\( \beta_2 \)-Microglobulin Determined by Radioimmunoassay with Monoclonal Antibody

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In this sensitive radioimmunoassay for \( \beta_2 \)-microglobulin (\( \beta_2 \)-M) involving a commercially available monoclonal antibody, we used a second monoclonal antibody, produced in our laboratory, for affinity-chromatographic puriﬁcation of \( \beta_2 \)-M. Both monoclonal antibodies bound to all of the charge isomers of \( \beta_2 \)-M identiﬁed by two-dimen- sional gel electrophoresis. Polyethylene glycol was used to separate the phases after incubation for 3 h at room temperature. Sensitivity was 0.25 ng of \( \beta_2 \)-M. Within-assay CVs were <5.4%, between-assay CVs <7.3%. Analytical recovery was 95–102%. The reference interval was 1.2–2.2 mg/L for 49 healthy subjects. The correlation coefﬁcient for comparison with a poly- clonal antibody assay was 0.997 for 44 patients. In a study correlating serum \( \beta_2 \)-M and creatinine concentrations with glomerular ﬁltration rate for 50 patients, correlation coefﬁcients for log-log transformed data were −0.94 and −0.95, respectively. Concentrations of \( \beta_2 \)-M were increased in serum of patients with impaired renal function and also in patients with normal renal function who had disorders involving the immunologic response. We found no clear-cut advantage of measuring serum \( \beta_2 \)-M over serum creatinine in the estimation of glomerular ﬁltration rate.

Additional Keyphrases: electrophoresis, two-dimensional • chromatography, afﬁnity • reference interval • glomerular ﬁltration rate • renal function

Since the biochemical characterization of \( \beta_2 \)-microglobulin (\( \beta_2 \)-M) in 1968 by Berggård and Bearn (1), this low-molecu- lar-mass protein has received considerable attention from basic scientists studying its biological role and from clinical scientists searching for clinical utility in its measurement (2–8).

Briefly, \( \beta_2 \)-M is a 11 800-dalton protein that is found on the surface of all cells except erythrocytes and the trophoblastic layer of the placenta (2). It is the smaller, invariant polypeptide of the HLA-A,B,C antigens, and it may also be bound to other proteins such as the histocompatibility Y-chromosome antigen and tumor antigens (2). Of the estimated 100 mg of \( \beta_2 \)-M per day shed into the plasma, about half originates from lymphocytes. The biological half-life of \( \beta_2 \)-M in plasma is about 40 min (3). It is eliminated from the body primarily by glomerular ﬁltration followed by tubular reabsorption and degradation to amino acids. Measurement of \( \beta_2 \)-M in plasma or serum has been suggested in the monitoring of malignancy, autoimmune disorders, and inﬂammatory diseases, and in the evaluation of renal function (4).

Many methods have been devised for measuring \( \beta_2 \)-M in serum (9–13), including radioimmunoassay (RIA) (12, 14–17).

These methods are adequately sensitive for use with serum, but some are too insensitive for use with urine. RIA generally is sensitive enough for use with all biological ﬂuids and is suitable for laboratories that already are handling radioiso- topes. However, each technique has disadvantages because of expense, precision, lack of commercial availability of anti- body, lot-to-lot differences in antibody, or long incubation periods.

Monoclonal antibodies directed against a number of anti- gens, including \( \beta_2 \)-M, have recently become commercially available, and there are several potential advantages to their use in RIA, including improved precision and interlaboratory comparability of results.

Using commercially available monoclonal antibody against human \( \beta_2 \)-M, and adapting the polyethylene glycol precipita- tion method of Plesner et al. (15), we developed an RIA for \( \beta_2 \)-M, which we describe here. To validate our monoclonal RIA, we established a reference interval, compared results with results from the widely used polyclonal “Phadebas \( \beta_2 \)-Micro Test” kit RIA (Pharmacia Diagnostics, Piscataway, NJ 08854), and compared plasma \( \beta_2 \)-M with plasma creatinine and iothalamate clearance in 50 patients with various renal disorders.

