Plasma levels of β 2-microglobulin in rheumatoid arthritis

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SUMMARY A simple and inexpensive method is described for the determination of β 2-microglobulin (β 2-MG) by enzyme-amplified single radial immunodiffusion. The values obtained with this method correlate well with those determined by means of a commercial RIA kit. Using the immunodiffusion method we have measured the plasma levels of β 2-MG in 135 patients with rheumatoid arthritis (RA) and normal serum creatinine levels. 33 % of the patients had increased concentrations of β 2-MG, but the levels were found to correlate poorly with the values of several variables generally used as indices of the degree of inflammatory activity in RA. Furthermore, in contrast to earlier claims to the contrary, β 2-MG correlated positively with age. The value of β 2-MG in plasma as an index of inflammatory activity in RA is questioned.

β 2-MG has since its discovery in 1968 been subject to numerous investigations. The association of β 2-MG with lymphocytes¹⁻² might explain the raised serum levels found in various lymphoproliferative disorders,³ infectious mononucleosis, and primary biliary cirrhosis.⁴

Moreover various rheumatic diseases are characterised by lymphocytic proliferation and infiltration. Raised concentrations of β 2-MG have been found in serum and joint fluid in patients with RA and in serum and saliva in patients with Sjögren's syndrome. Isolated keratoconjunctivitis sicca is not associated with raised serum levels of β 2-MG.⁵⁻⁷ In Sjögren's syndrome a significantly positive correlation between β 2-MG in serum and the degree of lymphocytic infiltration of the salivary glands has been observed.⁵ Weissel *et al.*⁸ reported slightly raised serum levels of β 2-MG in SLE without renal involvement. Orloff and co-workers recently described 21 patients with RA and normal creatinine clearance.⁹ They confirmed the finding of raised plasma levels of β 2-MG in a large proportion (50%) of these patients. Moreover they found a very good correlation between plasma β 2-MG and joint count.¹⁰ On the basis of these findings they considered that β 2-MG in plasma is a very useful index of inflammatory joint disease in rheumatoid arthritis.

The present study was undertaken in order to investigate further the relationship between plasma levels of β 2-MG and various clinical and laboratory variables in a larger, unselected population of RA patients. For the determination of β 2-MG a method based on an enzyme-amplified Mancini technique is described and compared with a commercially available RIA kit (Phadebas, Pharmacia).

Materials and methods

135 patients were included. All of them had a diagnosis of classical or definite RA according to the ARA criteria¹¹ and a normal serum creatinine level. Apart from cases excluded by these criteria the study included all patients seen at the Division of Rheumatology during a 2-month period. Clinical details are shown in Table 1.

Human body fluids. Blood samples were drawn

Table 1 *Patients: clinical data*

Number		135
Diagnosis	Definite RA	34
	Classical RA	101
Age (yr)	Range	25-83
	Median	61
Sex	Male	28
	Female	107
Duration of disease (yr)	Range	1-52
	Median	13
Functional class ²²	I	26
	II	69
	III	31
	IV	9

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from each patient on the same occasion as the physical examination. Urine from a patient with tubular proteinuria was used as β 2-MG standard.

Antisera. Monospecific rabbit antiserum against human β 2-MG was available at the laboratory. Preparation of the antiserum is described under 'Methods'. The purified IgG fraction was used. Peroxidase conjugated swine immunoglobulin against rabbit IgG was obtained from DAKO Immunoglobulins Ltd., Copenhagen, Denmark.

Miscellaneous. Phadebas RIA kit for the determination of β 2-MG was purchased from Pharmacia Diagnostics, Uppsala, Sweden. Agarose and plastic film with a hydrophilic surface was obtained from Marine Colloids, Rockland, Maine, USA; dimethylformamide from Mallinkrodt Inc., St Louis, Mo, USA; 3-amino-9-ethylcarbazole from Sigma Chemical Company, St Louis, Mo., USA; heparin 500IE/ml from Vitrum AB, Stockholm, Sweden; hydrogen peroxide 30% w/v as well as all other chemicals (reagent grade) from British Drug Houses Ltd., Poole, England.

Patient examination. Each patient was subject to a clinical examination that included an estimation of joint tenderness (Ritchie index¹²). After examination the physician gave his overall assessment of activity on a point scale from 0 (best) to 10 (worst). This was a subjective impression that took into consideration the recent history (including duration of morning stiffness) and the result of the physical examination of the joints.

