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Serum neopterin/creatinine values correlate with severity of symptoms caused by cytomegalovirus infection in renal transplant recipients

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Abstract. Serum neopterin/creatinine ratios were longitudinally measured in 86 renal transplant recipients from the day before transplantation until 4 months after transplantation, and the relationship to the clinical symptoms of cytomegalovirus (CMV) infection was studied. Infection with cytomegalovirus occurred in 23 patients, 11 cases of which were due to primary infection. Symptoms caused by CMV infection were more severe in male patients, in patients who had received prior antirejection treatment, and in patients with primary CMV infection. The measurement of serum neopterin/creatinine ratios proved to be a marker for the severity of symptoms caused by CMV infection, as peak values were significantly higher in eight patients with CMV disease than in patients with no or only mild symptoms of CMV infection ($P < 0.05$). Moreover, in seven out of eight cases of CMV disease, serum neopterin/creatinine ratios started to rise up to 2 weeks before CMV infection was proven by serology.

Key words: Kidney transplantation, CMV infection – CMV infection, kidney transplantation – Neopterin, CMV infection, kidney transplantation

Clinical transplantation is often complicated by infection with cytomegalovirus (CMV), either primary CMV infection or secondary infection, i.e., reactivation of endogenous CMV or superinfection with a different strain of CMV. Since CMV carrier status of donor and recipient are not matched and since renal transplant recipients are treated with immunosuppressive drug therapy and frequently receive blood transfusions, infection or reinfection with CMV frequently occurs. These infections or reinfections with CMV are not always accompanied by clinical symptoms, but they can sometimes lead to life-threatening disease, such as CMV pneumonitis.

Elevated levels of serum neopterin have been described during renal transplantation complicated by CMV infection [1, 2]. Neopterin is a product of gamma-interferon-stimulated monocytes and macrophages, which can be used as a measure of cell-mediated immunological activity, yet its physiological function is unknown [4]. In a previous study we have shown that the measurement of serum neopterin/creatinine ratios can be used for the differential diagnosis of periods of fever caused by CMV infection with periods of fever not caused by CMV infection [7].

In the present study, serum neopterin/creatinine ratios were longitudinally measured in 86 renal transplant recipients from the day before transplantation until 4 months after surgery, and they were correlated with the severity of clinical symptoms caused by CMV infection. We show that serum neopterin/creatinine ratios can be used as a measure of the severity of symptoms caused by CMV infection or reinfection and, in addition, that in seven of eight cases of CMV disease, serum neopterin/creatinine ratios were elevated up to 2 weeks before CMV infection was proven by serology.

Materials and methods

Patients

A group of 86 renal allograft recipients was studied longitudinally from the day before transplantation up until 4 months after surgery. Serum samples were obtained before transplantation, three times a week during hospitalization, and once a week after discharge from the hospital. Immunosuppressive therapy consisted of prednisolone (10 mg/day) orally and CyA orally (dosage based on whole blood trough levels of CyA: range 150–200 µg/l during the first 3 months, 100–150 µg/l thereafter). Patients did not receive any prophylaxis against CMV infection or reinfection. Blood was always drawn before the administration of immunosuppressive drugs. Serum samples were stored at −80°C.

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Table 1. Characteristics of patients with proven CMV infection. Group A: no symptoms; group B: mild symptoms, no hospitalization; group C: CMV disease, hospitalization. MPNS, Methylprednisolone

	Age	Weeks between transplantation and seroconversion	Sex (male/female)	Primary/secondary infection	Total no. of previous courses of antirejection therapy
Group A ($n = 8$)	46[a] (19–60)	12.5 (6–17)	1/7	1/7	4 (3 × MPNS, 1 × OKT3)
Group B ($n = 7$)	45 (37–53)	8 (2–13)	3/4	4/3	0
Group C ($n = 8$)	47.5 (28–64)	7.5 (5–13)	7/1	6/2	10 (7 × MPNS, 3 × OKT3)

[a] Median and (range)

CMV serology and conventional cultures of both urine and buffy coats were performed the day before transplantation and thereafter at intervals of 2 weeks (or more frequently when indicated by clinical symptoms). CMV infection was retrospectively confirmed by seroconversion or a fourfold rise in CMV antibody titer (hereafter also referred to as seroconversion) in the complement fixation test.

