

# Serum MMP-3 in rheumatoid arthritis: correlation with systemic inflammation but not with erosive status

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## Abstract

**Objective.** Metalloproteinases (MMP) play an important role in the remodelling of the extracellular matrix. However, evidence that they are responsible for tissue damage in pathological situations remains circumstantial. Stromelysin (MMP-3) production is increased in rheumatoid arthritis (RA), and has been proposed as a marker of joint damage. The relevance of serum levels of MMP-3 to erosions in RA was studied.

**Methods.** Fifty-three patients with active RA of >5 yr duration and with available X-rays were stratified according to disease duration. Hand X-rays were scored for erosions. Patients were then classified into upper and lower quartiles. Serum MMP-3 levels were compared between these two groups.

**Results.** No significant differences in serum MMP-3 were seen between high and low eroders. A statistically significant correlation was observed between sMMP-3 and erythrocyte sedimentation rate and C-reactive protein.

**Conclusions.** Serum MMP-3 is not an independent marker of joint damage, but is correlated with systemic inflammation. Its precise role in joint damage in RA remains to be elucidated.

**KEY WORDS:** Rheumatoid arthritis, Stromelysin, MMP-3, Inflammation, Erosion.

Stromelysin-1, or matrix metalloproteinase (MMP)-3, is an enzyme with a broad substrate specificity and which is capable of degrading connective tissue matrix components such as proteoglycan, laminin, fibronectin, collagen IV and other denatured collagens. In addition to matrix degradation, MMP-3 can activate other MMPs, such as collagenase (MMP-1), by proteolysis of the inactive proenzyme into its active state. Expression of MMP-3 with other metalloproteinases, such as MMP-1 and MMP-9, is considerably enhanced in inflammatory joint diseases, including rheumatoid arthritis (RA), seronegative arthritis and gout. Increased levels of MMP-3 were detected in synovial fluids [1, 2] and in sera of RA patients [3, 4]. It has been suggested that serum MMP-3 reflects systemic spillover from the increased amounts present in the inflamed joint, and therefore its measurement may be a useful marker for more destructive joint disease in RA [3]. The correlation between serum MMP-3 and radiological joint damage is controversial; some authors claimed a correlation with Steinbrocker grading [5], while others did not find one [6, 7].

Radiological progression of RA is correlated with genetic, clinical and inflammatory parameters [8]. The

most precise method of investigating the relevance of a particular marker to disease progression is by long-term prospective studies. In the absence of such data, we propose an alternative way of investigating the relevance of a particular marker in radiological progression, by comparing the level or prevalence of a marker(s) in patients who are located at the two extremes of the erosion spectrum. Long-term studies have shown that erosions progress more or less at a constant rate during the course of RA [9]. By comparing patients who have more or less erosive disease, if the marker has a direct relevance in the erosive process, any qualitative or quantitative differences of the marker in question will be highlighted.

We have performed a cross-sectional study of a cohort of RA patients with active disease who were classified as having 'more erosive' or 'less erosive' disease. Serum MMP-3 levels in these two populations were compared, as were standard demographic variables. Our results indicated that serum MMP-3 is a marker of systemic inflammation, and is not by itself a discriminator of cumulative joint damage.

## Methods

### *Patients*

Fifty-three consecutive RA patients who attended the rheumatology clinic of our service were identified. They all fulfilled the ACR criteria for RA [10] and had >5 yr

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of disease, and current hand X-rays were available for analysis. All patients had active disease as evidenced by swollen and tender joint counts and/or elevated erythrocyte sedimentation rate (ESR).

#### Radiological scoring

A modified Larsen score was used to score for joint damage in both hands. This method has been adapted by Rau, and has been validated [11, 12]. All films were antero-posterior (AP) views and were scored by two experienced rheumatologists (AS and AMC). The inter-observer correlation of the scoring method was high ( $r = 0.937$ ;  $P < 0.0001$ ).

