

# Comparison of serum and synovial fluid concentrations of $\beta_2$ -microglobulin and C reactive protein in relation to clinical disease activity and synovial inflammation in rheumatoid arthritis

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**SUMMARY**  $\beta_2$ -Microglobulin and C reactive protein (CRP) were measured in 33 and 57 matched pairs of serum and synovial fluid (SF) respectively, from patients with active rheumatoid arthritis (RA). Serum  $\beta_2$ -microglobulin concentrations were higher than in normal controls and the SF concentration was higher than the serum concentration on 25 of 33 occasions (76%), suggesting a local production of  $\beta_2$ -microglobulin within the synovial membrane. There was a correlation between serum and SF concentrations of  $\beta_2$ -microglobulin ( $r=0.50$ ). Patients' serum CRP concentrations in 57 samples were higher than in normal controls and were greater than in the matched SFs on 49 of the 57 paired samples (86%). In 18 samples CRP was absent in the SF, suggesting a local consumption or binding within the synovial membrane. Twenty four patients with RA given either sodium aurothiomalate or D-penicillamine for six months showed highly significant clinical improvements accompanied by reductions in serum and SF immunoglobulin concentrations and knee joint suprapatellar pouch synovial membrane T lymphocyte infiltrates. In this group of patients serum CRP, but not  $\beta_2$ -microglobulin, fell significantly, but there were no significant changes in SF  $\beta_2$ -microglobulin or CRP. These data suggest that serum and SF  $\beta_2$ -microglobulin concentrations are not a useful index for determining the therapeutic response to sodium aurothiomalate and D-penicillamine and that serum rather than SF CRP concentrations are more helpful. The persistent raised serum and SF concentrations of  $\beta_2$ -microglobulin probably reflect synovial inflammatory infiltrates, which are still considerable despite apparent clinical remission.

$\beta_2$ -Microglobulin, a polypeptide with a molecular weight of 11.7 kD, is found in serum and many biological fluids<sup>1</sup> as it is produced and secreted by both T and B lymphocytes.<sup>2,3</sup> It forms part of the light chain of the HLA class I molecule,<sup>4-7</sup> and the amino acid sequence shows close homology with the constant regions of immunoglobulin heavy and light chains.<sup>8-10</sup> In connective tissue diseases increased concentrations of  $\beta_2$ -microglobulin have been found within the peripheral blood and attempts have been made to show a correlation with disease activity.<sup>11-14</sup>

Few studies have compared the relative values of serum and SF concentrations of this molecule in rheumatoid arthritis (RA) or followed up its response to treatment.

C reactive protein (CRP) (105 kD) concentration rises in the serum after tissue injury, infection, or inflammation.<sup>15</sup> It is synthesised in the liver,<sup>16</sup> but the precise stimulus for its production is unknown. The value of measuring CRP in RA is established, and serum concentrations relate to disease activity.<sup>17-20</sup>

The purpose of this study was to compare serum and synovial fluid (SF) concentrations of  $\beta_2$ -microglobulin in patients with RA and determine the effect of six months' treatment with sodium aurothiomalate or D-penicillamine. We sought correla-

tions between those concentrations and clinical, haematological, immunochemical, peripheral blood immunohaematological, and synovial membrane immunohistochemical indices of disease activity. Serum and SF CRP were used for comparison.

### Patients and methods

#### PATIENT GROUPS

Patients entering the study were attending the rheumatology units of Southampton General Hospital or Queen Alexandra Hospital, Portsmouth, and fulfilled the criteria for classical or definite RA.<sup>21</sup>

All patients were judged to have active RA with knee joint synovitis and many were about to start treatment with either sodium aurothiomalate by intramuscular injection or D-penicillamine orally. Sodium aurothiomalate was given as a test dose of 10 mg and then 50 mg weekly until a clinical response was obtained. D-Penicillamine was started in a dose of 125 mg daily and increased monthly by 125 mg until a clinical response was obtained. The maximum dose required to achieve this effect was 750 mg.