Preparation of rabbit antiserum against human β 2-MG. β 2-MG was purified from the urine of a patient with tubular proteinuria. Rabbits were immunised with 0.5 mg of the purified β 2-MG in 1 ml 0.15 mol/l NaCl emulsified in 1 ml of complete Freund's adjuvant. The emulsion was administered subcutaneously in multiple sites twice at 3-week interval. Blood was collected every second week until the titres decreased. The nonabsorbed antiserum was monospecific with respect to human β 2-MG. Tests for specificity were carried out by immunoelectrophoresis and crossed immunoelectrophoresis. Serial dilutions of the antiserum produced one single precipitate line when tested against serial dilutions of human plasma (normal and uraemic).

β 2-MG determination by single radial immunodiffusion. The procedure was a modification of the one described by Mancini *et al.*¹³ Agarose (0.8% w/v) was dissolved in 0.025 mol/l barbital buffer pH 8.6. After cooling to +48°C the IgG-fraction

of the β 2-MG antiserum was added together with 2% (v/v) heparine solution (5000 IE/ml) to decrease nonspecific protein binding to the agarose gel (Grubb, personal communication). The gel was then cast on a plastic film as described by Jeppson *et al.*¹⁴ and 7 μ l plasma samples and serial dilutions of the standard applied to the wells. After 24 hours in a moist chamber at +4°C the gel was washed for 30 min in 0.15 mol/l NaCl and then pressed for 15 min. This alternating washing and pressing was repeated twice without allowing the gel to dry completely.

Visualisation of the immunoprecipitates. The technique was similar to the one used by Löfberg and Grubb¹⁵ based on the report by Ingild.¹⁶ The plastic film with the gel was placed on a moistened glass plate, which was put on top of 2 horizontal glass rods in a flat-bottomed container. The peroxidase-conjugated swine antirabbit IgG was diluted 1:20 (v/v) with PBS 0.1 mmol/l, pH 7.2, and poured on top of the gel (approximate volume 0.1 ml/cm²). The surface tension made the liquid stay on the plate, which was incubated for 60 min at +20°C. It was possible to use the antibody solution several times if merthiolate 0.005% (w/v) was added as preservative. The use of sodium azide should be avoided, since it interferes with the staining process. The gel was washed and pressed as described previously.

The peroxidase substrate was prepared for immediate use: 20 mg 3-amino-9-ethylcarbazole dissolved in 2.5 ml dimethylformamide was mixed with 25 μ l hydrogen peroxide (30% w/v) in 50 ml 0.05 mol/l acetate buffer, pH 5.0. The plastic film was placed horizontally as described above and the substrate solution poured on top of the gel (approximate volume 0.2 ml/cm²). The immunoprecipitates were stained wine red within 10–15 min. The staining process was discontinued by rinsing the gel in water before drying it in front of a hot-air fan.

Determinations of certain other proteins. The following proteins were determined in plasma samples at the Department of Clinical Chemistry by electroimmunoassay ('rocket'-technique¹⁷): orosomucoid, fibrinogen, C-reactive protein (CRP), and IgG.

Results

The Mancini method for β 2-MG was found to have an error of 0–10.4% (median 2.8%) in duplicate samples. It was compared with the Phadebas kit method on 17 samples (Fig. 1) and showed a good

Fig. 1 Comparison of Mancini- with RIA (Phadebas)-values of plasma β_2 -MG in 17 patients. One unit (Mancini technique)=1% of the concentration in the β_2 -MG standard.

correlation ($r=0.97$). The remainder of β_2 -MG determinations were performed with the Mancini method, and the result was expressed as a percentage of the concentration of the standard.

Table 2 shows some properties of the variables studied. Since the reference values for β_2 -MG in plasma refer to measurements with Padebas, our units were transformed to Phadebas units (mg/l) by means of the equation for the regression line (Fig. 1). Thus 33% of the patients had a raised β_2 -MG concentration in plasma. In contrast 84% had raised CRP, 79% increased orosomucoid, 60% increased fibrinogen, and 31% increased IgG levels. A very wide range was observed in ESR, Ritchie index, and examiner's assessment of disease activity, indicating the expected heterogeneity with regard to disease activity. In Fig. 2 β_2 -MG is plotted against the other variables analysed and the corresponding r values are given in the legend to each plot. The low values for the correlation coefficients show the absence of a close association between β_2 -MG

