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β_2-Microglobulinuria as an early sign of cytomegalovirus infection following renal transplantation

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Abstract. The frequency of cytomegalovirus infection was studied in a prospective study of 106 kidney recipients. The detection of cytomegalovirus-immediate-early-antigen and cytomegalovirus-immunoglobulin (IgM) antibodies in serum was used as the reference method and showed that 23.6% (25/106) of all patients were infected. In addition, four urinary proteins (IgG and transferrin as glomerular markers and α_1-microglobulin and β_2-microglobulin as tubular markers) were quantitatively measured in 24-h urine samples from all of the patients using an immunoluminometric assay (ILMA). In all cytomegalovirus infection cases a pronounced but isolated increase of urinary β_2-microglobulin excretion was observed. In 20 of 25 infected patients, the β_2-microglobulinuria occurred 1–21 days (median 5.0) earlier than the appearance of the cytomegalovirus-immediate-early-antigen in blood. Thus, it can be seen that the quantitative measurement of β_2-microglobulin in urine is useful for the early detection of cytomegalovirus infection following renal transplantation.

Key words: Renal transplantation – Cytomegalovirus – Proteinuria – β_2-Microglobulin – Cytomegalovirus-immediate-early-antigen

Florid disease in cytomegalovirus (CMV)-infected patients usually presents a much smaller diagnostic challenge than CMV infection in an early form, and the real value of a diagnostic test lies in its predictive value before the severe complications of CMV infection arise. The detection of CMV infection in patients who have successfully undergone a renal transplantation has been improved during the past few years because florid CMV infection is associated with the concomitant occurrence of CMV-immediate-early-antigen (CMV-IEA) in blood. This is important as in patients under immunosuppression with cyclosporin following renal transplantation, a significant increase of IgM antibodies is unlikely to occur. In such patients, the otherwise typical neutropenia is not a useful diagnostic criterion when cyclosporine, azathioprine, and prednisolone are used in combination to achieve immunosuppression.

Clinical criteria such as fever, myalgia, or arthralgia are not pathognomonic. The rapid and early diagnosis of CMV is of decisive importance because of the lethal complications (e.g., pneumonia) or irreparable damage (e.g., retinitis). In a few studies, tubular proteinuria accompanying CMV infection has been observed [4, 7]. However, this observation has not led to further prospective investigations.

Grundy et al. found that CMV is coated with β_2-microglobulin before renal excretion [5]. However, the diagnostic possibilities arising from this statement were not examined. It has been shown that a pathological β_2-microglobulinuria can both appear in patients with cyclosporin intoxication and accompany renal transplant rejection [1]. In previous studies, we have not been able to confirm these observations [9]. Here we report a prospective study performed to evaluate the diagnostic value of β_2-microglobulinuria in CMV-infected patients following renal transplantation.

Methods

In a prospective study started in July 1989 on 106 kidney recipients to date, the "glomerular" proteins immunoglobulin G (IgG) and transferrin as well as the selected "tubular" proteins α_1-microglobulin and β_2-microglobulin were measured in the urine. During hospitalization, these analytes were measured daily. Prior to analysis, the urine was centrifuged for 10 min at 3000 min^{-1}, and the proteins were measured immediately after centrifugation. Additionally, creatinine in urine and serum was evaluated colorimetrically using the Jaffe method without prior deproteinisation. Quantitative analysis of urinary proteins were made with immunoluminometric assays (ILMA) which have been developed in this laboratory. The assays function using the "sandwich technique" in which the liquid antibody is la-