Materials and Methods

Antigen Preparation

Isolation of \( \beta_2 \)-M. Antigen used for labeling was from the urine of patients who were undergoing renal-transplant re- jection; it was puriﬁed by the following modiﬁcation of the general method of Vincent and Revillard (12). Urine was concentrated on a P-10 ultraﬁltration membrane (Amicon Corp., Lexington, MA 02173); dialyzed vs distilled water in Spectropore 1 dialysis tubing (Spectrum Medical Industries, Los Angeles, CA 90054); and chromatographed through Sephadex G-100 equilibrated with a solution containing, per liter, 5 mmol of phosphate (pH 7.4) and 150 mmol of NaCl. Appropriate fractions were pooled, dialyzed vs Tris HCl (10 mmol/L, pH 7.8), and passed twice through a column of DEAE Sephadex A-50 equilibrated in the same buffer. The column was eluted with a 0–0.2 mol/L gradient of NaCl in the same buffer. Fractions were monitored at 280 nm and those corre- sponding to peaks were pooled, dialyzed vs a solution containing 10 mmol of phosphate (pH 7.4) and 150 mmol of NaCl per liter, and concentrated to give a 1 g/L solution of puriﬁed \( \beta_2 \)-M.

\(^{125}\)I-labeled \( \beta_2 \)-M. Purified \( \beta_2 \)-M was routinely iodinated by a modiﬁcation of the Chloramine T method of Hunter and Greenwood (18). We used 2.5 µg of \( \beta_2 \)-M, 300–500 µCi of carrier-free Na\(^{125}\)I (Amersham, Arlington Heights, IL 60005), 5 µg of Chloramine T, a reaction time of 30 s, and a total vol- ume of approximately 40 µL. The reaction was stopped with 5 µg of sodium metabisulphite. Free and bound \(^{125}\)I were sepa- rated by gel chromatography on a 0.5 × 15 cm disposable column packed with Sephadex G-10 equilibrated with a so- lution containing 10 mmol of phosphate (pH 7.4), 150 mmol of NaCl, and 1 g of bovine serum albumin per liter. Typically 60 to 70% of the \(^{125}\)I was present in the protein-bound frac- tions. However, precipitation of these fractions with a 100 g/L

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solution of trichloroacetic acid indicated that only 70 to 75% of the $^{125}I$ was actually protein bound, giving an estimated specific activity of about 70 Ci/g. The labeled protein was further purified by gel chromatography on a 0.9 x 30 cm column packed with Sephadex G-50, eluted with the same elution buffer. Peak fractions were pooled; precipitation of the pooled fractions with trichloroacetic acid indicated that 75 to 80% of the $^{125}I$ was protein bound. Typically, we pooled 100–150 μCi of labeled protein, providing sufficient label for 2000 to 3000 assay tubes. Such a pool was stable for at least 30 days at −20 °C.

**Antibody Preparation**

Monoclonal antibody production and affinity chromatography. BALB/c mice were immunized by injecting intraperitoneally 0.25 mL of an emulsion of 70 μg of purified $\beta_2$-M in complete Freund’s adjuvant. A booster injection of 70 μg of $\beta_2$-M in saline was given every two weeks. We tested sera from these immunized animals for antibody production by using an RIA similar to the one described below.

Three days after the first, second, and fourth booster injections, one mouse spleen was removed, and a suspension of single cells was prepared for lymphocyte fusion (19). These cells were fused to NS-1 mouse myeloma cells and aliquoted for initial selection of hybrids as previously described (20). The culture fluid was screened for specific antibody production. None was found in hybrids from the first two mice. In hybrids from the third mouse, fluid from one well was positive. The cells were cloned by limiting dilution, and plates showing clonal growth in less than 5% of the wells were assayed for anti-$\beta_2$-M. Positive clones were expanded in culture and grown as ascitic tumors in pristane oil-primed BALB/c mice as previously described (20). Nine to twelve days later, 4 to 7 mL of ascitic fluid was obtained from each tumor-bearing animal. The ascitic fluid protein, examined by two-dimensional gel electrophoresis, was estimated from relative spot density to contain about 35 to 50% of the total protein as monoclonal antibody.