#### CLINICAL METHODS

On entry a full clinical examination was performed, including measurements of a modified Ritchie articular index,<sup>22</sup> grip strength, visual analogue pain score,<sup>23</sup> and body weight. The duration and severity of early morning joint stiffness were noted. Synovial fluid aspirated from an affected knee joint was centrifuged, sodium azide (10 mM) added as a preservative, and the SF stored at 4°C. Blood stained SF was discarded.

A synovial biopsy specimen was taken blind under local anaesthesia from the suprapatellar pouch of the same knee joint, as previously described.<sup>24</sup> Patients were reviewed after 24 weeks when all investigations were repeated.

#### LABORATORY METHODS

##### Haematology/immunochemistry

Haemoglobin, peripheral blood white cell count, and erythrocyte sedimentation rate (Westergren) were measured. Serum and SF IgG, IgA, and IgM, and complement components C3 and C4 were measured by laser nephelometry (Hyland). Circulating immune complexes were measured by a modification of the nephelometric method used for the assay of immunoglobulin.<sup>25</sup> The sheep cell agglutination titre was determined by a standard method. An enzyme linked immunosorbent assay (ELISA) was used to measure  $\beta_2$ -microglobulin. The coating

antibody used was rabbit anti- $\beta_2$ -microglobulin (Dako Ltd) (1 in 10<sup>3</sup> dilution in carbonate buffer pH 9.5), and the detecting antibody was the same antibody coupled to horseradish peroxidase (Dako Ltd); this was used at a 1:500 dilution. The sensitivity of the assay was approximately 0.4 ng/ml. C reactive protein concentrations were measured by laser nephelometry (Hyland). Rheumatoid factor interference was eliminated by treatment of serum/SF with 20 mM 2-mercaptoethanol for 30 minutes at 37°C, followed by 22 mM iodoacetamide. This treatment did not affect concentrations of CRP or  $\beta_2$ -microglobulin.

##### Immunohistology

Peripheral blood lymphocytes and cryostat cut synovial membrane biopsy specimens were stained as previously described<sup>24</sup> with a panel of monoclonal antibodies directed against T lymphocyte differentiation antigens as follows: mature T cells (CD3+)—UCHT1,<sup>26</sup> T helper/inducer cells (CD4+)—Leu3a,<sup>27</sup> T suppressor/cytotoxic cells (CD8+)—OKT8,<sup>28</sup> and cells bearing the leucocyte common antigen with the pan-leucocyte marker (CD45+), WR6A.<sup>29</sup> Cells bearing immunoglobulin were detected by antibodies to  $\kappa$  and  $\lambda$  light chains (Dako Ltd). Major

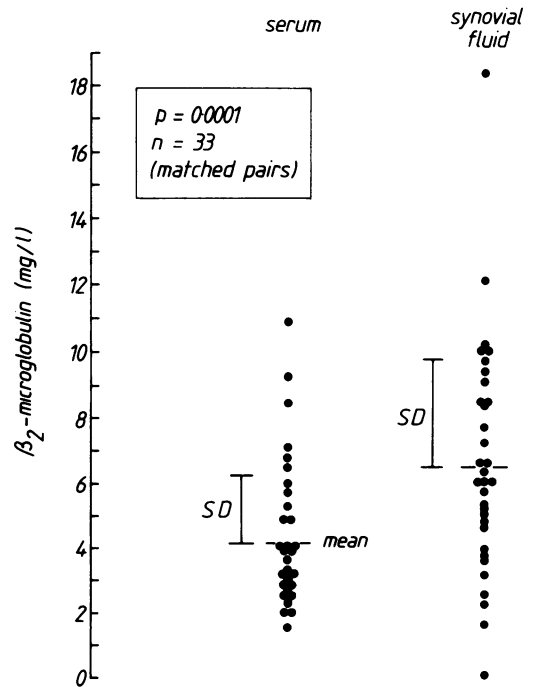


Fig. 1 Comparison of serum and synovial fluid  $\beta_2$ -microglobulin in rheumatoid arthritis.

