The significance of serum matrix metalloproteinase 3 in patients with early rheumatoid arthritis
Posthumus, Marcel Desiderius

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Chapter 4

Serum matrix metalloproteinase 3 in early rheumatoid arthritis is correlated with disease activity and radiological progression

Marcel D. Posthumus, Pieter C. Limburg, Johanna Westra, Miek A. van Leeuwen, and Martin H. van Rijswijk.

SUMMARY

Objective. To analyze the clinical significance of serial measurements of serum matrix metalloproteinase 3 (MMP-3) levels in relation to markers of disease activity and radiological progression in early rheumatoid arthritis (RA).

Methods. In a 3 year prospective study of 33 patients with early RA (symptoms < 1 year at entry) monthly measurements of serum MMP-3 were transformed into time-integrated values for 6 months periods for comparison with other markers of disease activity like swollen joint count (SJC), tender joint count (TJC), Ritchie articular index (RAI), the disease activity score (DAS), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and radiological progression, scored according to Sharp’s method, in which erosions and joint space narrowing are scored separately and combined to a total Sharp score.

Results. Significant correlations were found between serum MMP-3 and SJC, ESR, and CRP during all periods and between 6 and 30 months with the DAS. There were no correlations between serum MMP-3 and TJC or the RAI. During the first 12 months serum MMP-3 was only correlated with the item joint space narrowing of the Sharp score. After 12 months of follow-up it was also correlated with the total Sharp score and after 18 months it was correlated with all 3 items of the Sharp score. There was a wide inter-individual variation in the relation between serum MMP-3 and radiological progression but intra-individually this relation seemed to be rather constant.

Conclusion. Time-integrated values of serum MMP-3 are correlated with time-integrated values of other markers of disease activity such as joint swelling, ESR, CRP, and the DAS. Of the radiological scores, as outcome measures, especially joint space narrowing is closely correlated with cumulative serum MMP-3.

Key words: serum matrix metalloproteinase 3, stromelysin 1, early rheumatoid arthritis, disease activity, radiological damage.
INTRODUCTION
In rheumatoid arthritis (RA) cytokines play a major role in local joint inflammation and destruction as well as in the systemic acute phase response. The relationship between acute phase proteins, like C-reactive protein (CRP) and disease activity and radiological progression has been described. Although CRP appears to be a good parameter for prognostic purposes and for monitoring treatment effects, it is an indicator of inflammation in general which may be influenced by other stimuli of the acute phase response.

Matrix metalloproteinases (MMPs), which are locally produced and activated within the affected joint as a result of cytokine mediated stimulation, could be more specific markers for joint inflammation and especially destruction in rheumatic diseases. In RA matrix metalloproteinase 3 (MMP-3 = stromelysin 1) is of interest because it is thought to play a prominent role in the pathogenesis of matrix degradation in RA even though it is not the only key enzyme. MMP-3 is capable of degrading many components of the matrix in the synovial joint including proteoglycans, gelatins, laminin, fibronectin and collagen III, IV, IX, X. Moreover, MMP-3 is able to activate other matrix metalloproteinases like MMP-1, MMP-7, MMP-8, MMP-9 and MMP-13. The enzyme has been localized in the fibroblast-like synovial lining cells of rheumatoid synovium, and in RA cartilage. It is secreted as a latent pro-enzyme resulting in highly elevated MMP-3 levels in synovial fluid (SF). In RA serum MMP-3 is thought to originate from the inflamed joint because there are significant correlations between MMP-3 levels in serum and in SF, and between serum MMP-3 levels and the number of swollen joints and/or Lansbury articular index. Furthermore local therapies, like intra-articular corticosteroid injections cause significant reductions in serum MMP-3 levels.

In SF, levels of activated MMP-3 are also highly increased, but measurement of activated MMP-3 in serum is very difficult, if not impossible, because of the high serum levels of α2-macroglobulin that encloses the activated enzyme completely.