Ascites fluid was added to an equal volume of saturated ammonium sulfate and centrifuged to obtain the globulin fraction. This fraction was dissolved in distilled water and dialyzed extensively against sodium citrate buffer (0.2 mol/L, pH 6.4). Approximately 5.6 mg of globulin was coupled to 1 g of CNBr-activated Sepharose (Sigma Chemical Co., St. Louis, MO 63178) by the method of Cuatrecasas and Anfinsen (21), with 92% coupling efficiency. This column was washed extensively with two solutions: first with a solution containing 10 mmol of phosphate (pH 7.4) and 150 mmol of NaCl per liter (solution 1), then with a similar solution also containing 8 mol of urea per liter (solution 2), and finally with solution 1 again. Purified $\beta_2$-M was applied to this column. The bound material was eluted with solution 2, dialyzed extensively against solution 1, and analyzed by ultraviolet spectrophotometry (we used a Cary 118; Varian Instruments, Palo Alto, CA 94303). A scan of absorbance from 350 to 250 nm revealed a symmetrical protein peak at 278 nm. We determined the concentration of $\beta_2$-M by using a molar absorptivity value of 19 850 L mol$^{-1}$ cm$^{-1}$ (t) and calibrated the assay standards with this solution.

**Assay antibody.** Monoclonal anti-human $\beta_2$-M was purchased from Becton Dickinson FACS Systems, Sunnyvale, CA 94086. The antibody-producing clone, L385, was derived from hybridization of mouse NSI/1-Ab4 cells with spleen cells from a BALB/c mouse immunized with the human lymphoblastoid B cell line, RPMI 8866.

**Procedures**

Two-dimensional gel electrophoresis. Two-dimensional gel electrophoresis was performed by a modification of the ISO-DALT system of Anderson and Anderson (22). Briefly, resolution in the first dimension involved isoelectric focusing in 35 g/L polyacrylamide gels equilibrated in a solution containing sodium dodecyl sulfate and fused to the top of 10–18% exponential gradient sodium dodecyl sulfate slab gels. The pH gradient, when corrected for the presence of 8 mol of urea per liter, extended from pH 3.85 to 10. After electrophoresis, these gels were either stained with Coomassie Blue or dried and used for autoradiography at −70 °C with “Lithening” intensifying screens (Du Pont, Wilmington, DE 19888).

**Assay procedure.** All standards, controls, unknowns, label, and antibody were diluted in pH 7.00 assay buffer containing, per liter, 100 mmol of phosphate, 50 mmol of NaCl, 1 g of bovine serum albumin, and 0.5 g of Na$_3$P$_2$. For standards, we diluted purified $\beta_2$-M to give concentrations of 1.25, 2.5, 5, 10, 20, and 40 ng/mL. Normal serum was diluted 256-fold. Puriﬁed $^{125}$I-labeled $\beta_2$-M was diluted to 40 000–60 000 cpm/0.1 mL (approximately 4 ng/mL). Monoclonal anti-human $\beta_2$-M antibody was diluted 4000-fold.

Solutions were added to duplicate 12 x 75 mm disposable glass assay tubes in the following order: 0.1 mL of diluted standard, control, or unknown; 0.1 mL of diluted $^{125}$I-labeled $\beta_2$-M; and 0.1 mL of diluted antibody. Assay volume was therefore 0.3 mL, and final antibody dilution was 12 000-fold. For each standard curve, we included nonspecific-binding tubes containing 0.1 mL of diluted label and 0.2 mL of assay buffer. To each tube, after 3 h at room temperature, we added 30 μL of bovine serum, mixed the contents gently, added 1.0 mL of a 180 g/L solution of polyethylene glycol (Mr, 6000), and vortex-mixed vigorously for 10 s. We then centrifuged the assay tubes (3000 x g, 30 min, refrigerated centrifuge), aspirated and discarded the supernatant fluids, and counted the radioactivity of the precipitates (bound fractions), along with total-count tubes, for 1 min, in a gamma counter.

After subtracting nonspecific binding, we subjected counts for the standards to logit transformation: logit ($B/B_0$) = ln($B/B_0$)/(1 – $B/B_0$). Sample values were calculated from the slope and intercept of the logit–ln(dose) standard curve after it had been adjusted by weighted, least-squares linear regression. Results for a sample were accepted if the results for control serum were acceptable, if 0.20 ≤ sample $B/B_0$ ≤ 0.80, and if the difference between duplicate sample results was <15%. The 50% intercept and percentage nonspecific binding were also calculated, for quality control.

**Specimens.** Blood was collected from 49 apparently healthy subjects (28 men, 21 women, ages 19–59) whose serum creatinine concentration was less than 13 mg/L. Serum was stored frozen at −20 °C until assayed. Blood and timed urine specimens were also collected from 50 patients who were undergoing a 2-h isohalamic clearance test for renal evaluation. Plasma and urine were stored at −20 °C until assayed. Plasma creatinine concentrations were determined with an AutoAnalyzer (Technicon Instrument Corp., Tarrytown, NY 10591), with the Jaffé reaction.

