Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis

M. J. Green, A. K. S. Gough, J. Devlin, J. Smith, P. Astin, D. Taylor1 and P. Emery

Objective. Expression and activation of matrix metalloproteinases such as MMP-3 (stromelysin-1) and MMP-1 (collagenase-1) are increased in patients with rheumatoid arthritis (RA). Previous negative reports of their value as predictors of joint damage may be due to the lack of a large longitudinal study of early RA patients. This study evaluated their use in assessing early untreated patients with RA and predicting subsequent joint damage.

Methods. Ninety-eight patients with early untreated RA of less than 12 months duration and 20 normal controls had baseline serum samples tested with a double-antibody enzyme-linked immunosorbent assay for each of MMP-1 and MMP-3. The subsequent changes in Larsen score (ΔLarsen) and Health Assessment Questionnaire (ΔHAQ) over the first 12 months were recorded.

Results. Baseline serum levels of MMP-3 and MMP-1 correlated significantly with baseline C-reactive protein (CRP) (r = 0.42 and 0.49, P < 0.001), ΔHAQ (r = 0.32 and 0.30, P < 0.01) and ΔLarsen (r = 0.23 and 0.32, P < 0.05) respectively. Analysis of the group of patients with a normal CRP at presentation (n = 21) showed correlation of the baseline MMP-3 and MMP-1 with the presence of erosive disease during the first 12 months (r = 0.52 and 0.65 respectively, P < 0.05). Logistic regression analysis, in the patients who were non-erosive at presentation, showed that the strongest correlation with progression in Larsen score was the baseline MMP-3 level (r = 0.30, P = 0.01).

Conclusions. Baseline serum MMP-1 and MMP-3 levels correlate with disease activity and predict functional and radiographic outcome in early untreated RA. They may have a particular value in predicting the progression of erosive disease in patients who are not erosive at presentation.

KEY WORDS: Early, Arthritis, Prognosis, Outcome, Collagenase, Stromelysin.
studied for their ability to predict progression of RA in terms of joint damage. Early studies failed to show a correlation between serum MMP-3 and MMP-1 levels and the progression of RA in terms of joint damage [4–6]. However, these negative results may have been due to the study design as the studies were conducted in cohorts of patients with established disease, often tested in a cross-sectional manner, with relatively small numbers. Three more recent studies have suggested a role for the use of such markers in early disease [7–9]. The present study addressed this question and assessed the performance of these serum tests in subpopulations of patients who were either non-erosive or who had a normal acute-phase response at presentation. The study was performed using a large cohort of patients with early untreated RA who had been followed longitudinally and documented in a systematic way.

Patients and methods

Ninety-eight patients with untreated RA (satisfying the 1987 ACR classification criteria for RA) and with less than 12 months’ duration of symptoms had serum samples taken prior to the commencement of treatment. Patients were treated as clinically indicated by their physician during the course of the study. Twenty volunteers without clinical evidence of arthritis or any other inflammatory disease also had serum samples taken, which were used as controls. Patients were assessed according to a standard protocol, which included a complete history, physical examination, including a recording of swollen joint counts (SJC), tender joint count (TJC), Health Assessment Questionnaire (HAQ) calculated on a 0–24 scale, 10 cm visual analogue scale scores for patient assessment of disease activity and pain, and recording the observer assessment of disease activity (scored 0–4). Blood was taken for determination of the full blood count, plasma viscosity, erythrocyte sedimentation rate (ESR), electrolytes, glucose, liver enzymes, calcium, urate, thyroid function, rheumatoid factor (RF), C-reactive protein (CRP), antinuclear factor, and immunoglobulins, rubella and parvovirus IgM titres where clinically indicated. Blood was also tested for the HLA-DRB1*04 and *01 alleles. Radiographs of the hands and feet were taken.

Double-antibody ELISA for MMP-1 and MMP-3

Samples were stored at −20°C and later tested simultaneously with a double-antibody enzyme-linked immunosorbent assay (ELISA) for each of MMP-1 and MMP-3 according to the manufacturer’s instructions (The Binding Site, Birmingham, UK). It is to be noted that different commercially available enzyme assays have been raised against differing epitopes and may be measuring various serum constituents. The antibody used in both kits was raised to the inactive pro-MMP, which is the predominant form found in biological fluids [10]. The kits use polyclonal antisera and therefore recognize a range of the epitopes of the MMP antigen. Whilst there are no data on what proportion recognizes pro-MMP or active bound to TIMPs there is a close correlation with other kits. When this assay for MMP-3 was compared with another commercially available assay which uses two monoclonal antibodies (from Fuji, Japan) a correlation of r=0.98 was found for serum MMP-3 in 23 patients with RA (unpublished data, D. Taylor, The Binding Site). This indicates that, despite using different antibodies, both MMP-3 assays measure the same protein in the circulation.