Elevated levels of serum MMP-3 have also been found in patients with SLE, MCTD, systemic sclerosis, gout, ankylosing spondylitis, calcium pyrophosphate arthritis, and psoriatic arthritis. Nevertheless, in RA serum MMP-3 could be a useful parameter to evaluate disease activity and outcome. Especially in early disease the use of markers of joint inflammation (disease activity) and joint destruction (radiological damage) are of importance for prognostic and therapeutical reasons. Therefore we analyzed in a prospective study the clinical significance of serial measurements of serum MMP-3 levels in relation to...
other markers of disease activity and radiological progression in early rheumatoid arthritis.

PATIENTS AND METHODS

Patients
Thirty-three RA patients with radiological progression during a follow-up of 3 years were selected from a cohort of 149 patients with RA according to the 1987 ACR criteria with joint symptoms existing less than one year at presentation and who had not previously received disease modifying antirheumatic drugs (DMARDs). These patients participated in a prospective follow-up study at the department of rheumatology at the Groningen University Hospital. During follow-up patients were treated with non-steroidal anti-inflammatory drugs and DMARDs as indicated clinically. Guidelines for the sequence of the different DMARDs were as follows: hydroxychloroquine or sulphasalazine as first choice therapy, followed in order by intramuscular gold, D-penicillamine, azathioprine, or methotrexate. Low dose corticosteroids could be administered as adjuvant therapy. In this cohort of 149 patients 43% had normal X-rays at presentation.

Out of this cohort of 149 RA patients 33 consecutive patients were selected who showed radiological progression of at least 10 or more points according to Sharp’s method during a follow-up of 3 years.

Clinical and laboratory investigations were performed at monthly intervals during a follow-up of 3 year. Radiographs of hands and feet were obtained at study entry and every 6 months during follow-up.

Clinical markers of disease activity
Fifty-two peripheral joints were examined for tenderness and soft tissue swelling. The following articular indices were determined: Ritchie articular index (RAI), tender joint count (TJC), swollen joint count (SJC), and the disease activity score (DAS) according to Van der Heijde with 3 variables: Ritchie articular index, number of swollen joints, and erythrocyte sedimentation rate (ESR).

Radiological analysis
Radiological damage in hands and feet was assessed by Sharp’s method with some modifications as described by Van der Heijde et al. By this method joint space narrowing (JSN) and erosions (ER) are scored separately and combined to a total Sharp score (TSS) with a maximum TSS of 448 points. The radiographs were scored without knowledge of clinical and laboratory data in chronological order per patient.
by two observers. The inter-observer agreement was 0.90 and the intra-observer agreements were 0.96 and 0.99 for the two observers respectively.

Laboratory analysis
Serum MMP-3 levels were determined with a MMP-3 ELISA developed in our own laboratory. In short, 96 well plates were precoated with F(ab), fragment of goat-anti-mouse IgG, 1µg/ml (Jackson Immunoresearch Labs, West Grove, PN, USA). Next a mouse monoclonal antibody against human MMP-3, clone 55-2-A4 (clone B), (Oncogene, Cambridge, MA, USA) was coated, 0.2 µg/ml. Serum samples were analyzed in two-fold serial dilutions in high performance ELISA buffer (CLB, Amsterdam, NL) and incubated during 1 hour. After washing bound MMP-3 was detected with a biotinylated polyclonal sheep-anti-MMP-3 (The Binding Site, Birmingham, UK) in combination with streptavidin-polyHRP (CLB, Amsterdam, NL). Peroxidase activity was determined using tetramethylbenzidin as substrate. MMP-3 levels were calculated at the linear range of the assay from a standard curve (10-1500 ng/ml) using a RA synovial fluid, which was standardized against the BIOTRAK® MMP-3 ELISA kit (Amersham,’s Hertogenbosch, NL). The intra-assay coefficient of variation (CV) was 8.9%, the inter-assay CV 10.1%. With an immunoblot we demonstrated that both the monoclonal and the polyclonal antibody reacted with active MMP-3, pro-MMP-3 as well as with MMP-3 bound to tissue inhibitor of matrix metalloproteinases (TIMP). Treatment of the sera with p-aminophenyl mercury acetate (APMA) to activate pro-MMP-3 did not influence results in the ELISA. Furthermore it was demonstrated that rheumatoid factors do not react in this assay and do not interfere with measurement of MMP-3 (data not shown). For normal values of serum MMP-3 the 95 percentile in healthy bloodbank donors was used (female < 20 ng/ml, male < 60 ng/ml).