**Results**

Antigen and label purity. $\beta_2$-M prepared by gel filtration and anion-exchange chromatography was examined for purity by two-dimensional gel electrophoresis. Five discrete polypeptides having similar molecular masses but different isoelectric points were identified by Coomassie Blue staining (Figure 1A). Each of the major proteins was present in the $\beta_2$-M prepared by further purification on the affinity column (Figure 1B) and were thus assumed to be $\beta_2$-M. Several proteins, comprising <5% of the conventionally purified $\beta_2$-M preparation and differing in molecular mass from the five $\beta_2$-M proteins, were not present in the affinity-column-puriﬁed $\beta_2$-M.

Similarly, two-dimensional gel electrophoresis and auto-
radiography of the $^{125}$I-labeled $\beta_2$-M demonstrated the same major forms of $\beta_2$-M and minor contaminants (Figure 1D). All major forms of $\beta_2$-M were also present in the antigen–antibody complexes precipitated with polyethylene glycol, whether the monoclonal assay antibody was in excess (Figure 1E) or the antigen was in excess (data not shown). No cross reaction with the minor contaminating proteins was detected.

$\beta_2$-M was also isolated by directly applying plasma from an anephric patient on hemodialysis to the affinity column (3 mL of plasma, 45 mg/L $\beta_2$-M, stored three days at 4°C). The same major forms of $\beta_2$-M were identified on two-dimensional gel electrophoresis with Coomassie Blue staining (Figure 1C).

Assay antibody characterization. With antibody in excess, 40 to 65% of the radioactivity of the $^{125}$I-labeled $\beta_2$-M was precipitated with polyethylene glycol. Parallel precipitation experiments with polyclonal anti-$\beta_2$-M (Dako, Santa Barbara, CA 93103) demonstrated only an additional 5 to 8% binding of tracer. When affinity-chromatography-purified $\beta_2$-M was labeled by the same Chloramine T technique, 99% of the $^{125}$I was precipitable with trichloroacetic acid, 91% was precipitated by the monoclonal assay antibody, and 93% was precipitated by the polyclonal antibody. In other experiments, cross reactivity of the assay antibody with IgM, IgG, and IgA under our assay conditions was not detectable (<0.003%).

Variation of assay parameters. We studied the RIA incubation conditions by varying assay parameters and measuring the radioactivity of precipitated $^{125}$I-labeled $\beta_2$-M. Under the specified assay conditions, equilibrium was reached in 3 h at room temperature. The optimal pH was 6.8–7.2. We found a 10% loss of binding in buffers of low ionic strength (10 mmol/L phosphate and <25 mmol/L NaCl). In the polyethylene glycol precipitation step, bovine serum in excess of 30 μL or polyethylene glycol in concentrations exceeding 180 g/L increased the nonspecific binding without an increase in net binding of label.

Standard curves. When the standard curve was plotted as logit response vs ln dose, it was linear for at least 0.25 to 2.2 ng of $\beta_2$-M per assay tube, corresponding to $B/B_0$ of 0.8 and 0.2, respectively (Figure 2). Because all sera were diluted at least 256-fold, the sensitivity of the assay for serum was at least 0.64 mg/L. No serum sample had less than 0.64 mg of $\beta_2$-M per liter. The logit-In transformed standard curve had the following mean (and standard deviation) quality-control parameters for 82 assays: $B_0/T$, 0.34 (0.03); nonspecific binding, 2.7% (0.7%); slope, −1.19 (0.09); 50% intercept, 0.72 ng (0.05 ng); and correlation coefficient, −0.998 (0.001).

Precision. Precision within-run and between-run was estimated by assaying both a normal and an abnormal serum pool 10 times per run on 10 consecutive runs. The normal serum pool (1.59 mg/L) had a mean within-run CV of 3.4% (range, 1.5% to 5.4%) and a between-run CV of 6.3%. The abnormal serum pool (5.5 mg/L) had a mean within-run CV of 2.7% (range, 1.4% to 3.5%) and a between-run CV of 5.2%. Precision was also estimated by routinely using a frozen serum control at three different dilutions to monitor the high (2.2 ng), middle (0.8 ng), and low (0.3 ng) portions of the standard curve. CVs were 4.7%, 5.2%, and 7.3%, respectively (1 determination per run, 45 runs).

