Serum beta-2-microglobulin in the differential diagnosis of monoclonal gammopathies

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Summary

Serum beta-2-microglobulin (B_2m) concentrations were determined in 43 southern African black patients with multiple myeloma (MM), in 130 black patients with monoclonal gammopathy of undetermined significance (MGUS) and in 70 control subjects. The results showed median values for serum B_2m in patients with MM, MGUS and the control group to be 8,10 mg/l, 3,05 mg/l, and 2,35 mg/l, respectively; these values differed significantly from one another ($P < 0,01$), even when patients with normal renal function (serum creatinine value < 110 μmol/l) were considered separately. The median serum B_2m concentration for IgG MM (22 cases) was 4,3 mg/l, for IgA MM (8 cases) 7,3 mg/l, and 24,2 mg/l for Bence Jones MM (12 cases). These differences were also significant ($P = 0,001$), but not in the restricted group of MM patients with normal renal function. In the 43 MM patients serum B_2m concentrations had a significant positive correlation with serum creatinine ($r = 0,706$; $P < 0,005$) and a significant negative correlation with haemoglobin values ($r = -0,459$; $P = 0,006$). In 28 MM patients with normal renal function, serum B_2m values had a significant negative correlation with serum albumin ($r = -0,602$, $P = 0,003$). Sixty-five per cent of the 43 MM patients and 18,5% of the MGUS patients had raised serum B_2m values ($> 4,7$ mg/l). An optimum cut-off value for serum B_2m of 6,9 mg/l for differentiating MM from MGUS was determined using a classification rule. Despite lacking specificity, serum B_2m measurement was useful in differentiating MM from MGUS, and was the best second choice variable in relation to serum albumin and haemoglobin in patients with normal renal function.

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Beta-2-microglobulin (B_2m), a polypeptide with a molecular weight of 11,815 daltons, occurs as the free light-chain portion of the major histocompatibility complex (MHC) class I antigen of man (HLA) and other vertebrates.[1] It is present on the cytoplasmic membranes of nearly all nucleated cells, as well as in certain body fluids such as serum, urine, and cerebrospinal fluid.[2]

Increased levels of serum B_2m have been observed in association with lymphoproliferative disorders, such as chronic lymphocytic leukaemia, some lymphomas, as well as multiple myeloma (MM).[3] However, certain benign diseases, such as rheumatoid arthritis, systemic lupus erythematosus and viral infections, are also associated with raised serum B_2m concentrations.[4] Furthermore, serum B_2m levels are not only raised due to increased rates of synthesis, but also in patients with renal disease associated with a diminished glomerular filtration rate.[5]

Three reports[6-8] of serum B_2m concentrations in MM and monoclonal gammopathy of undetermined significance (MGUS) have appeared, although the total number of patients analysed was smaller than in this study. Only one study[8] analysed the concentrations of serum B_2m in MM patients in relation to both normal and abnormal renal function.

In the present study the usefulness of serum B_2m in differentiating MM from MGUS was evaluated, with particular emphasis on the influence of renal function in these patients. In addition, the correlation coefficients between serum B_2m and serum creatinine, serum albumin, and haemoglobin values were determined in both MM and MGUS patients, who were also divided between abnormal and normal renal status.

Patients with MM were all initially seen at advanced stages of this malignant disease and were analysed as one group. In consequence, a specific comparison between serum B_2m values in patients with low cell mass MM (grade I) and MGUS could not be made.

Patients and methods

The patient group consisted of 173 southern African black patients with monoclonal gammopathies. The differentiation between benign and malignant disease was made by accepted clinical, radiological, haematological and biochemical criteria.[9] Forty-three patients were diagnosed as having MM, of which 17 had renal impairment (serum creatinine level > 110 μmol/l). Out of 130 patients with MGUS, 32 had raised serum creatinine values (> 110 μmol/l). The control group consisted of 70 age-matched patients with normal renal function, attending an epilepsy clinic. Sera from MM patients were obtained before treatment.