CRP was measured by ELISA, ESR according to Westgren. IgM rheumatoid factor (RF) was measured by ELISA (normal value: < 10 IU/ml)).

Statistical analysis
As progression of radiological damage is a cumulative process, the production of MMP-3 was also expressed as cumulative values. Monthly levels of serum MMP-3 were plotted against time (weeks) and time-integrated values were obtained by calculation of the area under the curve (AUC). Cumulative values were calculated for each 6 month period concomitant with the X-ray intervals. Time-integrated values for the 6 month periods were indicated as δ-MMP-3, δ-TSS etc. In order to obtain comparable variables the serial data of CRP and the clinical variables were also transformed into time-integrated values.
Spearman’s rank correlation coefficient was used for the assessment of correlations between the different variables.

RESULTS
Baseline characteristics of the 33 patients with early RA are summarized in table 1.

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the 33 patients with early rheumatoid arthritis at study entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender, female/male (% female)</td>
</tr>
<tr>
<td>Disease duration (months)</td>
</tr>
<tr>
<td>IgM-RF positive (%)</td>
</tr>
<tr>
<td>Tender joint count</td>
</tr>
<tr>
<td>Swollen joint count</td>
</tr>
<tr>
<td>Ritchie articular index</td>
</tr>
<tr>
<td>Disease activity score</td>
</tr>
<tr>
<td>CRP (mg/l.)</td>
</tr>
<tr>
<td>ESR (mm/h.)</td>
</tr>
<tr>
<td>MMP-3 (ng/ml.)</td>
</tr>
<tr>
<td>Sharp score</td>
</tr>
<tr>
<td>Number of patients with normal X-rays (%)</td>
</tr>
</tbody>
</table>

Values are the median and range. Maximal scores: Tender and swollen joint count; 52, Ritchie articular index; 78, Disease activity score; 10.0, Sharp score; 448.

During follow-up 31 out of the 33 patients were treated with DMARDs. Mean values of serum MMP-3, CRP, joint swelling (of serum MMP-3, CRP, joint swelling etc) and radiological progression over 6 months periods are shown in figure 1.
FIGURE 1. Mean value over six months of monthly measured variables in an individual patient (●). The variables are MMP-3 (A), CRP (B), and swollen joint count (C). For radiological progression (D) the increment in total Sharp score over a period of six months in an individual patient is shown. The horizontal line represents the median.
Serum MMP-3 vs markers of disease activity

Correlation coefficients between time-integrated values for 6 months periods of serum MMP-3 (δ-MMP-3) and markers of disease activity, joint scores, DAS, ESR, and CRP are shown in table 2.