Radiographic data

A Larsen score was recorded for joint damage in both hands at baseline and at 12 months disease duration [11]. All films were scored blinded, in chronological order, on anteroposterior views by two experienced rheumatologists. The changes in Larsen score (ΔLarsen) and HAQ (AHAQ) over the first 12 months were calculated along with the mean and median progression in Larsen scores.

Statistical analysis

Significant differences between groups were determined by the Mann–Whitney U-test. The Pearson correlation coefficient was used to examine relationships between measurements. A stepwise linear regression analysis was performed using all of the potential predictors available at baseline. A further analysis of the data was carried out when the patients were divided dichotomously into those with a high rate of bone loss and those with a low rate. Patients with a ΔLarsen above the mean were classified as having the greatest loss and patients below the mean as having a low rate of loss. Further analyses were performed on two separate subpopulations of patients: (i) patients who were found to have a normal CRP at baseline (n=21); and (ii) those who were non-erosive on their baseline radiographs (n=81).

Results

The patients’ demographic details are shown in Table 1. They indicate a typical patient population with early RA. The details for the two subgroups (CRP ≤ 10 mg/dl

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole group (n=98)</th>
<th>CRP ≤ 10 mg/dl (n=21)</th>
<th>Non-erosive at baseline (n=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (days): mean (s.e.)</td>
<td>193 (13)</td>
<td>202 (22.5)</td>
<td>188 (14)</td>
</tr>
<tr>
<td>Age (yr): mean (s.e.)</td>
<td>58 (1.4)</td>
<td>55 (2.8)</td>
<td>57.8 (1.48)</td>
</tr>
<tr>
<td>HAQ: median (range)</td>
<td>12 (0–23)</td>
<td>9 (0–21)</td>
<td>11 (0–23)</td>
</tr>
<tr>
<td>CRP (mg/l): mean (s.e.)</td>
<td>35.5 (3.8)</td>
<td>7.76 (0.4)*</td>
<td>33.3 (4.1)</td>
</tr>
<tr>
<td>ESR (mm/h): mean (s.e.)</td>
<td>40.1 (2.7)</td>
<td>15 (2.0)*</td>
<td>38.57 (2.9)</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>66</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>RF-positive (%)</td>
<td>61</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>HLA-DRB1*01 or 04 (%)</td>
<td>61</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>DMARD-naive at entry (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*P < 0.001 compared with the whole group.
or non-erosive at baseline) are also shown in Table 1. They demonstrate that the only differences from the whole group are in the measures of acute-phase response, which were, as expected, lower in those patients selected with a normal baseline CRP. The mean disease duration at presentation was 6 months, with a mean age of 58 yr, 66% were female, 61% were seropositive for RF, 61% had either HLA-DRB1*01 or 04, 15% were erosive at baseline and all patients were DMARD-naive at study entry. The mean (s.d.) rate of progression in Larsen score over the 12 month period was 12.9 (13.6) for the total group, 24.6 (16.9) for patients who were erosive at baseline and 10.9 (11.9) for the non-erosive group (Table 2). The corresponding median (range) values were 7 (0–53), 27 (1–49) and 6 (0–53).

Mean serum MMP-1 and MMP-3 were significantly greater in RA patients than in controls (MMP-1, 41.8 vs 10.5 ng/ml, P < 0.001; MMP-3, 66.1 vs 22.6 ng/ml, P < 0.001). Patients were divided into those with a low and those with a high D\text{Larsen} (see Patients and methods). Patients with a high rate of X-ray progression were found to have significantly greater baseline serum levels of both MMP-1 and MMP-3 compared with patients with a low rate of progression (MMP-1, mean value 56.0 vs 39.0 ng/ml, P < 0.001; MMP-3, 60.0 vs 39.4 ng/ml, P < 0.001).

