

Solid phase radioimmunoassay for β_2 -microglobulin; a sensitive index for renal allograft evaluation

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β_2 -Microglobulin (β_2 M), a low molecular weight protein of unknown function, was first isolated from the urine of patients with renal tubular disorders.¹ It has been shown to be identical to a polypeptide noncovalently bound to the larger HLA molecule on the cell surface of nucleated cells and to have a close structural homology with the C_H3 region of the IgG heavy chain.^{2,3} This protein has been shown to be present on platelets, mononuclear cells and polynuclear white blood cells, but not on mature erythrocytes.⁴ It has also been found in human serum, cerebrospinal fluid, saliva, and colostrum.⁵ In serum it is present as a free monomer, which is filtered freely through the glomerular membrane, almost completely reabsorbed in the proximal renal tubules, and catabolized so that only relatively small quantities are excreted in the urine.^{6,7} Its diagnostic use in several types of renal disease has been reported.⁶⁻⁹

We report our experience with radioimmunoassay for β_2 M and its use in monitoring human renal allograft function. Because serum creatinine is commonly used for this purpose, we measured β_2 M and serum creatinine serially in eight renal allograft patients in an attempt to determine the relative sensitivity of these two tests of allograft function.

Materials and methods

Radioimmunoassay for β_2 M. The reagents for the assay were purchased from Pharmacia, Piscataway, New Jersey. The antibody was bound to Sephadex as the solid phase. In a typical radioimmunoassay protocol, 100 μ l of each standard 3 to 96 μ g/l was added to the appropriately marked tubes. Also, 100 μ l of serum specimen already diluted to 1:200 was added. The 100 μ l of antibody Sephadex complex was added while continuously stirring it. This was followed with 100 μ l of 125 I solution approximately 30,000 to 60,000 cpm. The tubes were tightly capped and rotated for 3 hours at room temperature. Then 2 ml of isotonic saline was added to each tube and centrifuged at 2000 rpm for 5 minutes. The supernatant was aspirated and the pellet was counted. The interassay variation obtained with this assay was well within 5% to 10% at three different concentrations. The analytical recovery experiments showed an average recovery of 93%, 100%, and 98%, respectively, at three different concentrations of β_2 M added to normal human plasma. In 45 normal subjects, the mean serum β_2 M was 1.44 ± 0.35 μ g/ml with a range of 0.74 to 2.1 μ g/ml. There were no significant differences in the levels obtained at different decades of age (up to age 50) or between the male and female groups.

Serum creatinine. This was measured by the Technicon Autoanalyzer System and the normal range with this procedure was 0.7 to 1.4 mg/dl.

Patients. To evaluate the usefulness of serial determinations of serum β_2 M in renal transplant patients, serum β_2 M was measured before and at intervals after the renal transplant in eight patients. At the same time a serum creatinine level was also obtained. The *Table* lists the age, sex, and status of the transplant in these patients. Of eight patients, two had irreversible rejection and six had a successful renal transplant, one from an HLA-identical sibling donor. All the patients had been treated with 1 g of methylprednisolone in divided doses and azathioprine at the time of transplantation; the methylprednisolone was given again at the time of graft rejection. All patients were maintained on a tapering dose of prednisone and up to 1.5 mg/kg of azathioprine according to clinical conditions.

Results

Case 1. *Figure 1* illustrates the β_2 M and creatinine levels in a 26-year-old woman with end-stage renal failure secondary to diabetes mellitus who received an HLA-identical kidney transplant from her brother which functioned well. Her pretransplant β_2 M and serum creatinine were 17 μ g/ml and 6 mg/dl, respectively. Within a week after transplantation both had decreased to

Table. Clinical characteristics of transplant patients

Case no.	Age/sex	Renal disease	Donor	Transplant status*
1	26 F	Diabetic nephropathy	Brother	Functioning
2	59 M	Chronic glomerulonephritis	Son	Functioning
3	35 F	Pyelonephritis	Cadaver	Functioning
4	30 M	Pyelonephritis	Cadaver	Functioning
5	47 M	Pyelonephritis	Cadaver	Functioning
6	34 F	Chronic renal failure (uncertain etiology)	Cadaver	Functioning
7	37 F	Diabetic nephropathy	Cadaver	Rejected
8	38 M	Chronic glomerulonephritis	Cadaver	Rejected

* Indicates one-year posttransplant status.

normal levels of 2.1 $\mu\text{g/ml}$ and 1.1 mg/dl , respectively. She was discharged but required readmission for a urinary tract infection and rejection. At this time, her serum $\beta_2\text{M}$ level was elevated (3.8 $\mu\text{g/ml}$) and the serum creatinine was normal (1.2 mg/dl). Over the next 3 days the serum creatinine progressively rose to 2.0 mg/dl . She was treated with additional prednisone and antibiotics with a subsequent decrease in $\beta_2\text{M}$ and creatinine to 2.8 $\mu\text{g/ml}$ and 0.8 mg/dl , respectively.