Assay methods. Sera obtained for B_2m estimation in the different groups were stored at −20°C until analyses were performed. The concentrations of B_2m were assayed in duplicate utilising a commercially available solid phase radioimmunoassay (Phadebas β_2-microtest, Pharmacia, Uppsala, Sweden). The normal reference range for the 70 control subjects was 0,7 - 4,6 mg/l (mean ± 2 SD). Serum creatinine concentrations were determined with a continuous flow analyser (SMA II, Technicon Corp., Tarrytown, N.Y.) utilising the Jaffé reaction. Serum albumin levels were determined by the same instrument using the bromocresol-green method. Haemoglobin levels were determined on the Technicon H1 (Technicon Corp.).

Statistics. The non-parametric Mann-Whitney *U*-test was used to test for a significant difference between the median B_2m values for MM, MGUS and control subjects. Sub-types of MM were tested for significant differences in median serum B_2m levels by means of the Kruskal-Wallis test. Spearman correlation coefficients were used to evaluate the correlation between serum B_2m levels and other serum analytes measured. The technique known as classification trees[10] was used to

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TABLE I. MEDIAN SERUM B_2m CONCENTRATIONS (mg/l) IN PATIENTS (MM AND MGUS) AND CONTROL GROUP

Disease	No. of patients	Median (range)	25th percentile	75th percentile
MM				
All cases	**43**	**8,10 (2,10 - 56,0)**	**3,55**	**18,8**
Serum creatinine (< 110 μmol/l)	**25**	**4,10 (2,60 - 22,5)**	**2,60**	**7,80**
MGUS				
All cases	**130**	**3,05 (0,40 - 16,0)**	**2,30**	**4,30**
Serum creatinine (< 110 μmol/l)	**98**	**2,80 (0,40 - 13,6)**	**2,30**	**3,70**
Control group	**70**	**2,35 (1,5 - 5,90)**	**2,0**	**3,0**

derive a rule for classifying MM and MGUS patients. Confidence intervals (CI) (95%) for serum B_2m concentrations in MM and MGUS patients were calculated based on the means.

Results

The range, median, 25th and 75th percentile values for serum B_2m in the patient and control groups are shown in Table I, and the 95% CI for serum B_2m values based on the means in both patient groups are shown in Table II. The differences between the median values for MM, MGUS and control patients were significant when considering all the patients ($P < 0,01$ for all comparisons), as well as those with serum creatinine levels < 110 μmol/l (normal renal function). In the restricted group with normal renal function MM patients had a significantly higher serum B_2m median value than the MGUS patients ($P = 0,003$) and the controls ($P < 0,0005$), and the MGUS patients, in turn, had a significantly higher median than the controls ($P = 0,009$).

The median serum B_2m values for patients with various myeloma sub-types (Table III) differed significantly ($P = 0,001$). However, when patients with renal impairment were excluded in this group, the difference in median values was not significant ($P = 0,174$).

TABLE II. 95% CI FOR SERUM B_2m CONCENTRATIONS (mg/l) (BASED ON MEANS)

	No. of patients	95% CI
MM		
All cases	**43**	**9,3 - 17,4**
Serum creatinine (< 110 μmol/l)	**25**	**4,1 - 8,1**
Serum B_2m (> 4,7 mg/l)	**28**	**13,8 - 24,0**
Serum B_2m (> 4,7 mg/l with serum creatinine < 110 μmol/l)	**11**	**7,2 - 13,3**
Serum B_2m (> 6,9 mg/l)	**25**	**15,1 - 25,9**
Serum B_2m (< 6,9 mg/l)	**18**	**2,8 - 4,1**
MGUS		
All cases	**130**	**3,3 - 4,2**
Serum creatinine (< 110 μmol/l)	**98**	**2,9 - 3,6**
Serum B_2m (> 4,7 mg/l)	**25**	**6,5 - 9,0**
Serum B_2m (> 4,7 mg/l with serum creatinine < 110 μmol/l)	**11**	**5,3 - 8,6**
Serum B_2m (> 6,9 mg/l)	**10**	**8,8 - 12,8**
Serum B_2m (< 6,9 mg/l)	**120**	**2,9 - 3,4**