Pearson correlation coefficients for baseline MMP-1, MMP-3 and CRP compared with baseline CRP, D\text{Larsen} and D\text{HAQ} are shown in Table 3. Baseline serum levels of MMP-3 and MMP-1 correlated significantly with baseline CRP (r = 0.42 and 0.49 respectively), D\text{HAQ} (r = 0.32 and 0.30) and D\text{Larsen} (r = 0.23 and 0.32) (P < 0.05 for all). The scattergrams in Fig. 1a and b show the distribution of MMP-1 and MMP-3 compared with the change in Larsen score over the first 12 months.

**Stepwise logistic regression analysis for all patients**

A stepwise logistic regression analysis was performed using the following variables available at baseline: MMP-3, MMP-1, presence of erosions (yes/no), ESR, CRP, RF latex, RF titre, Ritchie articular index, female sex (yes/no), HAQ, and the presence of HLA DRB1*01, *DW4, *DW10 or DW14. The cut-off for entry and removal from the model was 0.05 and 0.10 respectively. The result of this is shown in Table 4. The overall r value for the model was 0.50 and included, in order of strongest indicator, RF titre, erosive disease at presentation, HLA-DRB1*01 and baseline serum MMP-3 level.

**TABLE 2. Progression in Larsen score in patients erosive and non-erosive at baseline, and the total cohort**

<table>
<thead>
<tr>
<th></th>
<th>Mean (s.d.)</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erosive</td>
<td>24.6 (16.9)</td>
<td>27 (1–49)</td>
</tr>
<tr>
<td>Non-erosive</td>
<td>10.8 (11.9)</td>
<td>6 (0–53)</td>
</tr>
<tr>
<td>Total</td>
<td>12.9 (13.6)</td>
<td>7 (0–53)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Baseline CRP (r)</th>
<th>D\text{Larsen} (r)</th>
<th>D\text{HAQ} (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3</td>
<td>0.42 (94)</td>
<td>0.23 (98)</td>
<td>0.32 (94)</td>
</tr>
<tr>
<td>MMP-1</td>
<td>0.49 (95)</td>
<td>0.32 (94)</td>
<td>0.30 (95)</td>
</tr>
<tr>
<td>Baseline CRP</td>
<td>NA</td>
<td>0.31 (94)</td>
<td>0.43 (95)</td>
</tr>
</tbody>
</table>

P < 0.05 for all values shown.

**TABLE 4. Stepwise linear regression analysis for prediction of progression of radiographic damage (D\text{Larsen}) (n=91)**

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R²</th>
<th>Adjusted R²</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.330 a</td>
<td>0.109</td>
<td>0.099</td>
<td>11.7</td>
</tr>
<tr>
<td>2</td>
<td>0.403 b</td>
<td>0.162</td>
<td>0.143</td>
<td>11.4</td>
</tr>
<tr>
<td>3</td>
<td>0.456 c</td>
<td>0.181</td>
<td>0.181</td>
<td>11.15</td>
</tr>
<tr>
<td>4</td>
<td>0.500 d</td>
<td>0.215</td>
<td>0.215</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Dependent variable: D\text{Larsen} over 12 months.

aPredictors: (constant), RF titre.
bPredictors: (constant), RF titre, erosive.
cPredictors: (constant), RF titre, erosive, HLA-DRB1*01.
dPredictors: (constant), RF titre, erosive, HLA-DRB1*01, MMP-3.

![Fig. 1](https://academic.oup.com/rheumatology/article-abstract/42/1/83/1779749/101.777949)
Subanalysis of patients with normal CRP at baseline

Analysis of the group of patients with a normal CRP (≤10 mg/dl) at presentation (n=21) showed significantly lower levels of MMP-1 and MMP-3 than in patients with a high CRP (>10 mmol/l; n=74); MMP-1, 31.7 vs 52.6 ng/ml, P=0.002; MMP-3, 38.8 vs 52.9 ng/ml, P<0.001. A strong correlation was also found between the baseline MMP-1 and MMP-3 levels and the presence of erosive disease during the 12-month study (r=0.65 and 0.52 respectively, P<0.05) in this subpopulation. In addition, the baseline CRP correlated with the presence of erosive disease during the 12-month study (r=0.47, P<0.05), whereas other traditional predictors of X-ray damage, such as the RF titre (r=0.085, P<0.72) and the presence of the shared epitope (r=0.25, P=0.27), showed no significant correlation. There was no significant correlation with progression in radiographic damage (MMP-1, r=0.17, P=0.48; MMP-3, r=-0.339, P=0.13; CRP, r=-0.02, P=0.093; RF titre, r=-0.17, P=0.45). Stepwise regression analysis also revealed no significant associations. These latter results may have resulted from a type 2 error due to the small sample of patients in this subanalysis.