histocompatibility complex HLA class II surface antigens were detected using a monoclonal antibody directed towards HLA-DR—FMC4,<sup>30</sup> HLA-DQ—TU22,<sup>31</sup> HLA-DP—B7/21.<sup>32</sup> Peripheral blood positive lymphocytes were scored as a percentage of 200 cells counted. Positively stained lymphocytes on each synovial membrane biopsy section and for each monoclonal antibody and the intensity of HLA class II staining were assessed by the counting methods previously described.<sup>24</sup>

**STATISTICAL ANALYSIS**

The Wilcoxon matched pairs signed ranks test was used for non-parametric paired data and the Mann-Whitney U test for non-parametric unpaired data. Spearman correlation coefficients were used to assess the degree of correlation between serum and SF  $\beta_2$ -microglobulin and CRP concentrations and other indices of disease activity.

**Results**

**PATIENT DATA**

Fifty seven paired samples of serum and SF from patients with RA were available for analysis. Twenty five patients (12 male, 13 female) were selected to receive either sodium aurothiomalate or D-penicillamine, having never previously been treated with this type of drug. These patients had a mean age of 52.3 years (range 19.8–75.8), a mean disease duration of 3.1 years (0.25–10), and all patients had normal renal function. Fifteen received D-penicillamine and 10 sodium aurothiomalate. One patient receiving sodium aurothiomalate developed severe oral ulceration and a skin rash after eight weeks and was withdrawn from the study.

**COMPARISON OF SERUM AND SF  $\beta_2$ -MICROGLOBULIN AND CRP CONCENTRATIONS**

Matched pairs of serum and SF were available from 33 patients with active RA for the measurement of

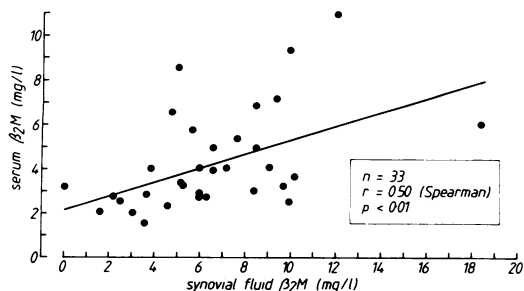


Fig. 2 Correlation between serum and synovial fluid  $\beta_2$ -microglobulin ( $\beta_2M$ ) in rheumatoid arthritis.

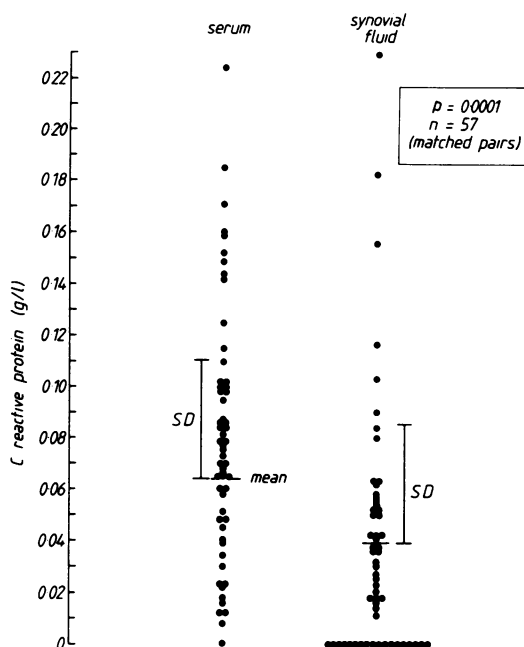


Fig. 3 Comparison of serum and synovial fluid C reactive protein in rheumatoid arthritis.

$\beta_2$ -microglobulin, and the results are shown in Fig. 1. There was a highly significant difference between the mean concentrations in the serum and SF ( $p=0.0001$ ). In 25 of the 33 paired samples (76%) the  $\beta_2$ -microglobulin concentration in the SF was higher than in the serum. On three occasions the concentrations were the same (9%), and in five the serum concentrations were higher (15%). There was a correlation between the serum and SF concentrations ( $r=0.50$ ,  $p<0.01$ ) (Fig. 2).