Patients with proven CMV infection were retrospectively divided into three groups: group A ($n = 8$): no clinical symptoms of CMV infection; group B ($n = 7$): mild symptoms of CMV infection, including tiredness, shivering, or subfebrile body temperatures (patients in this group were never admitted to the hospital); and group C ($n = 8$): CMV disease, defined as at least 3 days of fever ($\geq 38°C$), together with other such signs as pneumonitis, gastrointestinal symptoms, or disturbed liver function. All patients in this latter group had to be admitted to the hospital.

Neopterin assay

Serum neopterin was measured with a commercially available radioimmunoassay (IMMUtest Neopterin, Henning, Berlin, FRG). In brief, 100 µl of ^{125}I neopterin and 100 µl preprecipitating antibody complex (sheep anti-neopterin and donkey anti-sheep IgG) were added to 20-µl serum samples. The suspension was incubated for 1 hour at room temperature. Then, washing solution was added and samples were centrifuged for 10 min at 2000 *g*. Supernatant was removed and pellet radioactivity was counted in a gamma counter. Samples were related to a standard preparation of neopterin. Since neopterin is almost exclusively cleared through the kidney [8], all data are expressed as the ratio of serum neopterin and plasma creatinine to correct for renal dysfunction {[(nmol/l):(µmol/l)]*10^6}. The mean serum neopterin/creatinine ratio ($\pm$ SD) during stable transplantation (i.e., 4 months after transplantation, in the absence of complications) was 140 ± 92 ($n = 46$ patients).

Statistics

Statistical analysis was performed with the Mann-Whitney U-test. A P-value less than 0.05 was considered to be significant.

Results

The severity of symptoms caused by CMV infection correlated with the gender of the patients, as shown in Table 1. Seven of the eight cases of CMV disease (group C) occurred in male patients, whereas only one out of eight cases of CMV infection without clinical symptoms (group A) was in a male patient. Table 1 also shows that the severity of symptoms correlated with the nature of CMV infection: six out of eight cases of CMV disease (group C) were due to primary infection, whereas only one out of eight cases of CMV infection without clinical symptoms (group A) was due to primary infection. The age distribution and the number of previous renal transplantations were not significantly different between the three groups. The development of CMV disease was also related to prior antirejection treatment consisting of either methylprednisolone or OKT3, since 6 out of 8 patients with CMV disease (group C) were treated for acute rejection (3 of whom more than once), whereas only 3 out of 15 patients with no or mild symptoms of CMV infection received antirejection treatment (groups A and B).

The relationship between the highest values of serum neopterin/creatinine ratios and the severity of symptoms caused by CMV infection is shown in Fig. 1. Peak values of serum neopterin/creatinine ratios were significantly higher in patients with CMV disease (group C) than in patients with no or mild symptoms of CMV infection (groups A and B).

Figure 2 shows the time relationship between elevated values of serum neopterin/creatinine ratios and seroconversion in the eight patients who experienced CMV disease (group C). In these patients, serology and CMV culturing were performed every week. This figure shows that in seven out of eight patients, serum neopterin/creatinine ratios started to rise up to 2 weeks before seroconversion was detected, even when the difference between sampling

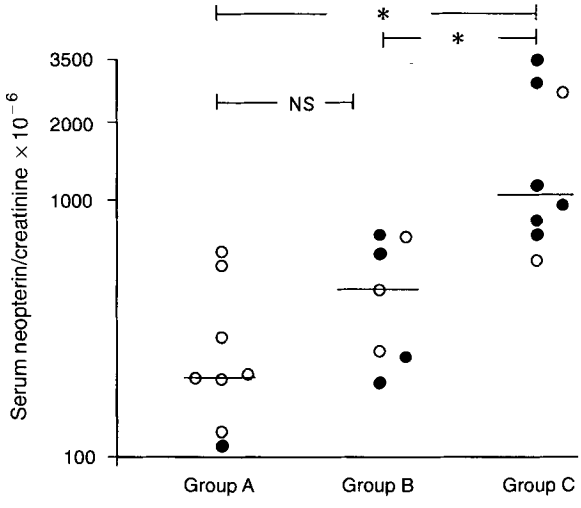

Fig. 1. Peak values of serum neopterin/creatinine during infection or reinfection with cytomegalovirus (CMV). Group A: no symptoms; group B: mild symptoms, no hospitalization; group C: CMV disease, hospitalization. ● Primary infection with CMV, ○ secondary infection with CMV, — median value. * $P < 0.01$ (Mann-Whitney U, test)