Subanalysis of patients non-erosive at presentation

Ninety-five of the patients had data recorded on whether they had erosions on their baseline X-ray. Of these 95 patients, 81 (85%) had no evidence of erosive disease and 14 (15%) had evidence of erosive disease. Stepwise logistic regression analysis in these patients found that the strongest correlation with progression in Larsen score was the baseline MMP-3 level (r=0.30, P=0.01) (Table 5). The only other significant predictor that came out in the model was the presence of HLA DRB1*01, which improved the overall r value to 0.4.

Discussion

Expression and activation of MMPs such as MMP-3 (stromelysin-1) and MMP-1 (collagenase-1) are considerably enhanced in the synovial fluid of patients with RA [11]. Secreted in the proenzyme form by synovial fibroblasts and chondrocytes, the majority of synovial fluid and serum MMP is found in this inactive form [11]. In vitro studies have demonstrated the ability of active MMPs to denature virtually all cartilage components (proteoglycan, laminin, fibronectin and collagen IV) [12] and hence they may promote joint destruction in vivo.

Concentrations of MMPs in synovial fluid of patients with RA are several hundred-fold higher than in serum, but a strong correlation between the two sites [13] has been demonstrated and serum MMP-3 is reduced by a single intra-articular steroid injection. In contrast, severe sepsis results in markedly elevated levels of CRP but not MMPs [13]. These data suggest that MMP production may be a more specific test for intrasynovial inflammation than CRP. The latter is produced in the liver, distant from the site of inflammation and under the action of many different messenger cytokines. There is thus considerable interest in the development of serum markers of activity of the MMPs which could predict the progression of X-ray damage. A few studies have been published previously with some conflicting results [4-9] and the potential reasons for this are discussed in the introduction. The serum levels of MMP-3 in the present study are similar to those in previous series [4, 13], although it is to be noted that the variation in published results has often been wide [14, 15]. The two published studies examining serum MMP-1 in patients with RA have shown levels of 10 ng/ml [14] and 11.2 ng/ml [9], compared with 41 ng/ml in the present cohort. MMP-1 has been less extensively researched and it therefore remains to be seen whether the range varies as much as with MMP-3.

One recent study by Cunnane et al. [9] examined serum levels of MMP-1, MMP-3 and also tissue inhibitor of metalloproteinase-1 (TIMP-1) as independent predictors of radiographic damage in terms of a total erosion score. Briefly, this study showed significant correlations between the baseline serum MMP-1 level and the erosion score at presentation. In addition, they found a correlation between the area under the curve of serum MMP-1 level and progression of erosion score but not for a serial measure of inflammation (area under the curve for CRP). The area under the MMP-3 curve did not correlate with progression of damage but did correlate with a measure of inflammation. The authors later discuss the possibility that these data indicate uncoupling of the mechanisms associated with joint inflammation and articular erosions. In contrast, the present study indicates reasonably strong correlations between the baseline levels of MMP-1 and MMP-3 and baseline CRP (r=0.49 and 0.42 respectively, P<0.05) and further significant correlation between the baseline MMPs and progression of radiographic damage (r=0.32 and 0.23, P<0.05). The baseline MMP-3 level appeared in all three logistic regression models when predicting damage. The absence of the MMP-1 level in the model probably reflects its duplicity (cf. MMP-3) when explaining the variation of damage score rather than any clear advantage of one over the other. There were differences in the way the two studies were performed and analysed, which may explain the variation in results. Cunnane et al. [9] examined patients with up to 2 yr of disease duration who were followed over 18 months, and they used the MMP levels as serial measures over time. The main form

Table 5. Stepwise linear regression analysis for prediction of progression of radiographic damage (ΔLarsen) in patients who were non-erosive at baseline (n=47)

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R²</th>
<th>Adjusted R²</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.301*</td>
<td>0.090</td>
<td>0.078</td>
<td>7.37</td>
</tr>
<tr>
<td>2</td>
<td>0.400b</td>
<td>0.160</td>
<td>0.138</td>
<td>7.12</td>
</tr>
</tbody>
</table>

Dependent variable: ΔLarsen over 12 months.