#### Biological measurements

The double-antibody ELISA for proMMP-3 was performed according to the manufacturer's instructions (Binding Site, Birmingham, UK) to assay for serum MMP-3 [4]. The assay detects MMP-3 in the range between 20 ng/ml and up to 480 ng/ml. Serum samples were stored at  $-70^{\circ}\text{C}$  prior to analysis. Measurements of rheumatoid factor and C-reactive protein (CRP) were performed by nephelometry.

#### Statistical analyses

Statistical analyses were performed using the program JMP (SAS Institute Inc., Cary, NC, USA). The Wilcoxon rank sum test was used to compare MMP-3 levels between populations. Correlation coefficients and significance were calculated between MMP-3 and ESR and CRP.

## Results

Patients were scored for radiological progression of RA on plain AP hand X-rays. In order to identify patients who were 'more erosive' or 'less erosive', they were stratified into three groups according to the duration of their disease. The three groups are: (1) disease duration between 5 and 10 yr; (2) disease duration between 10 and 15 yr; and (3) disease duration  $> 15$  yr. 'More erosive' patients were defined as patients in the upper quartile of erosion score in each group, while 'less erosive' patients represented patients in the lowest quartile. The upper quartile patients from the three groups were pooled, as were the lower quartile patients. In total, 13 patients were assigned as high eroders and 13 as low eroders. The demographics of the patient groups are shown in Table 1.

Figure 1A shows the difference in radiological erosion scores between the 'more erosive' and 'less erosive' patients. Measurements of serum MMP-3 levels in 9/13 of the high eroders and 10/13 of the low eroders were performed, and showed no significant difference in the levels of serum MMP-3 between the two groups (Wilcoxon,  $P = 0.39$ ), although there was a tendency for high eroders to have slightly higher serum MMP-3 levels. Comparison of the two groups in terms of their clinical variables confirmed well-described associations between the presence of rheumatoid nodules and more

TABLE 1. Demographics of RA patients in the study

	Total group ( $n = 53$ )	High eroders ( $n = 13$ )	Low eroders ( $n = 13$ )
Mean age at onset $\pm$ S.D. (yr)	42.3 $\pm$ 13.9	44.4 $\pm$ 13.4	43.0 $\pm$ 19.0
Mean duration of disease $\pm$ S.D. (yr)	12.6 $\pm$ 6.8	13.1 $\pm$ 8.9	14.6 $\pm$ 7.9
Female:male	4:1	2:1	12:1
Mean erosion score $\pm$ S.D.		51.4 $\pm$ 15.6	4.3 $\pm$ 3.5
Mean CRP (mg/l) $\pm$ S.D.		59.1 $\pm$ 54.2	49.5 $\pm$ 53.4
Median RF titre (IU/l, normal: $>20$ )		107	39

destructive disease (9/13 vs 3/13 in the less erosive group), as well as higher average titres of serum rheumatoid factor.

Serum MMP-3 was measured along with CRP and ESR for the total group of patients, including patients who were intermediate between the high and low eroder groups. The results showed a significant correlation between CRP and serum MMP-3 levels, as well as ESR and sMMP-3 levels (Fig. 2). The correlation between sMMP-3 and ESR was 0.484 ( $t = 3.71$ ,  $P = 0.0006$ ), and that between sMMP-3 and CRP was 0.43 ( $t = 2.82$ ,  $P = 0.008$ ). These results indicate that MMP-3 is a marker of systemic inflammation.

## Discussion

There is considerable evidence that MMPs are capable of mediating matrix degradation leading to joint destruction. Immunohistological and *in situ* hybridization studies revealed the abundant presence of MMP-3 in rheumatoid synovial membranes [13, 14], while expression of collagenase (MMP-1), matrilysin (MMP-7) and stromelysin-2 (MMP-10) is more focal [15]. Increased serum levels of MMP-3 have also been demonstrated, and raise the possibility that its measurement may serve as a useful surrogate marker of joint damage.

