2 weeks until 6 months after transplantation, and thereafter only if there was a strong clinical suspicion of CMV disease.

## Results

Included in this study were 175 patients (Fig. 1), 107 (61.1%) of whom were seropositive before transplantation. After transplantation 90 patients (51.4%) developed a CMV infection; most of these patients (75.5%) had previously been seropositive. Twenty-four patients developed CMV disease, 10 (41.7%) of whom were seropositive. Thus, 10 of the 68 infected seropositive patients and 14 of the 22 infected seronegative patients developed CMV disease ($P<0.001$). Sixteen of the 175 patients had received a living related kidney. In seven of these patients, a CMV infection could be diagnosed; three of them also suffered from CMV disease.

During the follow-up, 54 of the 90 patients (60%) with CMV infection were treated for an acute rejection as compared to 23 of the 85 patients (27%) without CMV infection ($P<0.001$). Both patient groups were fully comparable with regard to age, sex, percentage of retransplantations, percentage of living related donor transplantations, average DR, and AB match. Most of the patients (41/54) were treated for rejection before there was any clinical or laboratory evidence of CMV infection (mean interval between onset of rejection and the diagnosis of CMV infection $31\pm25$ days). Thirteen patients were treated for an acute rejection after the diagnosis of the CMV infection (mean interval between CMV infection and onset of rejection $44\pm27$ days). Of the patients with CMV disease ($n=24$), 16 were treated for rejection. Thirteen of these 16 rejections occurred before the onset of CMV disease. Although there were more rejections among patients with CMV infection, this did not lead to a decreased graft survival (Fig. 2). There was also no decreased graft survival in patients with CMV disease, as compared to those without CMV infection. There were 51 rejections in the 107 patients who were seropositive before transplantation and 26 rejections in the 68 patients who were seronegative before transplantation ($P=$ NS). Graft survival in patients who were seropositive before transplantation was equal to that in seronegative patients.

**Table 1.** The main clinical manifestations of 24 patients with cytomegalovirus (CMV) disease

| | Number of patients | Duration (days)[a] |
|---|---|---|
| Fever | 24 | 18.8 (3-62) |
| Leukopenia | 19 | 19.3 (1-70) |
| Thrombocytopenia | 6 | 14.6 (1-35) |
| Pneumonitis | 1 | 48 |
| Hepatitis | 14 | |

[a] Mean (range)

**Table 2.** Serological and virological parameters in patients with and without cytomegalovirus (CMV) disease

| | CMV disease[a] ($n=24$) | CMV infection (no disease) ($n=66$) | |
|---|---|---|---|
| IgM seroconversion | 16 | 13 | $P<0.001$ |
| IgA seroconversion | 23 | 29 | $P<0.001$ |
| IgG titer rise or seroconversion | 20 | 54 | NS |
| EA-positive | 16 | 45 | NS |
| CVI-positive | 14 | 44 | NS |
| Seronegative before transplantation | 14 | 8 | $P<0.001$ |

[a] The appearance of IgM or IgA antibodies and seronegativity before transplantation were strong indicators of CMV disease

Twenty-four patients had CMV disease. The interval between transplantation and the start of CMV disease was 74.5 days (range 28-206 days). The clinical manifestations are shown in Table 1. There was one patient with a biopsy-proven CMV pneumonitis for which artificial ventilation and therapy with DHPG were begun. There were no deaths attributable to CMV disease. In 12 patients the onset of clinical symptoms preceded the serological or virological diagnosis by a mean of 13.8 days (range 1-22 days). In 10 patients there had been a seroconversion or fourfold rise of the IgG titer or positive virus culture before clinical symptoms occurred. In the remaining 2 patients, the clinical symptoms and the laboratory tests concurred. In 18 patients there was a seroconversion or a rise of IgG titer before a positive CMV-EA or CVI; the opposite was true in 4 patients. In only 2 patients were both tests first positive on the same day.

The appearance of IgM or IgA antibodies or a negative serological status before transplantation was a strong indicator of CMV disease ($P<0.001$; Table 2). Using the same parameters it was not possible to distinguish any risk factor that could identify at an early stage patients at risk for severe CMV disease (fever lasting more than 10 days or pneumonia). Moreover, no difference was found in the severity of CMV disease in previously seronegative and seropositive recipients.

## Discussion

CMV infection and disease are recognized as the most frequent infectious complications after kidney transplantation, leading to serious morbidity and, at times, to mortality and graft loss. Strategies have, therefore, been developed to prevent CMV infection, including denying seropositive kidneys to seronegative recipients [21] and using CMV immune globulin [18]. However, most of the studies that have thus far been done on the incidence of CMV infection and disease have been performed with azathioprine as the main immunosuppressive drug. We decided to do a prospective study of patients on cyclosporin and low-dose prednisone without stratification for donor/recipient serological status before transplantation or use of CMV immune globulin.

Follow-up was done on 175 renal allograft recipients, using weekly serological and virological tests. There are only a few studies with comparable intensive follow-up on patients. Most studies are retrospective and evidence for CMV infection is only sought if there is a clinical suspicion of CMV infection or if the patient is hospitalized [10, 11, 20]. We found that 51.4% of our patients had evidence of CMV infection, but only 26.7% of those patients had symptoms of CMV disease (13.7% of all patients). These numbers are somewhat higher than the results reported by others [5, 7, 11], but they compare well with the results of the prospective study by Bia et al [1]. Infected seronegative patients were at serious risk of developing CMV disease. An important finding in our study is also that CMV disease had a rather benign course. There was only one patient who suffered from a pneumonitis, and this was able to be treated successfully with DHPG. Most patients had fever and some general malaise. However, the duration of the fever was rather long; in 18 patients it lasted for 10 days or more.

There was a very strong correlation between rejection and CMV infection. Most rejections (41/54) occurred before CMV infection, thereby confirming the well-known association between the use of additional immunosuppressive therapy and CMV infection. It does not seem likely that the CMV infection predisposed patients to rejection because most rejections occurred before there was any laboratory or clinical sign of CMV infection. Only 13 rejections occurred after CMV infection had been diagnosed. We found no evidence of increased graft loss after CMV infection or disease. Although it is frequently necessary to lower immunosuppressive therapy during CMV infection because of leukopenia or thrombocytopenia, we found that this can be done safely without increasing graft loss. These results are not in accordance with those of another study in cyclo-

sporin-treated patients [11] or with the results of two studies in azathioprine-treated patients [14, 19], in which there was increased graft loss after CMV infection. On the other hand, our results are comparable with those recently reported by Johnson [10] et al. on patients given cyclosporin.

We found no close time relationship between the onset of the clinical syndrome of CMV disease and the laboratory confirmation of a CMV infection. In half of the patients, the rise in antibody titers or the presence of a virus in the urine was delayed, leading to doubts about the correct diagnosis and to additional investigations. In this study only urine specimens were examined for the presence of CMV. Examination of additional patient specimens - in particular, blood - might have improved the sensitivity of laboratory tests for detection of CMV early in the course of the disease [2]. Sensitivity for CMV detection may be further increased by application of the polymerase chain reaction and subsequent DNA hybridization [17].

The third and final aim of our study was to determine which patients were at risk of developing severe CMV disease, this in the hope of identifying the group of patients that would benefit from early treatment with DHPG. While the appearance of IgM and IgA antibodies and seronegativity before transplantation were strong indicators of CMV disease, they could not discriminate at an early stage between serious and benign disease. Although most patients had longlasting fever, it must be concluded from our study that in almost all patients on cyclosporin and low-dose prednisone, CMV disease is a rather benign and self-limiting illness.

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