*Predictors: (constant), MMP-3.

bPredictors: (constant), MMP-3, HLA-DRB1*01.
of analysis was univariate, the main outcome measure being a change in erosion score. The present study recruited patients with a shorter disease duration (<1 yr) and used a single baseline measure of MMPs as a predictor, and the results include multivariate analysis, the main outcome measure being the change in Larsen score over a shorter period of 12 months.

As in previous studies, the present results demonstrate that the serum levels of MMP-3 and MMP-1 were greater in RA patients compared with normal controls. These levels correlate with measures of disease activity, such as CRP, and were predictive of outcome in terms of radiographic progression and function. They were also shown to add significantly to a model predicting outcome in terms of X-ray progression, which included predictive markers such as RF titre, erosive disease present at baseline and the presence of HLA-DRB1*01. It is to be noted in this analysis that HLA-DRB1*04 did not come out as a significant predictor of damage. This may be due to the presence of HLA-DRB1*01 in the model and a high overall level of HLA-DRB1*04 in the cohort, which would reduce the power to show a difference.

The $r$ value of the model is 0.50 and therefore explains only 25% of the variation in X-ray progression. Imaging techniques such as magnetic resonance imaging can improve the definition of patient populations [16] and it remains to be seen whether using such techniques can improve the predictive ability of such a model to a level that would make it applicable to clinical practice.

Currently, one of the best available serum predictors of progressive X-ray damage is the level of CRP [17]. It has also been demonstrated that loss of bone mineral density correlates with patients with an elevated CRP, and suppression to a normal level at least stabilizes this loss [18]. This is an easy and inexpensive test to perform and is available universally. Its drawbacks include the fact that CRP is released at a site distant to the joint (i.e. the liver), is under the influence of several cytokines [mainly interleukin (IL)-1, IL-6 and TNF-$\alpha$] and is thus a non-specific marker of inflammation anywhere in the body. Furthermore, the predictive ability of the baseline CRP remains less clear [19] and some patients noted to have a normal CRP continue to erode. The presence of erosive disease at baseline has been associated with a poor outcome [20]. However, only 15% of patients in this study were erosive at presentation. Whilst this demonstrates that these patients were at a particularly early stage of disease, it also highlights the poor sensitivity of this test. Therefore, separate analyses were performed in patients who either had a normal CRP or who were non-erosive at baseline to elucidate whether MMP levels offered any additional benefit.

In the group of patients with a normal CRP at presentation, MMP-1 and MMP-3 levels correlated strongly with the presence of erosive disease, measured by plain radiographs of the hands, producing $r$ values of 0.65 ($P < 0.05$) and 0.52 ($P < 0.05$) respectively. This is consistent with the current understanding of the evolution of RA, whereby damage begins early and usually in peripheral joints, often with a normal CRP, and then progresses to involve large joints, at which point disease mass generates an elevated CRP and the systematic features of RA. It is therefore understandable that markers of local damage such as MMP-1 and MMP-3 would be particularly useful at this early stage of disease and prior to the onset of an elevated acute-phase response. However, it must be noted that the number of patients in this subgroup may be too small ($n = 21$) to draw firm conclusions.

The patients who were non-erosive at presentation are of particular interest in clinical practice as it is in these patients that the diagnosis of RA remains most questionable. A positive serum IgM RF has been shown to be the only universally available marker that consistently predicts progression of erosive disease in this group [21]. It is therefore of interest that the MMP-3 level came out as the strongest predictive marker of progressive X-ray damage in a stepwise linear regression analysis of this subgroup, over and above the presence of RF. The only other significant predictive factor was the presence of HLA-DRB1*01. Further work will demonstrate if this can be reproduced in other cohorts of patients with early RA. If, in this population of patients who are non-erosive at presentation, the predictive ability can be proven, it is conceivable that therapy could then be targeted as preventative rather than to modify damage alone.

In summary, baseline serum MMP-1 and MMP-3 levels correlate with disease activity and predict functional and radiographic outcome in early untreated RA. Serum levels may be particularly helpful in situations where traditional markers are less accurate (e.g. patients with a normal CRP and who have non-erosive disease at presentation).

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References
15. Ishiguro N, Ito T, Obata KI, Fujimoto N and Iwata H. Determination of stromelysin-1, 72 and 92 kDa type IV collagenase, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. J Rheumatol 1996;23:1599–604.