Correlation between serum B_2m values and other measured analytes in monoclonal gammopathies

The Spearman correlation coefficient (r) for serum B_2m and serum creatinine levels in the MM patients was 0,706 ($P < 0,0005$), but only 0,141 in those patients with normal renal function (not significant). There was also a significant negative correlation in the 43 MM patients between serum B_2m and haemoglobin levels ($r = -0,459$; $P = 0,006$). In the 28 MM patients with normal renal function the only significant finding

TABLE III. SERUM B_2m LEVELS (mg/l) IN MM SUB-TYPES* (ALL CASES)

Myeloma sub-type	No. and light chain type	Median	25th percentile	75th percentile
IgG	**22 (12λ) (10λ)**	**4,3**	**2,5**	**8,4**
IgA	**8 (4λ) (4κ)**	**7,3**	**4,7**	**13,2**
Bence Jones	**12 (3λ (9κ)**	**24,2**	**13,6**	**35,0**
IgD	**1 (λ)**	**Single value of 51,0**		
Non-secretor IgA	**1 (κ)**	**Single value of 3,2**		

*Median values for myeloma sub-types studied differed significantly ($P = 0,001$).

was a negative correlation between serum B_2m and albumin ($r = -0,602$; $P = 0,003$).

In the 130 patients with MGUS, the only significant correlations were between serum B_2m and creatinine values ($r = 0,393$; $P = 0,0001$) and a negative correlation with serum albumin level ($r = -0,237$; $P = 0,008$).

Classification rule for differentiating MM from MGUS

The classification rule, using all the data for MM and MGUS patients (Fig. 1), indicated an optimum cut-off value for serum B_2m of 6,9 mg/l for differentiating between the two conditions (diagnostic sensitivity 58,1%, specificity 91,5%). When considering only patients with normal renal function, the haemoglobin and serum albumin levels were the preferred determinants (Fig. 2). Using this classification rule for differentiating MM from MGUS, a diagnostic sensitivity of 88% and a specificity of 70% for MM was obtained. B_2m, however, remains the best second choice variable for both haemoglobin and albumin in the classification tree as a predictor.

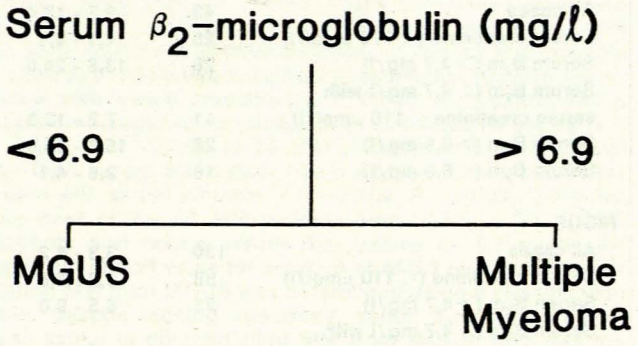

Fig. 1. Classification rule for differentiating MM and MGUS (all cases).

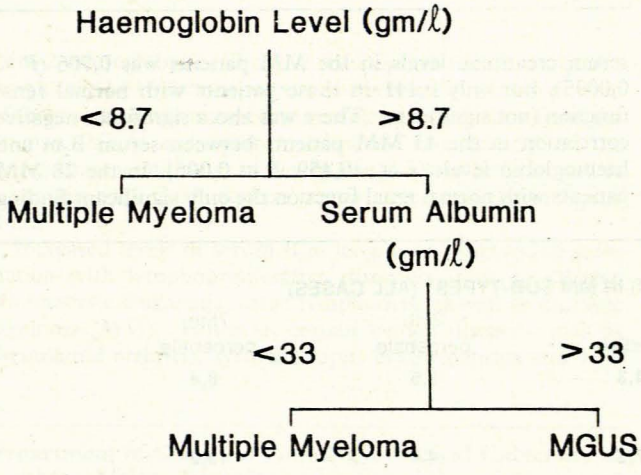

Fig. 2. Classification rule for differentiating MM and MGUS in patients with normal renal function.