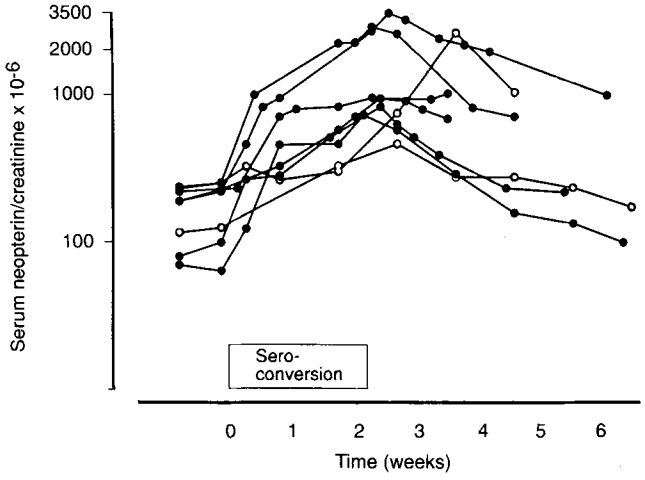

Fig. 2. Longitudinal course of serum neopterin/creatinine values in eight patients with CMV disease. Six cases of CMV disease were caused by primary infection (●) and two cases by secondary infection (○). The period in which seroconversion of these patients was first detected is also shown

frequencies of measurement of serum neopterin/creatinine ratios and serology was taken into account. The same results were obtained when comparing serum neopterin/creatinine ratios with the points in time at which CMV cultures became positive.

Discussion

CMV infection or reinfection is a complication that frequently occurs after renal transplantation. In 27% of the patients in our study population, infection or reinfection with CMV was proven within the first 4 months after transplantation. Admission to the hospital, due to the severity of symptoms caused by CMV infection or reinfection, was necessary in 8 out of 86 patients.

In the context of clinical transplantation, elevated levels of serum or urinary neopterin have been reported during acute rejection episodes [1, 2, 6, 8], viral infections such as CMV infection or herpes simplex virus infection [1, 2], and hemodialysis [5]. In a previous study, which was performed with the same group of renal transplant recipients, we showed that serum neopterin/creatinine ratios measured during CMV disease exceeded by far those obtained during bacterial infection, cyclosporin A toxicity, acute tubular necrosis, or acute rejection episodes [7]. This indicates a strong, probable gamma-interferon-mediated activation of monocytes and macrophages [4]. Another explanation may be that during CMV disease monocytes and macrophages contain a high load of CMV virus, which may result in high metabolic activity and increased neopterin production by these cells.

The lack of a significant difference between serum neopterin/creatinine ratios in group A (no symptoms) and group B (mild symptoms) is probably due to the fact that two patients in group A experienced oral-labial infection with herpes simplex virus, during which elevated values of serum neopterin/creatinine ratios were measured.

In the present study we show that the serum neopterin/creatinine ratios during CMV infection are significantly higher in patients who develop CMV disease (group C) than in patients with no or mild symptoms (groups A and B). Measurement of the serum neopterin/creatinine ratio may, therefore, be useful to distinguish CMV disease from less severe CMV infection, especially in patients at risk, e.g., CMV-seronegative patients who have received antirejection treatment(s).

The fact that in seven out of eight patients in group C (CMV disease) values of serum neopterin/creatinine ratios started to rise up to 2 weeks before seroconversion may indicate that measurement of serum neopterin/creatinine ratios may also be useful as an early marker of CMV disease. However, the relative contribution of the measurement of serum neopterin/creatinine ratios to the early diagnosis of CMV disease, as compared to other means of early diagnosis, such as the detection of immediate early antigen [3, 9], remains to be elucidated further.

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