Serum matrix metalloproteinase 3 levels in comparison to C-reactive protein in periods with and without progression of radiological damage in patients with early rheumatoid arthritis

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Abstract

Objective

To evaluate serum matrix metalloproteinase 3 (MMP-3) levels in comparison to C-reactive protein (CRP) in periods with and without progression of radiological damage in patients with early rheumatoid arthritis (RA).

Methods

Thirty-two patients with RA and radiological progression (≥ 5 points according to the Sharp/van der Heijde method) during 6 months followed by a 6-month period without radiological progression (≤ 1 point) were selected from a prospective follow-up study of early RA patients. Serum MMP-3 levels, CRP, the erythrocyte sedimentation rate (ESR), disease activity index (DAS), swollen joint count (SJC), tender joint count (TJC), and Ritchie articular index (RAI) were measured monthly and results were transformed into mean values for the 6-month periods.

Results

During the period with radiological progression the mean serum MMP-3 correlated significantly with the mean CRP (r = 0.68, p < 0.001), ESR (r = 0.54, p = 0.001) and swollen joint count (r = 0.48, p = 0.006). In the period without radiological progression the mean serum MMP-3 only correlated with the mean CRP (r = 0.44, p = 0.012). Individual changes – expressed in percentages (%) – between the two periods showed a decrease in both the mean serum MMP-3 and CRP in 19 and an increase in 3 patients, in parallel with other markers of disease activity in these patients (69% of cases). The individual change (%) in mean serum MMP-3 or CRP did not correlate with the difference in radiological progression between the two periods.

Conclusions

Serum MMP-3 and CRP are closely related and there seems to be no difference between serum MMP-3 and CRP with regard to the monitoring of the progression of radiological damage.

Key words

Serum matrix metalloproteinase 3, stromelysin 1, early rheumatoid arthritis, radiological damage.

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Introduction

Rheumatoid arthritis (RA) is characterised by chronic inflammation of the synovial tissue and in most cases progressive destruction of cartilage and bone (1). The matrix metalloproteinases (MMPs) are thought to play a critical role in the degradation of many components of the extracellular matrix in the synovial joint (2, 3).

Matrix metalloproteinase 3 (MMP-3, stromelysin 1) is of interest because this proteolytic enzyme is produced abundantly in the inflamed joints and plays a prominent role in the pathogenesis of matrix degradation in RA (2-4), even though it is not the only key enzyme (5). 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 (2, 4, 6, 7). Moreover MMP-3 is able to activate other matrix metalloproteinases like MMP-1, MMP-7, MMP-8, MMP9 and MMP-13 (8). The enzyme has been localised in the fibroblast-like synoviocytes of rheumatoid synovium (9-11), in RA cartilage (12,13), at sites of cartilage erosion (4), in synovial fluid (14-21) and in serum (20, 22-26).

Systemic MMP-3 levels are supposed to be a reflection of local synthesis induced by pro-inflammatory cytokines. As such, serum MMP-3 can be used as a systemic marker of local joint inflammation (16, 20, 24, 25, 27, 28) and/or destruction (26, 29, 30). In this respect serum MMP-3 may reflect joint inflammation and destruction more directly compared to C-reactive protein (CRP) which is produced indirectly by the liver after cytokine stimulation (31). In addition, it has been suggested that the pathophysiologic mechanisms of joint inflammation, as reflected by CRP, may be partially independent of destruction, which is also determined by the prominent local cytokine, protease and inhibitor environment (32-34). This possible uncoupling of inflammation and destruction could be one of the explanations for the broad inter-individual variation in radiological damage despite comparable inflammation as defined by, for example, CRP (35).

We hypothesise that serum MMP-3 is more closely related to the progression of radiological damage in comparison with CRP. Therefore, we analysed the mean serum MMP-3 levels over a 6-month period with and a consecutive 6-month period without progression of radiological damage and evaluated the differences between serum MMP-3 and CRP.

Patients and methods

Patients

Thirty-two patients with RA and progression of radiological damage (5 points according to the Sharp/van der Heijde method) during a 6-month period followed by a 6-month period with no radiological progression (1 point) were selected from a cohort of patients with RA according to the 1987 American College of Rheumatology criteria (36), who had joint symptoms existing for less than one year at presentation and who had not previously received DMARDs. These patients participated in a prospective follow-up study at the Department of Rheumatology at Groningen University Hospital. In accordance with the protocol, clinical and laboratory investigations were performed at monthly intervals and radiographs of the hands and feet were obtained every 6 months during the follow-up.

During follow-up patients were treated with non-steroidal anti-inflammatory drugs and DMARDs as indicated clinically. Guidelines for the sequence of the different second-line drugs 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.