<table>
<thead>
<tr>
<th>Time interval months</th>
<th>δ-CRP</th>
<th>δ-ESR</th>
<th>δ-DAS</th>
<th>(δ-SJC)</th>
<th>(δ-TJC)</th>
<th>δ-RAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>0.81 ***</td>
<td>0.62 ***</td>
<td>0.23</td>
<td>0.54 ***</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>6-12</td>
<td>0.90 ***</td>
<td>0.57 ***</td>
<td>0.45 **</td>
<td>0.56 ***</td>
<td>0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>12-18</td>
<td>0.88 ***</td>
<td>0.74 ***</td>
<td>0.43 *</td>
<td>0.57 ***</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>18-24</td>
<td>0.86 ***</td>
<td>0.69 ***</td>
<td>0.62 ***</td>
<td>0.78 ***</td>
<td>0.28</td>
<td>0.25</td>
</tr>
<tr>
<td>24-30</td>
<td>0.81 ***</td>
<td>0.76 ***</td>
<td>0.61 ***</td>
<td>0.71 ***</td>
<td>0.34</td>
<td>0.30</td>
</tr>
<tr>
<td>30-36</td>
<td>0.72 ***</td>
<td>0.68 ***</td>
<td>0.33</td>
<td>0.49 **</td>
<td>0.12</td>
<td>0.07</td>
</tr>
<tr>
<td>0-36</td>
<td>0.90 ***</td>
<td>0.74 ***</td>
<td>0.49 **</td>
<td>0.70 ***</td>
<td>0.20</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values are Spearman r.
* = P value < 0.05, ** = P value < 0.01, *** = P value < 0.001.

δ-MMP-3 was closely correlated with δ-SJC, δ-ESR and δ-CRP during all periods. Between 6 and 30 months δ-MMP-3 was also correlated with the DAS. There were no correlations between δ-MMP-3 and δ-TJC or δ-RAI. Time-integrated values of serum MMP-3 for the total follow-up period of 36 months showed the same pattern. In particular time-integrated values of serum MMP-3 and CRP were closely related. Figure 2 and 3 (see next page) show the relation between time-integrated values of serum MMP-3 and CRP in the first period of 6 months and over the total follow-up period of 36 months of the total group (n=33).
Serum MMP-3 in relation to disease activity and radiological progression

FIGURE 2. Time-integrated serum MMP-3 levels in relation to time-integrated CRP levels of 33 patients in the first period of 6 months. Spearman ($r = 0.81$, $p < 0.0001$).

FIGURE 3. Time-integrated serum MMP-3 levels in relation to time-integrated CRP levels over a 3 year follow-up period. Spearman ($r = 0.90$, $p < 0.0001$).

Also intra-individually there were significant correlations between serum MMP-3 and CRP in 25 of the 33 patients. In 8 patients there were no correlations between serum MMP-3 and CRP. In 2 of these 8 patients serum MMP-3 could not be detected.
Figure 4 shows serial serum MMP-3 and CRP levels of a patient with a close correlation between serum MMP-3 and CRP.

**FIGURE 4.** Patient data with significant correlations between serial serum MMP-3 (●) and CRP (○) \((r = 0.58, p < 0.001)\) and serial MMP-3 and swollen joint count (shaded area) \((r = 0.73, p < 0.001)\). Inset showing radiological progression.

**Serum MMP-3 vs radiological damage**

Table 3 shows the correlation coefficients between \(\delta\)-MMP-3 for 6 month periods and the progression in radiological scores: \(\delta\)-JSN, \(\delta\)-ER and \(\delta\)-TSS.

**TABLE 3.** Spearman correlations between time-integrated values for 6 months periods of serum MMP-3 with progression in joint space narrowing (JSN), erosions (ER) and total Sharp score (TSS) \((n=33)\).

<table>
<thead>
<tr>
<th>Time interval (months)</th>
<th>(\delta)-Joint space narrowing ((\delta)-JSN)</th>
<th>(\delta)-Erosions ((\delta)-ER)</th>
<th>(\delta)-Total Sharp score ((\delta)-TSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>0.46 **</td>
<td>0.19</td>
<td>0.33</td>
</tr>
<tr>
<td>6-12</td>
<td>0.45 **</td>
<td>0.12</td>
<td>0.34</td>
</tr>
<tr>
<td>12-18</td>
<td>0.73 ***</td>
<td>0.11</td>
<td>0.53 **</td>
</tr>
<tr>
<td>18-24</td>
<td>0.51 **</td>
<td>0.38 *</td>
<td>0.53 **</td>
</tr>
<tr>
<td>24-30</td>
<td>0.58 ***</td>
<td>0.52 **</td>
<td>0.64 ***</td>
</tr>
<tr>
<td>30-36</td>
<td>0.48 **</td>
<td>0.49 **</td>
<td>0.52 **</td>
</tr>
<tr>
<td>0-36</td>
<td>0.56 ***</td>
<td>0.31</td>
<td>0.49 **</td>
</tr>
</tbody>
</table>

Values are Spearman \(r\).