Case 2 (Fig. 1). A 59-year-old man who had kidney failure due to glomerulonephritis received a kidney transplant from his son. The high $\beta_2\text{M}$ (60 $\mu\text{g/ml}$) and creatinine (17.5 mg/dl) dropped posttransplant, and at the time of discharge the patient had $\beta_2\text{M}$ of 3.5 $\mu\text{g/ml}$ and creatinine of 1.3 mg/dl . The patient was readmitted later for rejection symptoms and was found to have a further increase in $\beta_2\text{M}$ (8.7 $\mu\text{g/ml}$) and serum creatinine (2.8 mg/dl). Treatment of the rejection was followed by a subsequent decrease in both $\beta_2\text{M}$ and creatinine.

Case 3 (Fig. 1). A cadaver kidney transplant was performed in a 35-year-old woman with kidney failure due to pyelonephritis. She had a benign postoperative course, and

2 weeks posttransplant, her $\beta_2\text{M}$ was 1.9 $\mu\text{g/ml}$ and creatinine was 1.3 mg/dl . A subsequent rise in $\beta_2\text{M}$ to 2.8 $\mu\text{g/ml}$ was observed and was followed by a rise in serum creatinine from 1.2 to 2.0 mg/dl and clinical rejection. She was treated with 2 g of methylprednisolone intravenously, which resulted in a drop of $\beta_2\text{M}$ to 2.1 $\mu\text{g/ml}$ and creatinine to 1.4 mg/dl .

Case 4 (Fig. 1). A 30-year-old man with a long history of chronic renal failure due to pyelonephritis received a cadaveric kidney transplant. Pretransplant $\beta_2\text{M}$ and serum creatinine levels (44 $\mu\text{g/ml}$ and 14 mg/dl , respectively) were very high. One week posttransplant, his serum creatinine returned to normal (1.5 mg/dl) but the $\beta_2\text{M}$ was still above normal limits (3.1 $\mu\text{g/ml}$). A further increase in $\beta_2\text{M}$ was followed by a mild clinical rejection with a rise in serum creatinine to 2.6 mg/dl in the second week posttransplant requiring treatment with 2 g of methylprednisolone. Rejection recurred in the third and also fourth weeks in association with sustained elevation of $\beta_2\text{M}$ between 4 and 5 $\mu\text{g/ml}$. These were reversed by appropriate therapy.

Case 5 (Fig. 2). A 47-year-old man re-

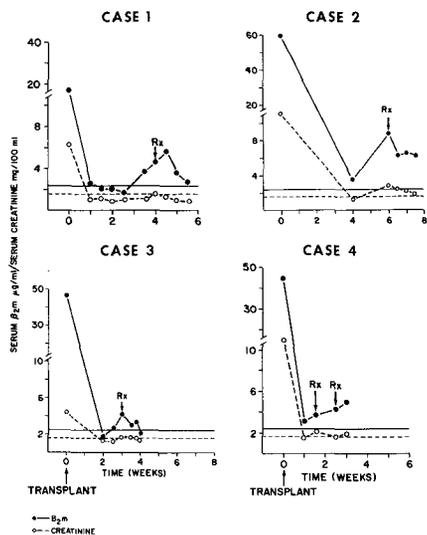


Fig. 1. Serial changes in serum $\beta_2\text{M}$ and serum creatinine in renal allograft patients (cases 1 through 4). Rx indicates the treatment for rejection with methylprednisolone.

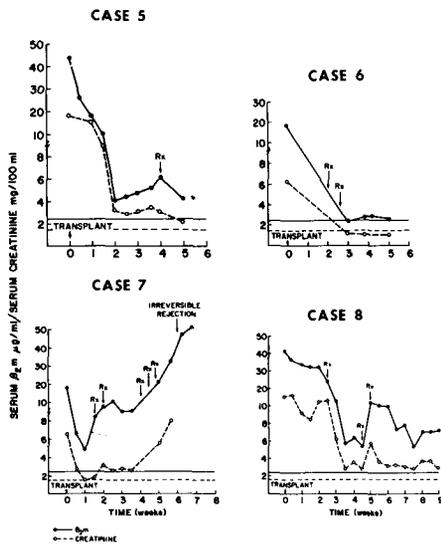


Fig. 2. Serial changes in serum $\beta_2\text{M}$ and serum creatinine in renal allograft patients (cases 5 through 8). Rx indicates the treatment for rejection with methylprednisolone.

ceived a cadaveric kidney transplant that functioned after a week of acute tubular necrosis. His $\beta_2\text{M}$ and serum creatinine decreased and stabilized in the second postoperative week at 4.1 $\mu\text{g}/\text{ml}$ and 3.2 mg/dl , respectively. Because of leukopenia, azathioprine was discontinued for 10 days. This led to a rise in $\beta_2\text{M}$ to 4.8 $\mu\text{g}/\text{ml}$ followed by a rise in serum creatinine from 2.7 to 3.8 mg/dl . Again, treatment with 2 g of methylprednisolone caused a significant drop in $\beta_2\text{M}$ from a peak of 6.0 to 4.2 $\mu\text{g}/\text{ml}$ and creatinine to 2.2 mg/dl . The patient is doing well on maintenance doses of immunosuppressive therapy.