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) (37), tender joint count (TJC), swollen joint count (SJC) and the disease activity score (DAS) according to Van der Heijde with 3

variables: RAI, number of swollen joints and erythrocyte sedimentation rate (ESR) (38). The maximal possible scores are: RAI 78, TJC 52, SJC 52 and DAS 10.0.

Laboratory analysis
Serum MMP-3 levels were determined by a MMP-3 ELISA developed at our laboratory (39). In short, 96-well plates were pre-coated with $F(ab)_2$ fragments of goat-antimouse IgG, 1 µg/ml (Jackson Immunoresearch Labs, West Grove, PN, USA). Next, a mouse monoclonal antibody against human MMP-3, clone 10D6 (R&D Systems, Abingdon, UK) was coated at 0.1 µg/ml. Serum samples were analysed in twofold serial dilutions in high performance ELISA buffer (CLB, Amsterdam, NL) and incubated for 1 hour. After washing, bound MMP-3 was detected with a polyclonal rabbit anti-human MMP-3 (AB 810, Chemicon, Temecula, CA, USA), followed by horseradish-peroxidase-labelled $F(ab)_2$ fragment of goat-anti rabbit IgG (Zymed, San Francisco, CA, USA). Peroxidase activity was determined using tetramethylbenzidin as substrate. MMP-3 levels were calculated at the linear range of the assay from a standard curve (3-400 ng/ml) using pro-MMP-3, purified from serum free supernatant of IL-1 stimulated rheumatoid arthritis synovial fibroblasts (40). The intra-assay coefficient of variation (CV) was 6.8%, the inter-assay CV 8.8%.

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 (TIMPs) (data not shown). 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 95th percentile in healthy bloodbank donors (n = 80) was used (female < 20 ng/ml, male < 60 ng/ml) (39).

CRP was measured by ELISA (41) (lowest limit of detection: 2 mg/l), and ESR according to Westgren. IgM rheumatoid factor (RF) was measured by a Dade/Behring BN-2 nephelometer (normal value: < 15 IU/ml).

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.* (42, 43). 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. Radiological progression was defined as 5 Sharp points and no radiological progression as 1 Sharp point in a 6-month period.

Statistical analysis
Monthly determined values of clinical and laboratory variables were transformed in a mean value over a 6-month period. Individual changes between the 2 periods were expressed in terms of percentage. For example:

$$\text{serum MMP-3} = \frac{\text{Individual mean serum MMP-3 in the non-progressive period}}{\text{Individual mean serum MMP-3 in the progressive period}}$$

Spearman's rank correlation coefficients were used for assessment of the correlation, and the paired T-test for the differences between periods. P values < 0.05 were considered significant.

Results
Thirty-two patients with RA and radiological progression (5 points according to Sharp's method) during a 6-month period followed by a 6-month period without radiological progression (1 point) were selected from a cohort of early RA patients. The median time between the moment of diagnosis of RA and such a period was 10 months, with a range of 0-31 months. The characteristics of these 32 patients with RA at the beginning of the period with radiological progression are summarised in Table I. The individual mean values of serum MMP-3, CRP, ESR and the disease activity score in the period with and without radiological progression are shown in Figure 1A-D. All of the mean values, including SJC, TJC and RIT decreased significantly (p < 0.05).

Table I. Characteristics of the 32 patients with RA at the beginning of the 6-month period with radiological progression.

Age	(years)	54	(20-78)
Gender	female/male (% female)	23/9	(72%)
Time until begin progressive period¶	(months)	10	(0-31)
IgM RF positive	(%)	28	(88%)
Tender joint count		7	(1-44)
Ritchie articular index		6	(1-40)
Swollen joint count		6	(1-31)
Disease activity score		2.9	(1.3-5.7)
CRP	(mg/l)	11	(2-117)
ESR	(mm/h)	25	(4-97)
MMP-3	(ng/ml)	54	(5-2370)
Sharp score†	T0	11	(0-65)
	T6	18.5	(7-71)
	T12	19	(8-71)