When the patients treated with sodium aurothiomalate and D-penicillamine were examined 10 paired samples of serum and SF were available for comparison before treatment with the drugs was started. In eight of these 10 samples (80%) the SF concentration of  $\beta_2$ -microglobulin was greater than that in the serum and on two occasions (20%) it was the same ( $p=0.01$ ). After the first six months of treatment with either sodium aurothiomalate or D-penicillamine the synovial effusion had disappeared in nine of these 24 patients.

For CRP determination 57 matched pairs of serum and SF from patients with RA were available for comparison (including all those in whom  $\beta_2$ -microglobulin was measured), and this is illustrated in Fig. 3. There was a highly significant difference between the serum and SF concentrations

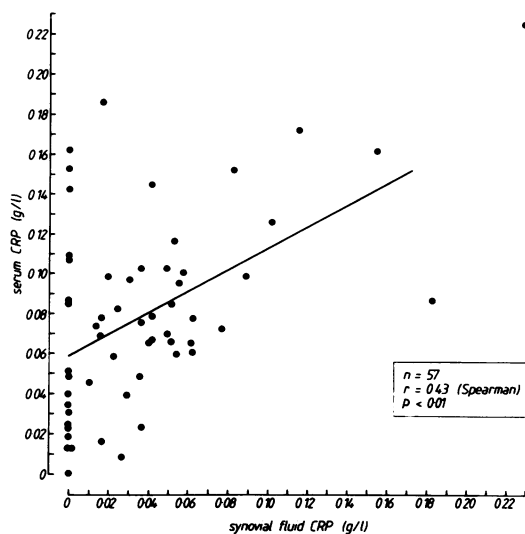


Fig. 4 Correlation between serum and synovial fluid C reactive protein (CRP) in rheumatoid arthritis.

( $p=0.0001$ ). On 49 occasions (86%) the serum concentration of CRP was higher than that in the SF. On two occasions (3%) the concentration was the same and on six occasions (11%) the SF concentration was higher than in the serum. In 18 of the 57 SF samples CRP was undetectable despite raised serum concentrations. There was a correlation between the serum and SF concentrations of CRP ( $r=0.43$ ,  $p<0.01$ ) (Fig. 4).

When serum and SF CRP concentrations were compared before treatment in the patients receiving sodium aurothiomalate and D-penicillamine 12 of the 14 available paired samples (86%,  $p=0.006$ ) showed higher serum concentrations. After six

months' treatment eight of the nine available paired samples (89%,  $p=0.02$ ) showed higher serum concentrations.

#### EFFECT OF SIX MONTHS' DISEASE MODIFYING ANTIRHEUMATIC DRUG TREATMENT ON DISEASE ACTIVITY

Twenty four patients given sodium aurothiomalate or D-penicillamine for six months showed highly significant improvements in body weight, morning stiffness, visual analogue pain score, grip strength, and Ritchie articular index, consistent with similar results reported previously.<sup>24</sup> There were no significant falls in peripheral blood lymphocytes expressing CD3 or CD8 surface markers,  $\kappa$  or  $\lambda$  light chains, or HLA-DR. Significant reductions occurred in the synovial membrane infiltrations of T lymphocytes (CD3+), the helper/inducer (CD4+), and suppressor/cytotoxic (CD8+) subsets, consistent with similar results reported previously.<sup>24</sup> The numbers of leucocyte common antigen positive cells fell but did not reach significance ( $p=0.06$ ).

Synovial membrane HLA class II antigen expression was reduced after treatment but only reached significance for HLA-DR ( $p=0.04$ ).