Ideally, the question of whether raised serum MMP-3 is an independent marker of radiological progression should be answered by a prospective study. As no such data are available at present, we compared patients at the two extremes of the radiological spectrum, controlling for duration of disease. As erosions progress as a function of disease duration, a disease duration of  $> 5$  yr was deliberately chosen in order to be certain that patients with 'less erosive' disease were not simply due to a shorter disease duration. The demographic profile of the two groups was similar, apart from the male preponderance in the 'more erosive' group. This finding is contrary to general experience, but is not exceptional [16].

Radiological progression in RA is a continual process, although it appears to be more rapid during the early years of disease [9, 17]. If MMP-3 were a direct marker of this process, then one may expect higher serum levels

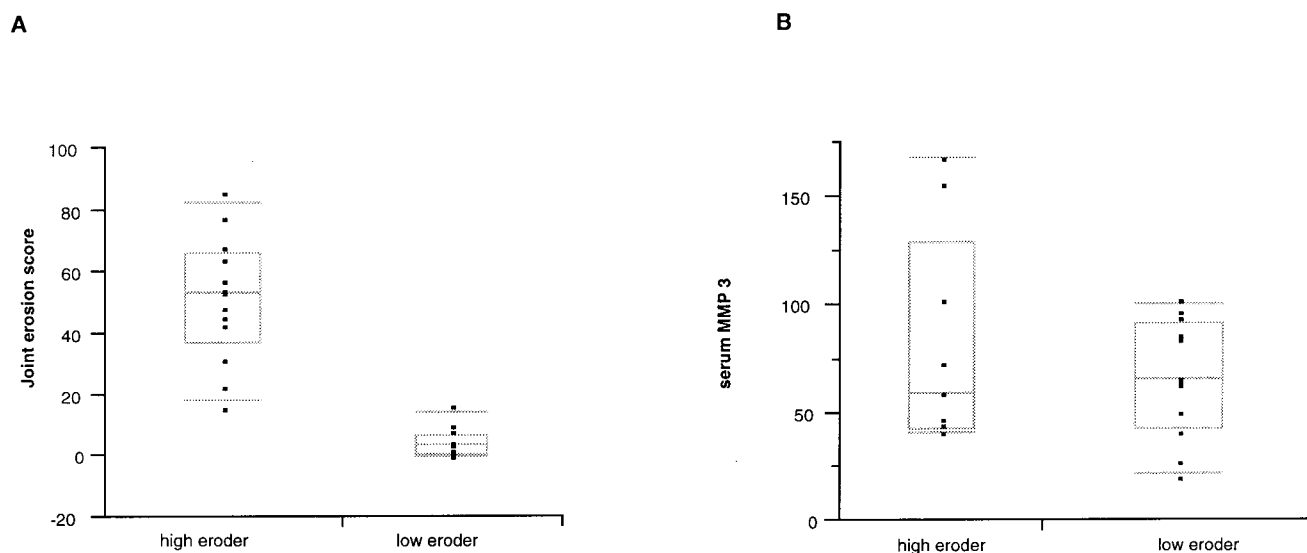


FIG. 1. (A) Box plot showing the median, upper and lower quartiles of the joint erosion scores of patients in the two subgroups. See the text for an explanation of the identification of subgroups. (B) Box plot showing median and quartiles of the serum MMP-3 levels (in ng/ml) in the two subgroups.