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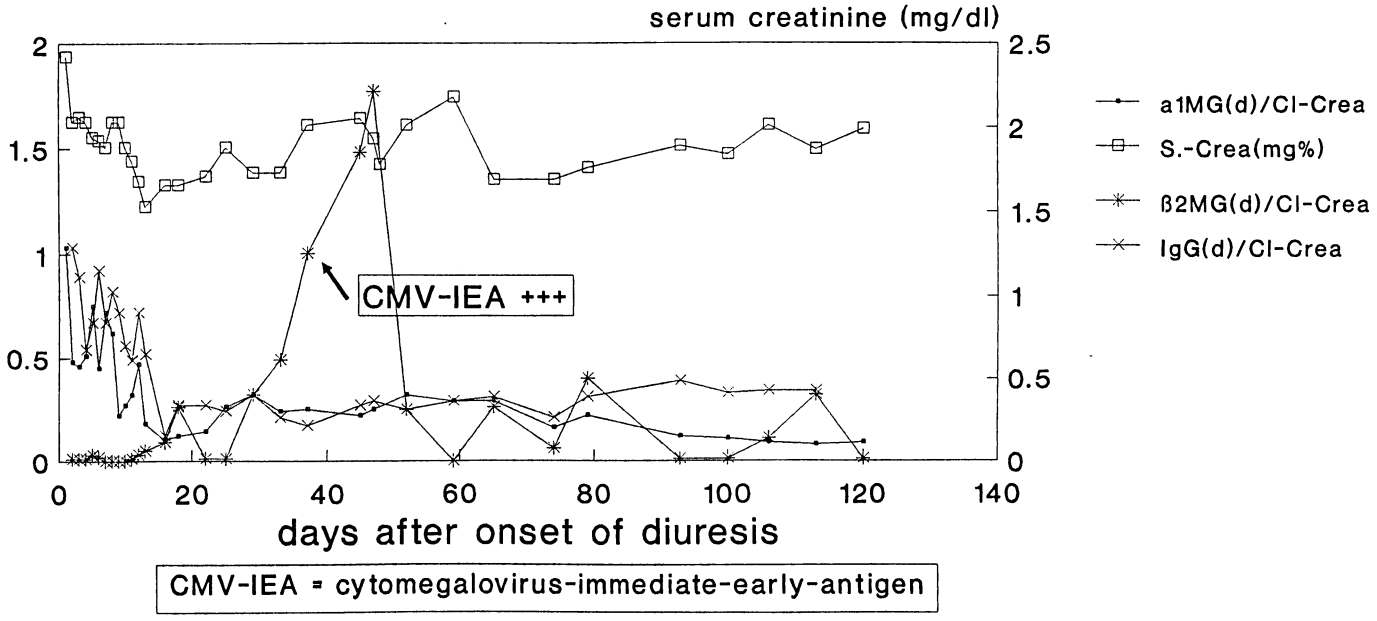

Fig. 1. Course of proteinuria in a 52-year-old patient with a cytomegalovirus (CMV) infection. After transplantation, at first typical mixed proteinuria without β_2-microglobulin excretion. On day 37 the detection of CMV-IEA was first positive. An isolated increase in β_2-microglobulin was seen, however, several days before. Other marker proteins (immunoglobulin IgG and α_1-microglobulin) showed little change

belled with a luminogen. All measurements were made in duplicate on a semiautomatic 250-sample luminometer (LB 952T/16; Berthold, Wildbad, FRG). Detailed descriptions of these assays have already been published [10, 11]. The adjustment of the urinary pH to above 7.0, which is often recommended for measurements of β_2-microglobulin [8], was not done for two reasons: Firstly, interference with other protein assays could not be excluded, and secondly, the β_2-microglobulin seems to be more stable in the urine of CMV-infected patients in general [5].

Patient-derived data were expressed as a quotient of the daily protein excretion (mg/day) to the actual individual creatinine clearance (ml/min) and was measured daily. Then the ratios between the actual quotient were compared with the previous quotient in the same patient and any significant increases or decreases logged for diagnostic purposes.

All patients received the same immunosuppressive therapy (prednisolone 25-7.5 mg/day, azathioprine 2 mg/kg daily, cyclosporin 3–5 mg/kg daily). Therapeutic cyclosporin levels in whole blood were normally between 80 and 120 ng/ml when measured with a specific radioimmunoassay (RIA; Sandoz). The CMV-IEA in the peripheral blood leukocytes was determined histochemically using the alkaline phosphatase/antialkaline phosphatase technique (APAAP) [2]. In our experience the APAAP method provides a higher diagnostic specificity and sensitivity for labeling CMV-IEA-positive leukocytes than the method described by van der Bij [3]. If at least 2 out of 400000 leukocytes were labeled, the CMV-IEA test was considered to be positive.

CMV-IgM antibodies were evaluated by a routine enzyme immunoassay. In hospitalized patients, the CMV-IEA and CMV-IgM antibody levels were determined weekly. In patients followed up in the outpatient clinic, the tests were made each time they attended.

Results

To judge cytomegaloviremia, the diagnostic criterion was a positive CMV-IEA test in blood. Out of 106 renal transplant recipients 25 were considered CMV infected (23.6%). One of these 25 patients showed a positive CMV-IEA test following treatment with methylprednisolone, which had been given to counteract acute renal transplant rejection. The other 24 patients had been treated with antithymocyte-globulin (ATG), either against renal transplant rejection (7 patients) or because of a high immunological risk accompanying repeated renal transplantation (17 patients).

