Albumin and $\beta_2$-Microglobulin Radioimmunoassays Applied to Monitoring of Renal-Allograft Function and in Differentiating Glomerular and Tubular Diseases

Jannie Woo, Michael Floyd, and Donald C. Cannon

Blood and urine were sampled daily from 11 renal-allograft recipients from one to six weeks after the transplant. Clearances of both albumin ($C_{\text{alb}}$) and $\beta_2$-microglobulin ($C_{\beta_2M}$) were significantly increased in all 11 patients. Five patients (Group I) with acute allograft rejection showed markedly increased $C_{\text{alb}}$ and moderately increased $C_{\beta_2M}$, concurrent with decreased creatinine clearance ($C_{\text{Cr}}$). Five other patients (Group II) with no evidence of rejection demonstrated episodes of grossly increased $C_{\beta_2M}$, with minimally increased but stable $C_{\text{alb}}$ and normal $C_{\text{Cr}}$. One patient had no evidence of rejection nor indications of glomerular or tubular proteinuria. While changes in serum $\beta_2$-microglobulin concentration closely paralleled those of serum creatinine in the Group I patients, the results diverged in the Group II patients because the increase in serum $\beta_2$-microglobulin exceeded that of serum creatinine and preceded the increase in creatinine by one to five days, suggesting that measurement of serum $\beta_2$-microglobulin might afford earlier indication of the nature and extent of renal damage in the allograft recipients.

Additional Keyphrases: proteins • clearances • renal damage • urine • reference intervals

The clinical laboratory evaluation of renal function typically involves any or all of a constellation of tests and measurements, including routine urinalysis, serum creatinine, creatinine clearance, blood urea nitrogen, urine osmolality, and total urinary protein. Recently, attention has focused on differential analysis for component proteins in urine as a more sensitive and specific index to renal damage, an index that would be of considerable value in monitoring kidney function in renal-allograft recipients (1). Of the proteins present in urine, albumin has been established as a sensitive indicator of renal glomerular injury (2) because its relative molecular mass ($M$, 68 000) is such that it only slightly exceeds the exclusion limit of glomerular basement membrane permeability (3, 4). On the other hand, $\beta_2$-microglobulin ($\beta_2M$)5, a protein of low molecular mass ($M$, 11 800) with a Stoke’s radius of 1.5 nm and a glomerular sieving coefficient close to unity, is almost totally reabsorbed by the proximal tubule and is subsequently catabolized in the kidney (2, 5, 6). The almost unhindered filtration of this protein through the glomerular basement membrane and its extensive renal tubular reabsorption provide the physiological basis for using the metabolism of $\beta_2M$ as a measure of renal tubular function and thus to explain the linear correlation between serum creatinine ($S_{\text{Cr}}$) and serum $\beta_2M$ ($S_{\beta_2M}$) concentrations that has been observed by several investigators (7–10). This relationship, however, is not entirely predictable in certain clinical situations, notably in renal transplantation, because endogenous production of this protein can change as a result of infection and increased immune activity, independently of renal function (7). Thus this variation in the relationship between $S_{\beta_2M}$ and $S_{\text{Cr}}$ appears to offer an opportunity to study endogenous production of $\beta_2M$ independent from its renal excretion and gain valuable information about concurrent infection or immunological activity (or both) in allograft patients. We previously described sensitive radioimmunoassays for albumin in urine (11), and $\beta_2M$ in both urine and serum (12). We report here the results of a study of serial clearances of albumin ($C_{\text{alb}}$) and $\beta_2M$ ($C_{\beta_2M}$) in 11 renal allograft patients during the first through the fifth week after the transplant. The protein clearances were correlated with renal function as represented by creatinine clearance ($C_{\text{Cr}}$), and the values of $S_{\beta_2M}$ and $S_{\text{Cr}}$ were also examined in relation to the clinical status of the patients.

Materials and Methods

Patients. We studied 11 patients—seven men and four women—who had received renal allografts. One patient, M.O., was the recipient of a kidney from a living relative. The rest of the patients received cadaver kidneys. Timed 12-h urines were collected during the period 0600 to 1700 hours from each recipient for the first one to five weeks after the transplant. The corresponding serum samples were collected from the same individuals at 0600 hours. These samples were stored frozen and were all assayed at one time.