\(* = P \text{ value} < 0.05, ** = P \text{ value} < 0.01 \text{ and } *** = P \text{ value} < 0.001\).
During the first 2 periods of 6 months $\delta$-MMP-3 was only correlated with $\delta$-JSN. After 12 months of follow-up it was also correlated with $\delta$-TSS and after 18 months it was correlated with all 3 items of the Sharp score. The intra-individual relations between $\delta$-MMP-3 and $\delta$-TSS during follow-up are shown in figure 5. Despite great differences between individuals, intra-individually the relation between $\delta$-MMP-3 and $\delta$-TSS appeared to be rather constant during follow-up.

FIGURE 5. Intra-individual relations between cumulative serum MMP-3 levels and radiological progression (total Sharp score) in 33 patients during a follow-up of 3 year.

**DISCUSSION**

In the present study we found that time-integrated values of serum MMP-3 are correlated with time-integrated values of other markers of disease activity as joint swelling, ESR, CRP and the DAS. Of the radiological scores especially joint space narrowing is closely correlated with cumulative serum MMP-3.

In rheumatoid arthritis a distinction must be made between process variables like joint tenderness, joint swelling, ESR, CRP etc. and outcome measures like radiological progression. The course of the disease is generally monitored by serial measurements of one or more process variables. As radiological outcome is essentially the result of what has happened during the course of the disease, theoretically the area under the curve of serially measured process variables meets the requirements of a proper outcome measure. Such a transformation into time-
integrated values enables the comparison of process variables with outcome measures\textsuperscript{1,41}.

As shown in table 2 time-integrated values for 6 months periods of serum MMP-3 were closely correlated with time-integrated values of joint swelling, ESR, and CRP and to a lesser extent with the DAS (as markers of disease activity). There were no correlations with the tender joint count and RAI, scores in which the variable pain plays a dominant role. Correlations between circulating MMP-3 and joint swelling, ESR, or CRP have also been reported by others\textsuperscript{6,7,20,27,28,42-44}. Our study with serial measurements and transformation of the data into time-integrated values confirms these findings.

Theoretically serum MMP-3 could have some advantages as a marker of disease activity in comparison to CRP. In RA MMP-3 is mainly locally produced and activated in the affected joints and in that way it is a more direct reflection of joint inflammation. The acute phase protein CRP is produced in an indirect manner by the liver after cytokine stimulation. This production can also be influenced by other stimuli of the acute phase response, as for example bacterial infections. Nevertheless, there are no stronger associations between serum MMP-3 and markers of disease activity in comparison with CRP, neither in our study nor in other studies\textsuperscript{28}. This confirms the value of CRP level as a good marker for disease activity of RA in clinical practice.

In contrast to parameters such as joint swelling, ESR, and CRP, no significant correlations were found between time-integrated values of serum MMP-3 and the RAI and tender joint count. This could also explain the less strong correlation with the DAS, which includes the item Ritchie articular index. In a recent study by Keyszer et al only a weak correlation was found between a visual analog scale of joint pain and serum MMP-3\textsuperscript{28}. Van Leeuwen et al found close correlations between CRP and joint swelling but not with the number of tender joints or the RAI\textsuperscript{1}. These results suggest different pathogenetic mechanisms of pain and joint inflammation. In addition nearly all patients used NSAIDs which change symptoms of pain and stiffness without changing the course of the synovitis, joint swelling or the acute phase response.