Table 1 gives detailed results of the serum immunochemistry, haemoglobin, and erythrocyte sedimentation rate after six months' treatment with sodium aurothiomalate or D-penicillamine. All patients had IgM rheumatoid factor (sheep cell agglutination titre), which fell significantly, as did serum CRP and IgG, IgA, and IgM. There were no significant changes in the serum complement components C3 and C4 or serum immune complexes. The mean concentration of  $\beta_2$ -microglobulin fell only slightly from 3.9 mg/l to 3.7 mg/l, and this was not significant. Before treatment the serum  $\beta_2$ -microglobulin showed a weak correlation with

Table 1 Laboratory findings: blood ( $n=24$ ). Changes in haemoglobin, erythrocyte sedimentation rate, and serum measurements after six months' treatment with gold or penicillamine

	SCAT* (log <sub>e</sub> )	CRP* (g/l)	$\beta_2$ M* (mg/l)	Immunoglobulins (g/l)			Hb* (g/l)	ESR* (mm/1st h)
				G	A	M		
On entry								
Mean	5.4	0.078	3.9	16.0	4.1	1.7	114	73
Range	2.8-8.5	0-0.225	0.8-9.2	8.7-25.5	1.3-8.2	0.5-4.0	79-142	29-135
After 6 months								
Mean	4.0	0.046	3.7	13.7	3.2	1.4	126	37
Range	0-9.2	0-0.185	1.9-7.0	8.6-19.3	0.9-7.9	0.6-3.8	79-154	5-90
p Value†	0.004	0.01	NS	0.02	0.02	0.02	0.001	0.00001

\*SCAT=sheep cell agglutination titre; CRP=C reactive protein;  $\beta_2$ M= $\beta_2$ -microglobulin; Hb=haemoglobin; ESR=erythrocyte sedimentation rate.

†Wilcoxon matched pairs signed ranks test.

Normal ranges: CRP 0-0.015 g/l;  $\beta_2$ M 1.5-2.2 mg/l; IgG 7.5-16.7 g/l; IgA 0.9-4.5 g/l; IgM 0.4-3.7 g/l.

Table 2 Laboratory findings—synovial fluid (eight matched pairs). Changes in synovial fluid immunochemistry after six months' treatment with gold or penicillamine

	CRP* (g/l)	$\beta_2$ M (mg/l)	Immunoglobulins (g/l)			Complement (g/l)	
			G	A	M	C3	C4
On entry							
Mean	0.064	6.1	11.5	2.6	1.0	0.63	0.13
Range	0-0.231	2.5-10.0	7.3-16.4	1.6-4.0	0.4-1.4	0.45-0.91	0.04-0.38
After 6 months							
Mean	0.024	5.0	5.0	0.8	0.4	0.25	0.05
Range	0-0.052	1.6-6.7	1.3-10.1	0.5-1.6	0.2-0.8	0.2-0.26	0.05-0.05
p Value†	NS	NS	0.02	0.02	0.01	0.02	NS

\*CRP=C reactive protein;  $\beta_2$ M= $\beta_2$ -microglobulin.

†Wilcoxon matched pairs signed ranks test.

serum CRP ( $r=0.41$ ,  $p=0.04$ ) but with none of the other immunochemical variables. After six months' sodium aurothiomalate or D-penicillamine treatment there was still a correlation between the serum  $\beta_2$ -microglobulin and serum CRP ( $r=0.63$ ,  $p=0.01$ ).

Table 2 shows the detailed results of the SF indices before and after six months' treatment (where matched pairs of SF were available). There were significant falls in the SF IgG, IgA, and IgM, and C3 component of complement, and downward but insignificant changes in CRP,  $\beta_2$ -microglobulin, and immune complexes. No significant correlations were found between the SF  $\beta_2$ -microglobulin concentrations and the other SF immunohistochemical variables before or after six months' sodium aurothiomalate or D-penicillamine treatment, but SF CRP correlated with SF IgG ( $r=0.71$ ,  $p=0.03$ ) and IgA ( $r=0.79$ ,  $p=0.01$ ). Pretreatment serum  $\beta_2$ -microglobulin showed a correlation with the visual analogue pain score ( $r=0.62$ ,  $p=0.009$ ) and a negative correlation with grip strength ( $r=-0.45$ ,  $p=0.03$ ). Synovial fluid  $\beta_2$ -microglobulin showed no relevant correlations. After six months' treatment the duration of early morning stiffness correlated weakly with serum  $\beta_2$ -microglobulin ( $r=0.42$ ,  $p=0.03$ ) and SF  $\beta_2$ -microglobulin ( $r=0.42$ ,  $p=0.03$ ). Serum and SF CRP concentrations showed no correlations.