Table 2 Variables; characteristics of the distributions (protein concentrations refer to plasma)

Variable	N	Range	Median	Normal range	Percentage above upper normal limit
Examiner's assessment (0-10)	129	0-10	3		
Joint count ¹²	135	0-39	7		
ESR (mm/h)	135	2-149	39		
Orosomucoid (%)	134	70-310	180	70-135	79
Fibrinogen (g/l)	125	2.3-7.8	4.8	2.0-4.4	60
CRP (mg/l)	135	0-300	26	<5	84
IgG (g/l)	135	7-30	13	7-15	31
β_2 -MG (mg/l)	135	0.6-6.1	2.0	0.8-2.4	33

Fig. 2 Comparison of plasma β_2 -MG with other variables. The corresponding correlation coefficient is shown in parentheses. (a) Examiner's overall assessment (0.29).

Fig. 2 (b) *Ritchie index (0-34).*

Fig. 2 (c) *ESR (0-35).*

Fig. 2 (d) *Orosomucoid (0-34).*

Fig. 2 (e) Fibrinogen (0.17).

Fig. 2 (f) CRP (0.32).

Fig. 2 (g) IgG (0.15).

Fig. 3 Comparison of plasma β 2-MG with age. $r=0.45$

and these variables. The highest correlation was found with ESR ($r=0.35$) and the lowest with IgG ($r=0.15$).

Fig. 3 is a plot of β 2-MG against age. The positive correlation is obvious ($r=0.45$). A weaker positive correlation was demonstrable between β 2-MG and serum creatinine ($r=0.34$).

Discussion

The method described for measuring β 2-MG seems to be a simpler and less expensive alternative to the commercial RIA kit. It gives reproducible results that correlate very well with the values obtained by the RIA method. Results are obtained on the same day with RIA method, whereas the immunodiffusion method requires $2\frac{1}{2}$ days but with a small total working time.

With regular Coumassie staining it was not possible to obtain visible precipitates of sufficient magnitude from any of the plasma samples. Thus we were justified in using the somewhat more complicated amplification technique described. This is in accordance with Ingild,¹⁶ who has reported a significantly increased sensitivity of her method when compared with ordinary direct staining procedures for various immunoprecipitates.

The potential practical usefulness of plasma β 2-MG in characterising the state of RA patients seems rather doubtful because of the obvious but hitherto unobserved age dependency in patients with normal

serum creatinine levels. Of course this might, at least in part, be explained by the known reduction of glomerular filtration rate (GFR) with increasing age. Thus it may be necessary to make corrections for GFR in every instance, which is clearly not feasible.

Disregarding age, we found raised levels of plasma β 2-MG in about the same proportion of RA patients as reported by other authors. However, β 2-MG was raised in a lower proportion of patients than conventional biochemical variables of tissue damage such as CRP, orosomucoid, and fibrinogen. Thus the sensitivity of β 2-MG as an indicator of disease activity in RA is probably low.

Only weak correlations were obtained between plasma β 2-MG and clinically and biochemically assessed variables of joint activity. Exclusion of patients with pathological GFR would hardly change this result for the following reasons: A decreased GFR almost invariably gives rise to an increase in plasma β 2-MG.¹⁸ Consequently all patients with a possibly reduced GFR must be included in the group with elevated plasma β 2-MG. However, it is evident from Fig. 2 that exclusion of some patients with high β 2-MG would effect the degree of association only to a minor extent.

Our results did not confirm those of Manicourt *et al.*,⁹ who based their conclusions on only 21 cases. Their results might have been influenced by selection or chance. Our material on the other hand was much larger and included the whole

clinic population of RA seen during a 2-month period.

Brauman *et al.*¹⁹ found the ratio between β 2-MG in joint fluid and in plasma to be higher in RA than in gout or osteoarthritis. They also found that in RA the levels of β 2-MG correlated with lymphocyte counts in joints. β 2-MG may thus be of value in distinguishing RA from other conditions with joint effusion, though this requires confirmation.

The problem of quantitating 'disease activity' in RA still awaits a satisfactory solution. None of the plasma proteins known to be 'acute phase reactants' show a closer correlation with clinically assessed variables of joint activity than ESR,²⁰ though CRP was recently claimed to be correlated with joint erosions.²¹ Whether the subset of RA patients with high β 2-MG in plasma differ in type of illness or prognosis remains to be evaluated.

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