Figure 1 shows the typical development of the β_2-microglobulinuria and its temporal course following the first positive CMV-IEA test in the blood. Note here that neither the "tubular" marker protein α_1-microglobulin nor the "glomerular" marker protein IgG showed significant alterations.

In all of the CMV-IEA-positive cases, an isolated (at least three fold) increase of β_2-microglobulin excretion was observed. Only in 4 cases were slight increases in IgG and in 6 cases, slight increases in α_1-microglobulin seen.

Table 1 shows the time course of the first appearance of β_2-microglobulinuria, CMV antibodies in serum, and CMV-IEA in blood (day 0). With the exception of 5 cases, increases of β_2-microglobulin in urine were found considerably earlier than or at the same time as CMV-IEA in the blood. The CMV-IgM antibody in the serum appeared much later.

Only in CMV infections is an *isolated* increase of β_2-microglobulinuria to be found. β_2-microglobulinuria was also observed in patients with renal transplant rejections and during urosepsis; however, in these patients the β_2-microglobulinuria was associated with general "tubular" proteinuria (α_1-microglobulinuria). In patients with renal transplant rejection, initial "glomerular" proteinuria was followed by extensive "tubular" proteinuria. In renal transplant recipients suffering from urosepsis, extensive

Table 1. The time relation between the appearance of CMV-immediate-early-antigen in blood (day 0 = CMV-IEA detection) and the detection of CMV-IgM antibody in serum and β_2-microglobulinuria expressed in days before (−) or after (+) appearance of CMV-IEA

Patient	β_2-MG	CMV-IgM	CMV-IgG Donor/recipient
1	−3	+3	+/−
2	−5	+9	+/−
3	−4	+4	+/−
4	−9	>10	+/−
5	−1	+2	+/+
6	−5	>10	+/−
7	−1	>10	+/−
8	−8	>10	+/−
9	−15	>10	+/+
10	−6	>10	+/−
11	0	>10	+/−
12	−8	>10	+/−
13	+4	+9	+/+
14	−7	+8	+/−
15	−6	>10	+/−
16	−9	>10	+/+
17	0	>10	+/−
18	−4	>10	−/−
19	−21	>10	+/−
20	−3	>10	+/−
21	−8	+6	+/+
22	0	>10	+/−
23	−5	>10	+/−
24	+2	>10	+/−
25	−9	>10	+/−

The CMV-antibody constellation of donor and recipient at the time of transplantation is shown in the last column

"tubular" proteinuria was observed. In patients with acute cyclosporin nephrotoxicity, no changes in proteinuria were seen [9].

The specificity of an isolated β_2-microglobulinuria during CMV infection is 100%. The sensitivity is 80%, when related to the occurrence of CMV-IEA in blood.

Discussion

Our study results found no general tubular proteinuria during CMV infection. More interestingly, an *isolated* (at least three fold) increase of β_2-microglobulin excretion only occurred in CMV-infected patients. Moreover, in 20 out of 25 CMV-infected patients, the β_2-microglobulinuria occurred up to 21 days (median 5.0) earlier than the appearance of CMV-IEA in the blood. Thus, the detection of isolated β_2-microglobulinuria is specific for CMV infection and is useful in the early diagnosis of CMV infection in renal transplant recipients.

The underlying pathomechanisms causing β_2-microglobulinuria in CMV infection are not clear. Grundy and coworkers [5] reported that the β_2-microglobulin coats the CMV, resulting in a stable complex. It could be shown that β_2-microglobulin was at higher concentrations in the sediment than in the supernatant, which supports the results of Grundy et al.

Thus, it can be concluded that the CMV-β_2-microglobulin complex is to be found in the sediment because of the higher weight of the viral complex and that it can be measured in a β_2-microglobulin assay [5].

It seems to be an advantage not to alkalinize the urine because most of the "unspecific" and instable "tubular" β_2-microglobulin is not supposed to be detectable in acid urine, whereas the more stable "CMV-specific" β_2-microglobulin can be measured.

Measuring β_2-microglobulin alone is not sufficient to diagnose a CMV infection, as β_2-microglobulinuria can also be found after successful antirejection therapy as well as following bacterial infections of the urinary tract [9, 11]. Nevertheless, in such cases, a general tubular proteinuria, but not an isolated increase of β_2-microglobulin, is to be observed [9].

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