Discussion

The serum B_2m concentration in healthy white subjects was reported to be 1,1 - 2,4 mg/l (mean ± 2 SD) in one study[6] and 0,84 - 2,76 mg/l in a second.[7] The upper limit of normal for serum B_2m level was taken as 2,4 mg/l and 3,0 mg/l, respectively, in each of these earlier reports compared with 4,7 mg/l in the southern African blacks in the present study. The explanation for the higher circulating B_2m in black subjects is not known, but it may be related to increased chronic exposure to certain infectious agents, particularly *Mycobacterium tuberculosis*, resulting in lymphocyte or macrophage stimulation and increased B_2m synthesis.[4]

In this study 28 of the MM patients (65%) had raised serum B_2m values (> 4,7 mg/l) and of these, 11 had normal renal function. Of the MGUS patients, 24 (18,5%) had raised values, of which 8 patients had normal renal function. This agrees well with the results of previous investigations where between 39% and 74% of MM patients were reported to have increased B_2m levels.[6,11,12] In our MM patients 39,5% had raised serum creatinine concentrations, compared with 29% in Morrell and Riesen's[6] study. The higher incidence of renal impairment in our MM patients may partly explain the higher median serum B_2m value (8,1 mg/l) obtained, which is also related to the fact that our patients present at a much later stage of disease.

In the present study a significant difference was noted between the median serum B_2m levels in MGUS patients and the control group. Furthermore, a significant difference in the median serum B_2m concentrations for the MM different immunoglobulin classes analysed was observed, but not when comparing MM patients with normal renal function as a separate group. Various studies have failed to establish such a statistically significant difference,[3,6,11,12] but only Kin *et al.*[12] clearly defined their MM patient group as having normal renal function. The highest serum B_2m concentrations in our 43 MM patients were associated with Bence Jones MM, followed by IgA and IgG MM. This may be explained by the fact that some Bence Jones MM cases are associated with a more rapid onset of tubular and ultimately glomerular damage,[13] resulting in a reduced glomerular filtration rate and diminished clearance of B_2m. Serum B_2m concentrations for single cases of an IgDλ and an IgAκ non-secretor MM were 51,0 mg/l and 3,2 mg/l, respectively. These values are in accordance with the relatively short survival time for patients with IgD MM[14] and the longer survival time for non-secretor MM patients who have a lower tumour mass at presentation.[15]

Serum B_2m and creatinine levels showed a significant correlation in all MM and MGUS patients, reflecting the effect of renal impairment on lowering the glomerular filtration rate, thereby raising serum B_2m and creatinine concentrations. A significant negative correlation between serum B_2m and haemoglobin was observed in the 43 MM patients, but not in MM patients with normal renal function. This finding is obscure, but could imply that the anaemia associated with MM in the more advanced stages of disease is more significantly related to renal complications, such as low erythropoietin production, than to increased tumour cell mass and bone marrow replacement alone. The significant negative correlation between serum B_2m and the serum albumin was observed only in the MM patient group with normal renal function. This observation seems paradoxical, since one would have also expected a significant correlate when considering all 43 MM patients. There is no apparent explanation for this finding.

Using a cut-off value for serum B_2m concentrations of 4,6 mg/l, the diagnostic sensitivity for MM in our patient population group was 65,5%, but 24 out of 130 MGUS patients (18,5%) would be falsely classified as MM. By means of the classification tree rule an optimum cut-off value of 6,9 mg/l for serum B_2m was derived for differentiating MM patients (irrespective of clinical stage and renal function) from MGUS patients. The resultant sensitivity using this value for MM was 58,1%, but the specificity rose to 91,5%.

In conclusion, it can be seen that serum B_2m value lacks sensitivity and specificity as a marker for MM. However, it

can be useful in differentiating MM patients considered globally from MGUS in southern African black patients. When considering MM patients with normal renal function, this differentiation can be better accomplished by measuring serum haemoglobin and albumin values with serum B_2m as the best second choice variable. Furthermore, the various MM immunoglobulin classes show a significant difference in the median serum B_2m values when patients with abnormal renal function are included in the analysis.

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