There was a wide variation in the absolute values of serum MMP-3 and CRP between patients conceivably due to inter-individual differences in extent of disease, involved joints, used medication and differences in locally produced cytokines and cytokine inhibitors. Despite these variations we found significant intra-individual correlations between serum MMP-3 and CRP in 25 of 33 patients (data not shown). In 8 patients there were no correlations between serum MMP-3 and CRP. In 2 of these 8 patients serum MMP-3 could not be detected possibly due to a limited
sensitivity of the test or to the fact that MMP-3 was not produced at all. In the remaining 6 patients documented infections resulted in clear discrepancies between serum MMP-3 and CRP but there were also several CRP peaks in all 33 patients that could not be explained with the available data.

Long-term follow-up studies that link serum MMP-3 to radiological progression are rare. In a recent study by So et al. in patients with long standing RA no correlations were found with erosive disease. Recently our group evaluated the prognostic significance of serum MMP-3 in relation to the development of radiological damage in patients with early RA without radiological damage at presentation. At a cut-off point of 80 ng/ml (the upper limit of controls and osteoarthritis patients) a positive predictive value of 91% was found for the development of radiological damage within 2 years. In addition serum MMP-3 at presentation was correlated with the total Sharp score after 6 and 12 months of follow-up. This correlation was almost exclusively determined by the item joint space narrowing in the Sharp score. As shown in table 3, time-integrated values of serum MMP-3 in patients with early RA are closely correlated with the total Sharp score, but in particular with joint space narrowing, representing destruction of cartilage. Although cause and effect remains to be elucidated, these results fit with the fact that the main targets of MMP-3 are localized in the matrix of cartilage, like proteoglycans and the fact that in animal models MMP-3 was inducible and detectable in the chondrocytes in a very early phase of the arthritis. These findings are of importance for the unraveling of the pathogenesis of RA but also for analyzing mechanisms of drug therapy. For example specific MMP-inhibitors may uncouple the relation between CRP and radiological damage. Radiological progression could be stopped by these agents although inflammation and acute phase response may continue. In that case new markers, not only stromelysins (MMP-3), but also upregulated collagenases or gelatinases could become essential.

As shown in figure 5 there were great inter-individual differences in the relation between serum MMP-3 level and radiological progression. Intra-individually this relation seemed to be rather constant over time. The inter-individual differences might be explained by differences in involved joints. For example, large joints, like the knee, producing much MMP-3, could have a great influence on the serum MMP-3 level while in Sharp’s method only small hand and feet joints are scored. Another explanation could be that different RA patients have different profiles of cytokines and cytokine inhibitors resulting in different responses of MMP production, activation and joint destruction. Furthermore, in RA MMP-3 is produced in the affected joint as pro-MMP-3, which is locally activated by other proteinases. After activation it can degrade many components of the matrix or it is inactivated by tissue
inhibitors of matrix metalloproteinases (TIMPs) and/or by α2 macroglobulin. Inter-individual differences in production site and/or activation level of pro MMP-3 and levels of inhibitors could explain the wide inter-individual variations. Finally, MMP-3 is not the only important enzyme. Inter-individual differences in the upregulation of other MMPs, like collagenases could also have an influence on the individual relation between serum MMP-3 and radiological damage.

The rather constant intra-individual relation between serum MMP-3 and radiological progression resembles the relation between CRP and radiological progression \(^2,45\), a result which could be expected because of the close relation between CRP and MMP-3.

In conclusion time-integrated values of serial serum MMP-3 measurements showed close correlations with other markers of disease activity as joint swelling, ESR, CRP, and the DAS in patients with early RA. Of the radiological scores, as outcome measures, especially the item joint space narrowing was closely correlated with cumulative serum MMP-3. We therefore propose that serum MMP-3 may be an additional serological marker indicative of joint inflammation and cartilage degrading activity in rheumatoid arthritis.

**ACKNOWLEDGEMENTS**

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