Accuracy. Analytical recovery was evaluated by mixing solutions containing 50 to 1280 μg of $\beta_2$-M per liter with equal volumes of normal serum diluted to give a $\beta_2$-M content of 9.64 μg/L. Mean recovery for five experiments was 98% (range, 95–102%). Potentially interfering substances were added to normal serum to give the following final concentrations per liter (and assayed percentage of original concentration $\beta_2$-M): 0.3 g of bilirubin (99%), 1.0 g of creatinine (104%), 3.0 g of urea (105%), and 0.2 g of ascorbic acid (98%). In serum with hemolysis produced by mechanical disruption of the clot, the assayed concentration of $\beta_2$-M was 97% of that for the original serum. Serum obtained from three normal volunteers during postprandial lipemia had a concentration of $\beta_2$-M that was 86–94% of the value found during fasting. Serial twofold dilutions over similar concentration ranges in a single run gave, after logit-In transformation of the dose–response curves, slopes for purified $\beta_2$-M, normal serum, and an abnormal serum of −1.08, −1.12, and −1.08, respectively. After analysis of covariance, the hypothesis that the three lines had a common slope (null hypothesis) could not be rejected (0.25 > p > 0.10).

Reference interval. Results of $\beta_2$-M determinations in
serum of 49 apparently healthy volunteers are shown in Table 1. The mean was 1.53 mg/L, the range 1.10–2.19 mg/L. By Student's t-test, there was no significant difference between samples from men and women, but there was a significant difference between samples from persons up to age 30 and those older than 30 years.

Comparison of monoclonal and polyclonal assays for plasma. Plasma samples from 44 of the 50 patients undergoing renal evaluation were assayed for $\beta_2$-M by both the monoclonal and the Phadebas $\beta_2$-Micro Test methods. The correlation of results was excellent. Figure 3 shows a comparison of results from the 38 patients with plasma $\beta_2$-M concentrations <6 mg/L. The correlation coefficient was 0.986; the slope, 0.807; the intercept, 0.106; and standard error of the estimate, 0.184. When six additional pairs of results ranging from 6 to 27 mg/L were included, the correlation coefficient was 0.997; the slope, 0.804; and the intercept, 0.157.

Comparison of plasma $\beta_2$-M by monoclonal assay with corrected iothalamate clearance. Plasma specimens from the 50 patients undergoing renal evaluation by iothalamate clearance and Phadebas $\beta_2$-M concentration were assayed for $\beta_2$-M by monoclonal assay. On comparison of log$_{10}$ ($\beta_2$-M) and log$_{10}$ (corrected iothalamate clearance), there was a good inverse correlation of results (Figure 4). The correlation coefficient was -0.938; the slope, -0.82; the intercept, 1.90; and the standard error of the estimate, 0.11. Twenty-two of these 50 patients had been transplanted. These conditions, $\beta_2$-M may be increased in plasma without a decrease of glomerular filtration rate. When these 22 patients were excluded, the correlation coefficient was -0.971; the slope, -0.86; the intercept, 1.95; and the standard error of the estimate, 0.09. The upper limit of normal for plasma $\beta_2$-M selected for Figure 4 was 2.2 mg/L, although the reference interval varies with age (23, 24). The lower limit of normal for iothalamate clearance also varies with age; 80 mL/min per 1.73 m$^2$ body surface was selected for Figure 4.

Comparison of plasma creatinine with corrected iothalamate clearance. Figure 5 shows a comparison of log$_{10}$ (plasma creatinine) and log$_{10}$ (corrected iothalamate clearance) for the same 50 patients. The correlation coefficient was -0.946; the slope, -0.73; the intercept, 2.38; and the standard error of the estimate, 0.09. It is well known that the plasma creatinine concentration tends to be less in women than in men because

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**Table 1. Serum $\beta_2$-Microglobulin Concentrations in Normal Subjects**

<table>
<thead>
<tr>
<th>No.</th>
<th>Age yr</th>
<th>Serum $\beta_2$-M mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>------------------------</td>
</tr>
<tr>
<td>All subjects</td>
<td>49</td>
<td>33.1</td>
</tr>
<tr>
<td>Women</td>
<td>21</td>
<td>34.0</td>
</tr>
<tr>
<td>Men</td>
<td>28</td>
<td>32.4</td>
</tr>
<tr>
<td>$\leq$ age 30</td>
<td>25</td>
<td>25.6</td>
</tr>
<tr>
<td>&gt; age 30</td>
<td>24</td>
<td>40.9</td>
</tr>
</tbody>
</table>

$^a$ No significant sex-related difference (p > 0.5). $^b$ Significant age-related difference (p < 0.001).