The patients were classified according to their graft function. The assessment was based on a constellation of clinical observations and laboratory results including $S_{\text{Cr}}$, $C_{\text{Cr}}$, total urine protein, blood urea nitrogen, electrolytes, nonspecific immunological monitoring (active rosette-forming cells and spontaneous blastogenesis) and regular isotopic evaluation of renal function with $^{99m}$Tc-labeled diethylenetriaminepentaacetic acid (DTPA) and $^{131I}$-labeled hippuran. Rejection was diagnosed on the basis of an increase of $S_{\text{Cr}}$ equal to or greater than 2 mg/dL or a decline of $C_{\text{Cr}}$ equal to or greater than 20% observed during two consecutive determinations within 24 h, or a defined change in renal handling of one or both radiopharmaceuticals.

Of the 11 patients studied, five (Group I) demonstrated clinical episodes of acute allograft rejection based on a constellation of clinical and laboratory results including serial immunological monitoring by spontaneous blastogenesis and active rosette forming T-cells. Five patients showed no clinical evidence of rejection. Patient M.O., the only recipient of a living donor allograft, had an extremely rapid and uneventful recovery and was considered separately.

Clearance of albumin. Urinary albumin excretion ($U_{\text{alb}}$) was determined by radioimmunoassay as described elsewhere (11). Briefly, aliquots of urine, diluted 50- and 1000-fold, were incubated at room temperature for 1 h with $^{125I}$-labeled al-
Table 1. Maximum Clearances of Albumin and β2-Microglobulin in Renal-Allograft Recipients 1–5 Weeks after Transplant

<table>
<thead>
<tr>
<th>Patient</th>
<th>CCr</th>
<th>µL/min</th>
<th>CAlb</th>
<th>CAlb/CCr</th>
<th>Cβ2µ/CCr</th>
<th>Cβ2µ/Alb</th>
<th>Cβ2µ/CCr</th>
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<tbody>
<tr>
<td><strong>Group I</strong></td>
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</tr>
<tr>
<td>C.O.</td>
<td>22.7</td>
<td>248.6</td>
<td></td>
<td>10.58(1058)</td>
<td>6.51</td>
<td>0.186(155)</td>
<td></td>
</tr>
<tr>
<td>B.O.</td>
<td>7.8</td>
<td>60.0</td>
<td></td>
<td>12.69(1269)</td>
<td>0.90</td>
<td>0.231(193)</td>
<td></td>
</tr>
<tr>
<td>B.R.</td>
<td>5.1</td>
<td>57.1</td>
<td></td>
<td>11.19(1119)</td>
<td>3.38</td>
<td>0.663(553)</td>
<td></td>
</tr>
<tr>
<td>N.O.</td>
<td>19.0</td>
<td>27.6</td>
<td></td>
<td>1.45(145)</td>
<td>0.60</td>
<td>0.032(27)</td>
<td></td>
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<tr>
<td>C.U.</td>
<td>31.0</td>
<td>25.6</td>
<td></td>
<td>0.83(83)</td>
<td>0.64</td>
<td>0.021(18)</td>
<td></td>
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<tr>
<td><strong>Group II</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.R.</td>
<td>95.8</td>
<td>2.00</td>
<td></td>
<td>0.021(21)</td>
<td>22.10</td>
<td>0.231(193)</td>
<td></td>
</tr>
<tr>
<td>K.I.</td>
<td>91.0</td>
<td>2.76</td>
<td></td>
<td>0.030(30)</td>
<td>12.20</td>
<td>0.134(112)</td>
<td></td>
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<tr>
<td>P.R.</td>
<td>70.6</td>
<td>3.00</td>
<td></td>
<td>0.042(42)</td>
<td>8.13</td>
<td>0.115(95)</td>
<td></td>
</tr>
<tr>
<td>A.R.</td>
<td>83.0</td>
<td>3.23</td>
<td></td>
<td>0.039(39)</td>
<td>9.22</td>
<td>0.111(93)</td>
<td></td>
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<tr>
<td>L.E.</td>
<td>75.4</td>
<td>4.91</td>
<td></td>
<td>0.065(65)</td>
<td>9.78</td>
<td>0.130(109)</td>
<td></td>
</tr>
<tr>
<td><strong>Patient M.O.</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.O.</td>
<td>91.0</td>
<td>2.11</td>
<td></td>
<td>0.023(23)</td>
<td>0.88</td>
<td>0.010(9)</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>90–120</td>
<td>0.018–0.105</td>
<td></td>
<td>0.001* (1)</td>
<td>0.008–0.13</td>
<td>0.0012* (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Reference intervals for CAlb/CCr and Cβ2µ/CCr were calculated by using the upper limits of the reference intervals for CAlb (0.105 µL/min, Cβ2µ (0.13 mL/min), and CCr (105 mL/min).