No strong correlations were found between serum and SF  $\beta_2$ -microglobulin and peripheral blood leucocytes and subsets.

## Discussion

$\beta_2$ -Microglobulin has previously been investigated as a serological marker to assess disease activity in connective tissue disorders. Talal *et al* found increased concentrations of  $\beta_2$ -microglobulin in the plasma and SF of patients with RA when compared

with normal controls,<sup>33</sup> and Crisp *et al* found raised concentrations of  $\beta_2$ -microglobulin in the plasma of patients with RA compared with patients with osteoarthritis.<sup>34</sup> Manicourt *et al* found significantly increased concentrations of  $\beta_2$ -microglobulin in the SF from patients with active RA (mean 4.86 mg/l) when compared with SF from patients with gout or pseudogout (mean 2.0 mg/l) and osteoarthritis (mean 1.2 mg/l).<sup>12</sup> Latt *et al* found raised serum concentrations of  $\beta_2$ -microglobulin in seropositive patients with RA treated with a non-steroidal anti-inflammatory drug when compared with normal individuals, but not when compared with patients with RA treated with gold.<sup>35</sup> We too have found increased concentrations of  $\beta_2$ -microglobulin in the serum of our patients with active RA before sodium aurothiomalate or D-penicillamine treatment (mean concentrations in the patients with RA 3.9 (SD 1.9) mg/l compared with the normal range of 1.5-2.2 mg/l). The mean SF concentration of  $\beta_2$ -microglobulin in our patients was 6.1 (2.2) mg/l, and although we have not examined the concentrations in SF from other arthritides, our results in RA SF are consistent with those published previously.

In theory, it seems reasonable to speculate that if disease activity in RA is paralleled by raised tissue and blood levels of lymphocytes, and  $\beta_2$ -microglobulin is a product of these cells, then there might be a correlation between serum or SF  $\beta_2$ -microglobulin concentrations and synovial membrane immunohistology. Moreover, suppression of disease activity and reduced tissue lymphocyte infiltration might be associated with a concomitant fall in both serum and SF  $\beta_2$ -microglobulin concentrations. Manicourt *et al* found that in patients with RA there was a linear correlation between the SF  $\beta_2$ -microglobulin concentrations and the 'joint count', erythrocyte sedimentation rate, latex fixation test, and the SF lymphocyte count ( $r=0.89$ ,  $p<0.001$ ).<sup>12</sup> The

same authors found that plasma concentrations of  $\beta_2$ -microglobulin in RA paralleled the peripheral blood lymphocyte count and suggested that the concentrations reflected the total lymphoid mass or membrane turnover of the lymphoid tissue in RA.<sup>11</sup> On the other hand, Sjoblom *et al* found a poor correlation between serum  $\beta_2$ -microglobulin and clinical or laboratory parameters, including Ritchie articular index, erythrocyte sedimentation rate, orosomucoid, fibrinogen, CRP, and IgG.<sup>14</sup> Strom and Evrin studied 50 patients with active RA and found a tendency towards higher serum  $\beta_2$ -microglobulin concentrations in the more severely affected but also a poor correlation with serum acute phase reactants.<sup>13</sup>

Rheumatoid arthritis is a disease characterised by lymphocytic proliferation within the synovial membrane and examination of Fig. 1 clearly shows that the mean concentration of  $\beta_2$ -microglobulin was greater in SF than in serum; this was true for 25/33 (76%) of the matched pairs, pointing strongly to a local production of  $\beta_2$ -microglobulin within the synovial membrane of inflamed joints. This is not unexpected as  $\beta_2$ -microglobulin forms part of the HLA class I light chain and is actively produced and secreted by lymphocytes,<sup>2, 3, 6</sup> which are abundant within the RA synovial membrane.