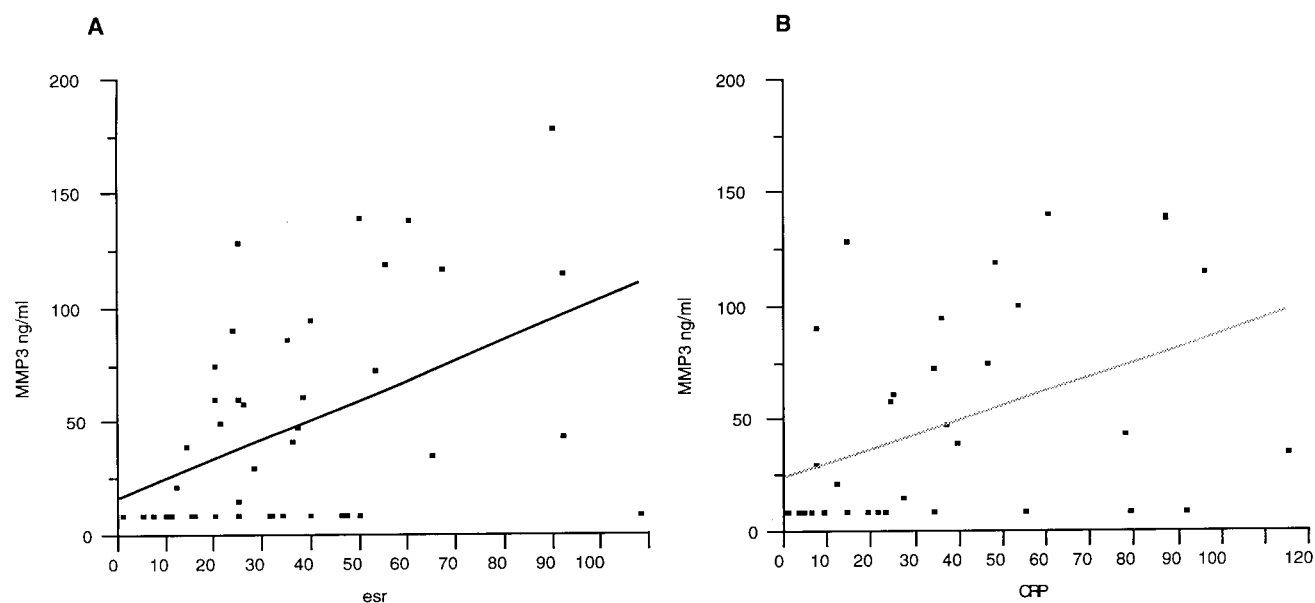


FIG. 2. (A) Correlation of serum MMP-3 level (ng/ml) and ESR (mm/h). (B) Correlation between serum MMP-3 level and CRP level (mg/l).

in patients with more destructive disease, as serum levels reflect joint production of MMP-3 to an extent. Our findings do not support this hypothesis. We found no significant difference in serum MMP-3 levels between the more and less erosive disease patients, although serum MMP-3 was slightly higher in the more erosive group. A lack of correlation between MMP-3 levels and erosions has also been noted in other studies [7, 18]. In neither of these studies were patients stratified by disease duration.

A correlation between serum MMP-3 levels and ESR and CRP was also observed by other authors [5, 7].

Our findings suggest that serum MMP-3 is a marker of systemic inflammation in RA. The absence of serum MMP-3 differences in this cohort of established (>5 yr of disease evolution) RA and its positive correlation with inflammatory markers may indicate that systemic inflammation may not be quite so important in established erosive disease. It also raises the question of the site of production of MMP-3 found in the serum. Although overspill from the joint is one cause, it is more likely that the source of circulating MMP-3 is the endothelial cell, which is known to be capable of synthesizing MMP-3 under stimulated conditions.

Although there is indirect evidence from animal studies to indicate that MMPs are involved in arthritis [19], the role of individual MMPs is less clear. Recently, results from experimental arthritis in MMP-3 knockout mice suggest that this enzyme is not necessary for joint damage, as deficient mice showed just as much bone and cartilage destruction as their wild-type controls [20]. Thus, joint damage seems to be mediated by a large number of different enzymes which are augmented in chronic inflammation, and MMP-3 may only have a minor role in this process. These results do not support the measurement of serum MMP-3 as an independent marker of joint damage in RA.

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