albumin and a rabbit antiserum monospecific for human albumin. The radiolabeled albumin was prepared by a modification of the procedure of Hunter and Greenwood (13) in which 5 µg of highly purified albumin and 500 µCi of carrier-free 1251 were incubated for 1.5 min. The preparation was then purified by gel-filtration chromatography, and the specific activity of the purified material was adjusted to 1 Ci/g. The separation of antibody-bound and free fractions was achieved with use of a goat anti-rabbit gamma globulin serum. The standard curve covered an absolute range of 15–1000 ng of albumin. Serum albumin concentration (SAlb) was measured by the bromcresol green procedure with a centrifugal analyzer (Centrifichem System 400, Union Carbide, Rye, NY 10581).

Clearance of β2-microglobulin. Both serum and urinary β2µ were determined by radioimmunoassay as described previously (12). Briefly, aliquots of fivefold diluted serum and five- and 500-fold diluted urine were incubated for 1 h at room temperature with 125 labeling β2µ and a rabbit antiserum specific for human β2µ. The β2µ used as the standard was isolated from the urine of a patient with Fanconi’s syndrome and purified. It was iodinated by a modified Hunter/Greenwood procedure (13) in which 2.5 µg of β2µ, 500 µCi of carrier-free Na1251, and 30 µg of Chloramine T were allowed to react for 1 min before the labeled product was purified by gel-filtration chromatography. The specific activity was adjusted to 10 Ci/g. The antibody-bound and free fractions were separated by the double antibody technique with use of a goat anti-rabbit gamma globulin serum. The standard curve covered an absolute range of 2–64 ng β2µ.

Clearance of creatinine. Serum and urinary creatinine were both measured by the Jaffe reaction by an automated kinetic method with the centrifugal analyzer (14).

Results

Reference Interval

The reference intervals for UAlb, CAlb, Sβ2µ, and Cβ2µ were established by use of serum and 24-h urine specimens collected from 25 ostensibly healthy laboratory individuals known to be taking no drugs. The reference intervals for UAlb and CAlb were 2.2–12.6 mg/24 h and 18–105 mL/min, respectively. The reference intervals for Sβ2µ and Uβ2µ were 1.1–2.3 mg/L and 40–360 µg/24 h, and the corresponding β2µ clearance range was 8–130 µL/min. The reference intervals for SAlb, SCr, and UCr, each established from data on no fewer than 50 healthy laboratory individuals, were 35–50 g/L, 5–14 mg/L, and 1.0–1.8 g/24 h, respectively.

Clearance of Albumin and β2-Microglobulin

In comparison with the 25 controls studied, all patients showed significantly increased CAlb and Cβ2µ at all times. Analysis of the clearance status of albumin and β2µ in association with changes in CCr in these three groups of patients revealed distinctly different patterns.

Group I. The patients in Group I all showed episodes of extremely increased CAlb accompanied by relatively moderate increased Cβ2µ (see Table 1). The clearance profile of patient C.O. (Figure 1) serves as a typical example. A rapid decline in CCr, beginning on day 14, was accompanied by a moderate increase in Cβ2µ (maximal on day 15, Cβ2µ = 6.51 mL/min) and a marked increase in CAlb (maximal on day 18, CAlb = 248.6 µL/min). During this period, CCr declined from 88 mL/min to 8.0 mL/min on day 15, then remained relatively unchanged at about 25 mL/min. Similar clearance patterns in association with reduced renal function were observed also in the other four patients: B.O., B.R., N.O., and C.U. As shown in Table 1, the maximum CAlb for this group of patients ranged from 26.5 to 248.6 µL/min. When corrected for variations in glomerular filtration, the values ranged from 0.83 to 12.69 µL of CAlb per milliliter of CCr, or 83–1269 times the mean clearance ratio for normal individuals. The maximum clearance of β2µ ranged from 0.60 to 4.22 mL/min. When corrected for variations in glomerular filtration, the values ranged from 0.021 to 0.186 mL of Cβ2µ per milliliter of CCr, or 18 to 553 times the mean clearance ratio for normal subjects. In all five patients, progressive loss of renal function, i.e., decreased CCr, correlated well with increasing clearances of both albumin and β2µ. The range of CCr values at maximum protein clearances was 5.1–31.0 mL/min.