Figure 2 shows the correlation between serum and SF  $\beta_2$ -microglobulin concentrations. In view of the heavy lymphocytic infiltrate within the RA synovial membrane we postulate that this may represent diffusion of the molecule out of the synovium and into the peripheral blood.

Figure 3 shows that the mean CRP concentration was greater in serum than in the SF, which might be expected as CRP is primarily produced by the liver.<sup>16</sup> Rowe *et al* found that SF CRP concentrations were lower than would be predicted if the molecule were simply diffusing in from the serum.<sup>36</sup> They suggested a specific binding or consumption of CRP within the synovium, and it is known that after the hepatic synthesis of CRP the molecule moves to the site of injury or inflammation<sup>37</sup> and can bind to lymphocytes or phagocytic cells.<sup>38</sup> In 18 of 57 SF samples from our patients CRP was undetectable despite considerably raised serum concentrations, and this is consistent with these previous observations.

We determined the effect of the drugs sodium aurothiomalate and D-penicillamine upon serum and SF concentrations of  $\beta_2$ -microglobulin and CRP in 24 patients with active RA, in relation to clinical, haematological, immunochemical, and histological indices of inflammation. There was a good clinical and laboratory response to treatment. Where paired samples were available the SF  $\beta_2$ -micro-

globulin was measured but showed no significant fall. It is important to note, however, that nine of the 24 patients treated with sodium aurothiomalate or D-penicillamine lost their knee joint effusions after six months and were therefore not included in the analysis. This loss of effusion implies a good clinical response to these drugs and perhaps only partial improvement in those patients in whom the effusion persisted. This may be explained by our observations that despite clinical improvement there were still significant synovial membrane infiltrates of leucocytes and T lymphocytes present in the patients treated with sodium aurothiomalate or D-penicillamine. Also, large numbers of cells bearing HLA class II antigens remained, which in the RA synovial membrane probably represent macrophages, other antigen presenting cells, B lymphocytes, and activated T lymphocytes. The persistence of this marked, albeit reduced, cellular infiltrate despite an apparently good clinical response may be the explanation for the continued increase of both serum and SF concentrations of  $\beta_2$ -microglobulin. After six months' treatment serum CRP concentrations fell significantly, but although the mean value of SF CRP fell by more than threefold and the median value by almost as much, this did not reach statistical significance.

In summary, our experience is similar to that of Sjoblom *et al*<sup>14</sup> and Strom and Evrin.<sup>13</sup> Thus although we showed a correlation between SF  $\beta_2$ -microglobulin concentrations and the visual analogue pain score and a negative correlation with grip strength, there was a poor correlation with the clinical and laboratory variables overall. Nevertheless, this was better than for CRP. Serum  $\beta_2$ -microglobulin did show a correlation with CRP, probably only reflecting the fact that both these markers are raised in rheumatoid inflammation. Although SF  $\beta_2$ -microglobulin showed some correlations with the synovial membrane infiltrate of cells bearing the leucocyte common antigen, HLA-DQ, and HLA-DP, this was inconsistent as the improvement in immunohistology after treatment was not reflected by a fall in the SF  $\beta_2$ -microglobulin. In our study serum and SF  $\beta_2$ -microglobulin proved to be of limited value in assessing improvement of disease activity after the first six months of treatment with sodium aurothiomalate or D-penicillamine in the patients with RA, who had shown considerable clinical, immunochemical, and synovial membrane improvement. Possibly, a longer period of time is required before a fall in the serum or SF  $\beta_2$ -microglobulin concentration becomes apparent. On the other hand, serum CRP concentrations did fall significantly and SF concentrations appreciably after six months' treatment, and thus in the short term

CRP seems to be a more useful index than  $\beta_2$ -microglobulin in reflecting overall disease activity in RA.

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