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**Fig. 3. Comparison of plasma $\beta_2$-M results by polyclonal (Phadebas) and monoclonal assay for 38 patients.**

The equation of the regression line is $\beta_2$-M (monoclonal) = 0.807 $\beta_2$-M (Phadebas) + 0.106 (r = 0.988). The standard error of the estimate is 0.184. When six additional data pairs in which $\beta_2$-M exceeds 6 mg/L are included, r increases to 0.997, and slope and intercept are not significantly changed.

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**Fig. 4. Comparison of plasma $\beta_2$-M by monoclonal assay with corrected iothalamate clearance ($C_{OTH}$) for 50 patients.**

The equation of the regression line is log$_{10}$ $\beta_2$-M = -0.82 log$_{10}$ $C_{OTH}$ + 1.90 (r = -0.938). The standard error of the estimate is 0.11. Subjects with known immunologic disorder or malignancy (n = 22); O, all other patients (n = 28). When only the latter 28 patients are considered, the regression becomes log$_{10}$ $\beta_2$-M = -0.86 log$_{10}$ $C_{OTH}$ + 1.95 (r = -0.971), with standard error of the estimate = 0.09. ULN, upper limit of normal; LLN, lower limit of normal for plasma $\beta_2$-M selected for Figure 4 was 2.2 mg/L, although the reference interval varies with age (23, 24). The lower limit of normal for iothalamate clearance also varies with age; 80 mL/min per 1.73 m$^2$ body surface was selected for Figure 4.

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**Fig. 5. Comparison of plasma creatinine with corrected iothalamate clearance ($C_{OTH}$) for 50 patients.**

The equation of the regression line is log$_{10}$ creatinine = -0.73 log$_{10}$ $C_{OTH}$ + 2.38 (r = -0.946). The standard error of the estimate is 0.09. O, male (28); $\beta_2$-M = -0.76 log$_{10}$ $C_{OTH}$ + 2.40; r = -0.946. O, woman (22); log$_{10}$ creatinine = -0.68 log$_{10}$ $C_{OTH}$ + 2.34; r = -0.962. UlN, upper limit of normal; LLN, lower limit of normal.
Table 2. Clinical Data for Subjects with Misleading Results for Plasma $\beta_2$-M

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Sex</th>
<th>Creatinine, mg/L</th>
<th>$\beta_2$-M, mg/L</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with false-positive plasma $\beta_2$-M$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>F</td>
<td>125</td>
<td>2.8</td>
<td>7.8</td>
</tr>
<tr>
<td>40</td>
<td>F</td>
<td>100</td>
<td>2.4</td>
<td>7.8</td>
</tr>
<tr>
<td>59</td>
<td>F</td>
<td>91</td>
<td>2.4</td>
<td>7.8</td>
</tr>
<tr>
<td>42</td>
<td>M</td>
<td>90</td>
<td>2.3</td>
<td>10.5</td>
</tr>
<tr>
<td>63</td>
<td>M</td>
<td>75</td>
<td>4.8</td>
<td>10.5</td>
</tr>
<tr>
<td>Subjects with false-negative plasma $\beta_2$-M$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>66</td>
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<td>14.5</td>
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<tr>
<td>25</td>
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<td>77</td>
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<td>74</td>
<td>M</td>
<td>69</td>
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<td>10.8</td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>53</td>
<td>1.72</td>
<td>11.3</td>
</tr>
<tr>
<td>47</td>
<td>F</td>
<td>74</td>
<td>1.36</td>
<td>10.5</td>
</tr>
</tbody>
</table>

$^a$ False positive: $\beta_2$-M > 2.2 mg/L and iothalamate clearance exceeding age-adjusted lower limit of normal. $^b$ False negative: $\beta_2$-M < 2.2 mg/L and iothalamate clearance below age-adjusted lower limit of normal.