Group II. The patients in Group II showed episodes of extremely elevated β2µ clearance accompanied by rather moderate increases in CAlb. The clearance curve (Figure 2) of patient E.R. exemplifies the clearance pattern characteristic of
this group. During the entire course his creatinine clearance, which was 56 mL/min on day 1, steadily increased and remained relatively unchanged after attaining 92 mL/min on day 8. While albumin clearance was extremely stable, fluctuating barely between 1.6–2.2 μL/min, two episodes of increased β2μ clearance took place, the first peaking on day 4 (maximal Cβ2μ = 15.65 mL/min) and the second forming a major peak on day 15 (maximal Cβ2μ = 22.10 mL/min). Similar clearance patterns were observed with the four other patients in this group. As shown in Table 1, the maximum clearances of albumin ranged from 2.00 to 4.91 μL/min. When corrected for changes in glomerular filtration, the values ranged from 21 to 65 mL of Cαβ per milliliter of Ccr, or 21–65 times the clearance ratio of normal subjects. On the other hand, maximum Cβ2μ ranged from 8.13 to 22.10 mL/min. Corrected for changes in glomerular filtration, the values ranged from 111 to 231 μL of Cβ2μ per milliliter of Ccr, a 93- to 193-fold increase as compared with normal subjects.

Patient M.O. This recipient of an allograft from a living relative showed no clinical evidence of rejection, and was the only individual whose protein clearance pattern was completely unremarkable (Figure 3). Renal function was stable within two days after the transplant, and both Cαβ and Cβ2μ decreased from 27.86 to 2.11 μL/min (day 3) and 77.37 to 0.82 mL/min (day 2), respectively. After correcting for variation in glomerular filtration, these clearances are equivalent to 23 nL of albumin and 10 μL of β2μ cleared per milliliter of creatinine, or 23- and ninefold the normal clearances of albumin and β2μ, respectively.

Serum β2-Microglobulin and Serum Creatinine

Group I. Typical variations in Sα and Sβ2μ for the Group I patients are shown in the protein curve for patient C.O. (Figure 1). While Sα rose initially on day 14 to a peak value of 38 mg/L on day 16, an initial increase in Sβ2μ of 0.5 mg/L was observed on day 12, two days before the increase in Sα. At this point, a divergence was noted: Sβ2μ rose much more rapidly, reaching its peak value of 9.0 mg/L on day 15, coincidental
with the maximal S\textsubscript{Cr}. When a between-day S\textsubscript{BPM} increase equal to or greater than 0.5 mg/L was used as an indication of allograft rejection, this characteristic pattern became evident also in three other Group I patients who had demonstrated acute allograft rejection during their clinical course. With patient B.R. (Figure 4), a significant S\textsubscript{BPM} increase preceded both the initial increase in S\textsubscript{Cr} by two days and the decline in C\textsubscript{cr} by one day. Similarly, a significant increase in S\textsubscript{BPM} preceded both the initial S\textsubscript{Cr} elevation and the C\textsubscript{cr} fall by four days and three days in patient N.O. (Figure 5), and by five days and four days in patient C.U. (Figure 6).

Group II. Closely parallel changes in S\textsubscript{BPM} and S\textsubscript{Cr} were observed for the five patients classified in this group. An example is shown in Figure 2. During the first week after the transplant, deterioration in renal function was accompanied by concurrent increase in the concentrations of both these substances. As glomerular filtration improved, S\textsubscript{Cr} approached normal values and S\textsubscript{BPM} was correspondingly decreased.

Patient M.O., who showed no evidence of rejection and no increase in clearances of either albumin or β\textsubscript{2}μ, was the only patient who attained both normal S\textsubscript{Cr} and S\textsubscript{BPM} 10 days after the transplant (Figure 3).

**Discussion**

This study demonstrates for the first time sequential changes of albumin and β\textsubscript{2}μ clearances in renal allograft recipients one to five weeks post-transplantation.