Case 6 (Fig. 2). A 34-year-old woman with end-stage renal disease of uncertain etiology did not have nephrectomy prior to transplantation. No tissue diagnosis was made for her original disease. She received a cadaveric kidney transplant, which functioned immediately, but two urinary tract infections developed and a biopsy specimen showed evidence for cellular rejection. She was treated with 4 g of methylprednisolone. This led to a decrease in serum $\beta_2\text{M}$ to 2.5 $\mu\text{g}/\text{ml}$ and creatinine to 1.1 mg/dl within 4 weeks after transplantation.

Case 7 (Fig. 2). This 37-year-old woman with chronic renal failure secondary to diabetic nephropathy received a cadaveric kidney transplant. Her $\beta_2\text{M}$ remained elevated (4.8 $\mu\text{g}/\text{ml}$) one week posttransplant, though the creatinine had fallen to 1.5 mg/dl . A further rise in $\beta_2\text{M}$ to 8.4 $\mu\text{g}/\text{ml}$ was followed by elevation of the serum creatinine to 3.2 mg/dl . Despite improvement in the serum creatinine levels to 2.2 mg/dl after rejection therapy, there was only a minimal decrease in $\beta_2\text{M}$. Subsequently, there was a continuous rise in $\beta_2\text{M}$ and irreversible rejection unresponsive to a total of 6 g of methylprednisolone.

Case 8 (Fig. 2). A 39-year-old man with end-stage renal disease due to nephrosclerosis received a cadaveric renal transplant that had a prolonged period of acute tubular necrosis and cellular rejection within the first several weeks after transplantation. While on hemodialysis the patient had an extremely slow rate of decrease in $\beta_2\text{M}$ during

the first 2 weeks posttransplant; the levels for $\beta_2\text{M}$ and creatinine dropped to 32 $\mu\text{g}/\text{ml}$ and 12.4 mg/dl , respectively, from pretransplant levels of 42 $\mu\text{g}/\text{ml}$ and 15.8 mg/dl . As the diuretic phase began in the third week and with treatment for rejection, the $\beta_2\text{M}$ and creatinine decreased to 5.7 $\mu\text{g}/\text{ml}$ and 2.9 mg/dl , respectively. A subsequent rise in serum creatinine was followed by further treatment for rejection. In the eighth week posttransplant, his $\beta_2\text{M}$ and creatinine were stabilized but were elevated (7.3 $\mu\text{g}/\text{ml}$ and 2.8 mg/dl , respectively). Although 3 months later the serum creatinine was as low as 2.1 mg/dl , the $\beta_2\text{M}$ at that time was 8.6 $\mu\text{g}/\text{ml}$. The allograft eventually underwent irreversible rejection 8 months after transplantation.

Discussion

Elevated $\beta_2\text{M}$ levels have been reported in end-stage kidney failure,⁷ and a direct correlation between $\beta_2\text{M}$ and serum creatinine has been observed pretransplant.^{6,7} In the present study, very high pretransplant $\beta_2\text{M}$ levels were associated with high levels of serum creatinine. Studies utilizing serial determinations of serum $\beta_2\text{M}$ measured by a radioimmunoassay method^{8,9} have demonstrated a significant decrease in serum $\beta_2\text{M}$ after transplant even in the anuric phase. The decrease that occurs with viable cadaver allografts having oliguric tubular necrosis indicates the catabolic role of transplanted kidneys on $\beta_2\text{M}$. However, the serial evaluation of both $\beta_2\text{M}$ and serum creatinine after transplantation has not been described. In our experience, increase in $\beta_2\text{M}$ often preceded the increase in serum creatinine and clinical evidence of graft rejection. A considerable decrease in $\beta_2\text{M}$ and serum creatinine was observed following treatment for rejection in all cases except two in which irreversible rejection developed. In some cases $\beta_2\text{M}$ never reached the normal range, although the serum creatinine was near or

less than 2.0 mg/dl. All these patients showed a further rise in β_2 M followed by a rise in serum creatinine that necessitated treatment for rejection. Sustained elevations of β_2 M greater than 6 μ g/ml even in the presence of near normal serum creatinine levels, in retrospect, was a predictor of chronic progressive rejection. Also, patients with irreversible rejection showed a relatively slow rate of decline in serum β_2 M. These features indicate the significance of β_2 M in monitoring renal allograft function and suggest the increased sensitivity that β_2 M may have over serum creatinine measurements.

Summary

A radioimmunoassay for serum β_2 M was evaluated for its clinical usefulness in monitoring renal transplantation patients. In eight patients, β_2 M was measured serially before and after transplantation and was correlated with serum creatinine. The pretransplant levels of β_2 M were high (mean 36.6 ± 16.5 μ g/ml), but decreased after successful renal transplantation at variable rates. In three patients, β_2 M decreased to a normal range and subsequently rose. This increase preceded the increase in serum creatinine and clinical evidence of acute rejection. In five patients posttransplant, β_2 M was above normal despite a normal serum creatinine level in three and showed a progressive increase, which was followed by a later rise in

serum creatinine. A sustained decrease in serum β_2 M was observed after rejection therapy with methylprednisolone in all these patients except two in whom irreversible rejection developed.

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