Table 3. Clinical Data for Subjects with Misleading Results for Plasma Creatinine

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Sex</th>
<th>Creatinine, mg/L</th>
<th>$\beta_2$-M, mg/L</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with false-positive plasma creatinine$^a$</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>F</td>
<td>104</td>
<td>1.96</td>
<td>10.5</td>
</tr>
<tr>
<td>Subjects with false-negative plasma creatinine$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>F</td>
<td>73</td>
<td>2.7</td>
<td>8.3</td>
</tr>
<tr>
<td>49</td>
<td>F</td>
<td>67</td>
<td>2.4</td>
<td>8.0</td>
</tr>
<tr>
<td>46</td>
<td>M</td>
<td>69</td>
<td>3.1</td>
<td>11.5</td>
</tr>
<tr>
<td>46</td>
<td>M</td>
<td>76</td>
<td>3.2</td>
<td>10.2</td>
</tr>
<tr>
<td>55</td>
<td>M</td>
<td>72</td>
<td>2.8</td>
<td>12.0</td>
</tr>
<tr>
<td>74</td>
<td>M</td>
<td>69</td>
<td>2.0</td>
<td>10.8</td>
</tr>
</tbody>
</table>

$^a$ False positive: creatinine > 9 mg/L (women) or 12 mg/L (men) and iothalamate clearance exceeding age-adjusted lower limit of normal. $^b$ False negative: creatinine < 9 mg/L (women) or 12 mg/L (men) and iothalamate clearance below age-adjusted lower limit of normal.

Table 4. Correlation of Plasma Creatinine and $\beta_2$-Microglobulin with Iothalamate Clearance

<table>
<thead>
<tr>
<th>No. subjects with iothalamate clearance (mL/min per 1.73 m²)</th>
<th>&gt;80</th>
<th>50–80</th>
<th>&lt;50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased creatinine $^a$</td>
<td>0</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Increased $\beta_2$-M $^b$</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Normal $\beta_2$-M</td>
<td>4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Normal creatinine</td>
<td>15</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Men > 12 mg/L; women > 9 mg/L. $^b$ > 2.2 mg/L.

Discussion

Monoclonal antibodies have many potential uses in the clinical laboratory. Freedom from cross reactivity with contaminant antigens in the preparation used for immunization is achieved by clonal selection. The possibility of high specificity for biologically active or otherwise clinically important
forms of an antigen could give a better predictive value for a test. The monoclonal antibody obtained commercially \( (K_d \sim 2 \times 10^{-11} \text{ mol/L}) \) proved adequate for RIA; the antibody that we developed \( (K_d \sim 10^{-7} \text{ mol/L}) \) was very useful for purification of \( \beta_2 \text{-M} \) by affinity chromatography.

Two forms of \( \beta_2 \text{-M} \), differing in isoelectric point, have been previously described (25, 26). Our \( \beta_2 \text{-M} \) preparation was clearly resolved by two-dimensional electrophoresis into at least five forms, with similar molecular masses but differing in isoelectric point. Proteolytic degradation of \( \beta_2 \text{-M} \) is an unlikely cause of the multiple forms because the molecular mass dimension of the two-dimensional gel electrophoresis system is sensitive to the loss of only a few amino acids in this range. A more likely explanation is the progressive denaturation of glutamine and asparagine residues, which would decrease the isoelectric point (shift to the left in Figure 1). The isoelectric point of the main form of \( \beta_2 \text{-M} \) in the 8 mol/L urea isoelectric-focusing gel was approximately 7.1, as compared with 5.8 previously reported under non-denaturing conditions (2). In the one case of renal failure examined by isolating \( \beta_2 \text{-M} \) directly from serum by affinity chromatography, two-dimensional gel electrophoresis demonstrated at least four of the five major forms of \( \beta_2 \text{-M} \). Previous reports have shown that multiple forms of \( \beta_2 \text{-M} \) are not present in all subjects. A monoclonal antibody with high affinity for one form of an antigen might have low (or no) affinity for another form. Both monoclonal antibodies used in this study appeared to combine with all forms of \( \beta_2 \text{-M} \) seen in serum.

This RIA makes use of commercially available monoclonal antibody, and it equals or exceeds previously published methods in sensitivity, specificity, and precision. The sensitivity obtained is greater than that necessary for \( \beta_2 \text{-M} \) determinations in most body fluids, including serum and cerebrospinal fluid. This degree of sensitivity is required, however, for its accurate determination in urine. Although the results presented here validate this assay only for serum, further data indicate that it is valid for the other body fluids (manuscript in preparation).