As shown in Table 1, all five patients classified in Group I showed episodes of markedly increased C\textsubscript{ALB} (25.6–248.6 µL/min) and moderately increased C\textsubscript{BPM} (0.60–4.22 mL/min) concurrent with decreased glomerular filtration rate as evidenced by a decreased C\textsubscript{cr}. Each of these episodes was closely correlated with the clinical impression of acute allograft rejection.

On the other hand, all five patients categorized in Group II with no evidence of clinical rejection demonstrated episodes of grossly elevated C\textsubscript{BPM} (8.13–22.1 mL/min) with minimally increased but stable C\textsubscript{ALB} (2.0–4.9 µL/min) and normal renal function. Our findings of extremely increased C\textsubscript{ALB} in association with acute rejection are consistent with the observation of Laterre et al. (15) of early glomerular injury after transplantation, and serve as further evidence for the clinical value of albumin assay as an indicator of basement membrane integrity. The episodes of markedly increased C\textsubscript{BPM} accompanied by minimally increased C\textsubscript{ALB} under conditions of normal glomerular filtration rate are almost certainly the result of a proximal tubular defect, a conclusion based on both our present knowledge of β\textsubscript{2}μ metabolism and similar findings reported by other investigators (2, 16). The smaller increase in C\textsubscript{BPM} in the patients demonstrating clinical rejection (Group I) would appear to be explained largely by a decrease in the filtered load of β\textsubscript{2}μ in patients with predominantly glomerular injury. This would also explain the relatively higher concentration of S\textsubscript{BPM} in the patients demonstrating acute allograft rejection.

The concentrations of β\textsubscript{2}μ in serum of patients with either glomerular or tubular proteinuria, or both, invariably exceed the reference interval. The closely parallel changes of S\textsubscript{BPM} and S\textsubscript{Cr} in patients with primarily tubular injury are entirely predictable when the renal function is stable, because the sieving coefficient is close to unity. A similar relationship between these two substances has also been observed by others (7–10). Linear correlations between S\textsubscript{BPM} and S\textsubscript{Cr}, on a logarithmic scale, were reported separately by Vincent et al. (7), Brauman et al. (8), and Evrin et al. (9). In our study, this parallel relationship was not as close in patients whose urinary protein patterns demonstrate primarily glomerular injury. In this group, S\textsubscript{BPM} and S\textsubscript{Cr} diverge somewhat because the increase in S\textsubscript{BPM} exceeded that in S\textsubscript{Cr}. Furthermore, the increase in β\textsubscript{2}μ in
the serum of these patients preceded that of Scr by one to five days and the decline in Ccr by one to four days (see Figures 4–6). This phenomenon, observed also by Vincent et al. (7), was attributed by them to severe multiple infections of bacterial, fungal, or viral origin. However, no example of infection was demonstrated in any pre-rejection period in the patients that we studied. It is predictable that deteriorating glomerular filtration alone should result in a simultaneous increase in Scr and Sgrp if the sieving coefficient of both substances remains unchanged and renal functional loss is determined by a decrease in glomerular filtration rate alone. A reduction in sieving coefficient to account for the disparity between β2µ and Scr is unlikely, because increased glomerular permeability evidenced by a high-molecular-mass proteinuria frequently accompanies acute rejection.

Thus an increase in Sgrp that precedes an increase of Scr is most reasonably interpreted as a result of extrarenal events, notably an increase in synthesis. Stimulation of the lymphoid mass by infection and immunization has been shown experimentally to increase the elaboration of β2µ by plasma cell lymphocytes. In each instance of a premature rise in serum β2µ, renal allograft rejection was subsequently observed and is attributed to the effects of alloimmunization.

Our data demonstrate conclusively that the determination of daily clearances of both albumin and β2µ in patients recovering from renal allograft transplantation is an effective means of assessing renal damage—i.e., differentiating between glomerular injury and renal tubular dysfunction—and that data on the concentration of β2µ in serum can be a valuable predictor of acute renal allograft rejection. Our study thus supports the use of differential analysis of urinary proteins in monitoring kidney function of renal transplant recipients. Such analysis of urinary proteins merits further investigation to determine its applicability in the diagnosis and prognosis of renal diseases of other etiologies.

We thank Ms. Mary Ann Longely for her valuable technical assistance.

References