Comparison of results by the monoclonal assay with results by the polyclonal Phadebas \( \beta_2 \text{-M} \) Micro Test assay showed an excellent correlation but a slope of 0.81 ± 0.02. However, the reference interval determined for normal volunteers with the monoclonal assay was 1.1–2.2 mg/L compared with the range 1.1–2.4 mg/L suggested in the kit insert. This small difference in reference ranges is probably attributable to a narrower age range for the younger volunteers used in our study. The low slope may be attributed to loss of potency of the lyophilized standard provided by this particular kit lot. The reconstituted standard did not completely dissolve, and it remained cloudy even after several days.

Further to validate the monoclonal assay, we studied 50 patients with various types and degrees of renal impairment, comparing plasma \( \beta_2 \text{-M} \) and plasma creatinine with iothalamate clearance. This study repeated and corroborated, in part, the original work of Wibell et al. (27), who used inulin clearance as the measure of glomerular filtration rate. Our correlation coefficient, slope, and intercept of (log plasma \( \beta_2 \text{-M} \)) vs log (corrected clearance) compared well with the values found by Wibell et al. (27). When we excluded data from patients with conditions known to be associated with increased or decreased plasma \( \beta_2 \text{-M} \), the inverse correlation further improved.

When we compared log (plasma creatinine) vs log (corrected iothalamate clearance), the correlation coefficient and slope were -0.95 and -0.73, respectively. In the study of Wibell et al., these parameters were -0.92 and -0.70. When we calculated these parameters for men and women separately, the inverse correlation was even stronger. Thus, both assays dently measure \( \beta_2 \text{-M} \) in a comparable manner. These data also suggest that, although \( \beta_2 \text{-M} \) appears to have clearance characteristics that more closely approach those of an ideal endogenous substance than does creatinine, the correlation of plasma \( \beta_2 \text{-M} \) with glomerular filtration rate is nearly the same as the correlation of plasma creatinine with glomerular filtration rate in a young or middle-aged population, particularly when the reference range is adjusted for patient gender.

In their case-by-case analysis, Wibell et al. found a possible superiority of serum \( \beta_2 \text{-M} \) over serum creatinine for patients with glomerular filtration rates of less than 80 and more than 35 mL/min. Of 24 patients in this group, they found six to have increased serum \( \beta_2 \text{-M} \) with normal serum creatinine by their criteria. Five of these six cases, however, were women with serum creatinine values of 10 to 12 mg/L, which in our laboratory would be considered increased. Thus, the apparent superiority of serum \( \beta_2 \text{-M} \) over serum creatinine largely disappears when criteria for increased serum creatinine include the patient's sex, as is shown in Table 4.

The advantages of serum \( \beta_2 \text{-M} \) over serum creatinine for estimating glomerular filtration rate further decrease when false positives are considered. Although our upper limit of normal for serum \( \beta_2 \text{-M} \) was somewhat lower than that proposed by others, the false positive cases in Table 2 were generally related to autoimmune or inflammatory disorders, which are known to be correlated with increased serum \( \beta_2 \text{-M} \) without renal disease. For patients with malignancy (especially hematologic malignancy), autoimmune disease, or other diseases or therapies associated with stimulation or suppression of the immune system, the estimation of glomerular filtration rate from serum \( \beta_2 \text{-M} \) would be inappropriate. However, the difference between the serum \( \beta_2 \text{-M} \) estimated from glomerular filtration rate and that actually determined could have clinical significance by suggesting increased or decreased production of \( \beta_2 \text{-M} \). In fact, our experience and that of others (7, 16) indicate that in certain settings characterized by increased \( \beta_2 \text{-M} \) production and decreased renal function—such as during renal transplant rejection—measurement of \( \beta_2 \text{-M} \) is quite useful.

In several recent papers the use of serum \( \beta_2 \text{-M} \) in the diagnosis or detection of renal disease is favored (28–30), but convincing evidence of a clear-cut advantage of serum \( \beta_2 \text{-M} \) over serum creatinine in predicting renal disease is still lacking. Each has disadvantages in sensitivity, specificity, or cost. It seems likely that continued careful clinical studies will define those situations in which determination of serum \( \beta_2 \text{-M} \) is indicated.

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References