Values are expressed as the median and range.
¶: Time between the moment of diagnosis of rheumatoid arthritis and the beginning of the period with radiological progression.
†:T0 is the Sharp score at the beginning and T6 the Sharp score at the end of the 6-month period with radiological progression. T12 is the Sharp score after the subsequent 6 months without radiological progression.
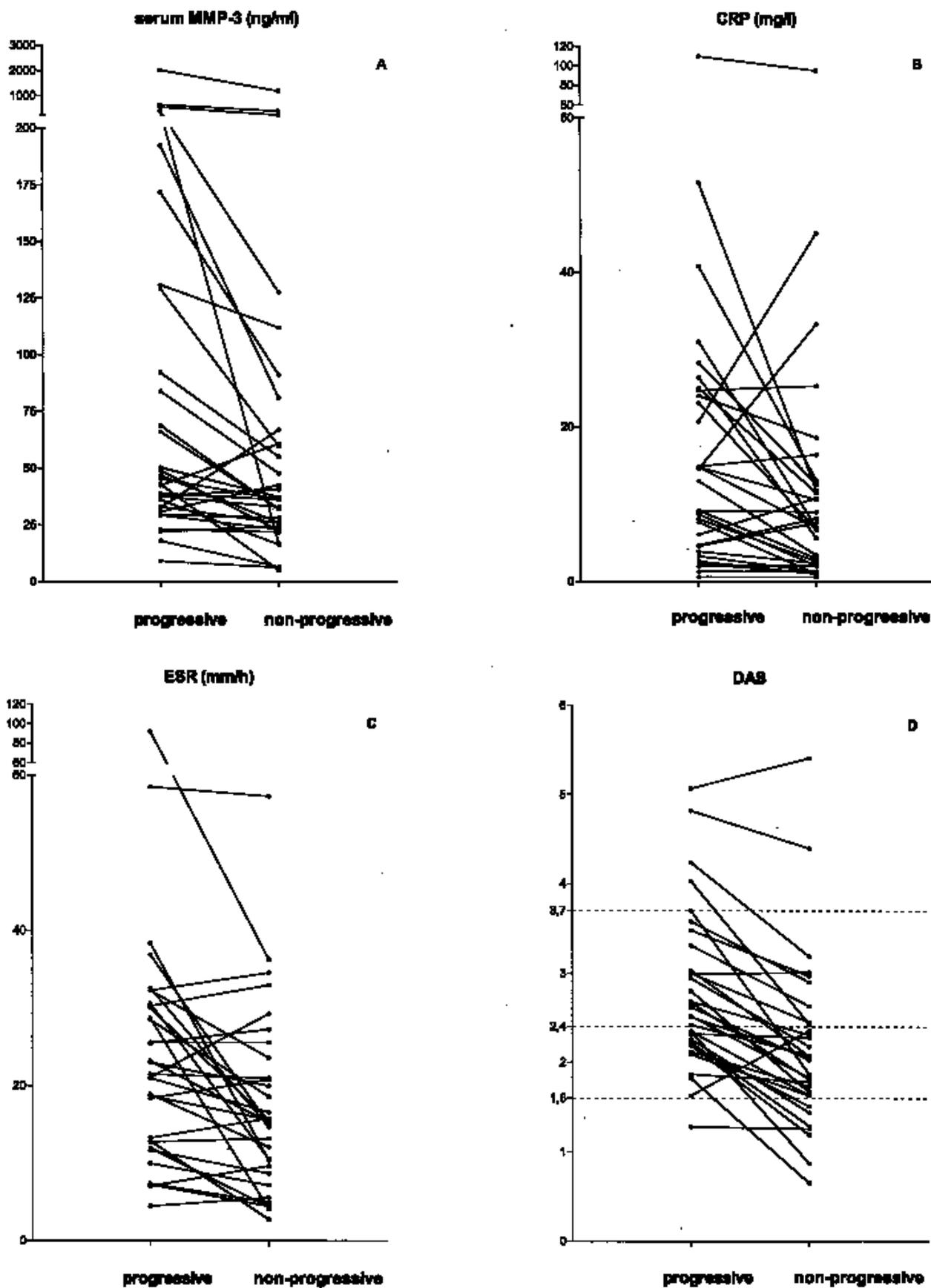
Fig. 1. Individual mean values for serum MMP-3 (**A**), CRP (**B**),ESR (**C**), DAS (disease activity score (**D**) in the 6 months period with vs. the consecutive 6 months period without progression of radiological damage. Each dot represents 1 patient (n=32). All mean values decreased significantly (p < 0.05).
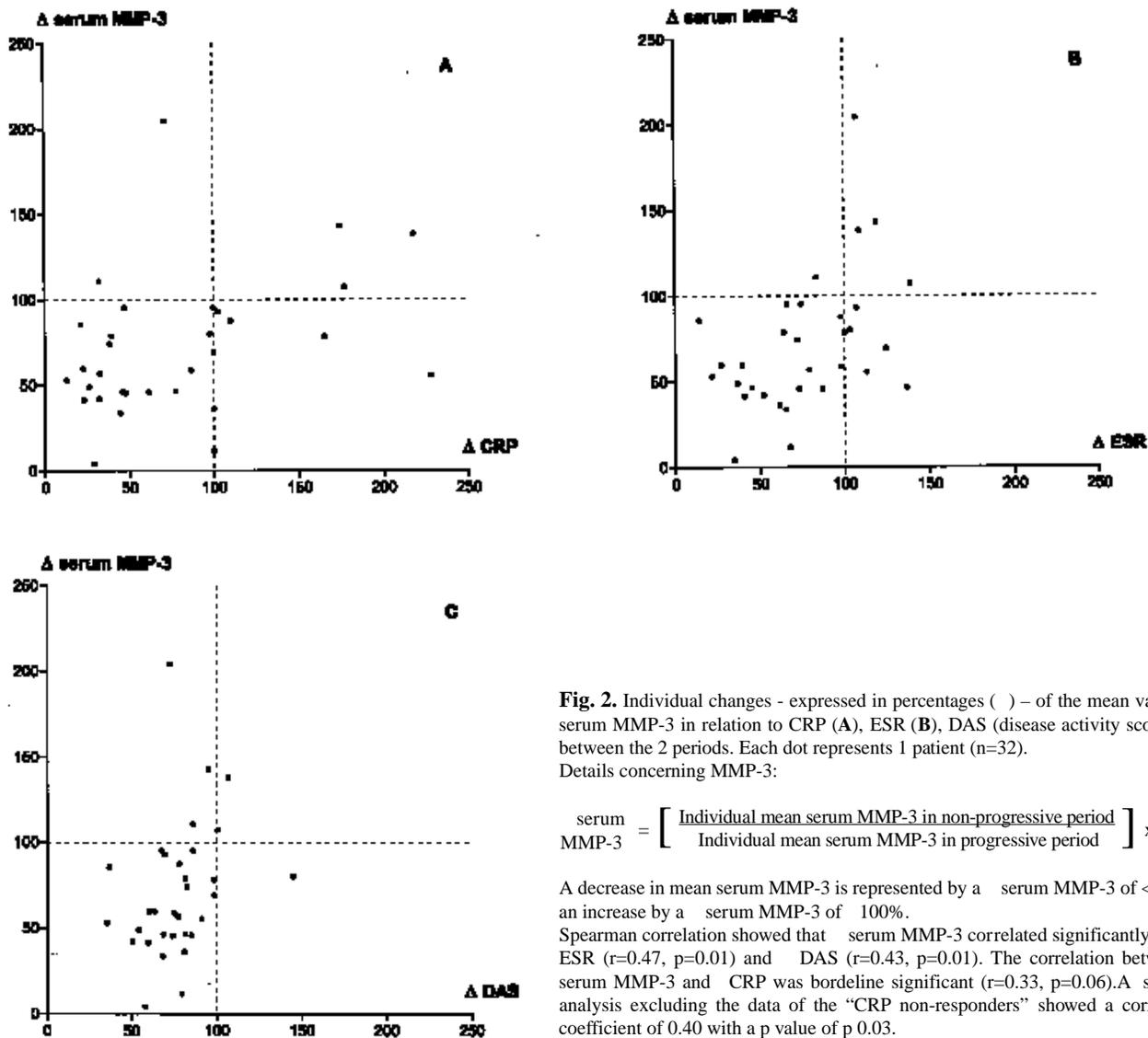
Fig. 2. Individual changes - expressed in percentages ( ) – of the mean values of serum MMP-3 in relation to CRP (**A**), ESR (**B**), DAS (disease activity score (**C**)) between the 2 periods. Each dot represents 1 patient (n=32).
Details concerning MMP-3:

$$\text{serum MMP-3} = \left[\frac{\text{Individual mean serum MMP-3 in non-progressive period}}{\text{Individual mean serum MMP-3 in progressive period}} \right] \times 100\%$$

A decrease in mean serum MMP-3 is represented by a   serum MMP-3 of < 100%, an increase by a   serum MMP-3 of   100%.
Spearman correlation showed that   serum MMP-3 correlated significantly with   ESR (r=0.47, p=0.01) and   DAS (r=0.43, p=0.01). The correlation between   serum MMP-3 and   CRP was bordeline significant (r=0.33, p=0.06).A separate analysis excluding the data of the "CRP non-responders" showed a correlation coefficient of 0.40 with a p value of p 0.03.

In the period with radiological progression 3 patients (9%) had a mean serum MMP-3 within the normal range and 4 patients (12%) a CRP of < 2 mg/ml. In the period without radiological progression 7 patients (22%) had a normal mean serum MMP-3 and 8 patients (25%) a CRP of < 2 mg/ml.

During the period with radiological progression the mean serum MMP-3 correlated significantly with the mean CRP (Spearman r = 0.68, p < 0.001), ESR (r = 0.54, p=0.001) and swollen joint count (r = 0.48, p = 0.006). In the period without radiological progression the mean serum MMP-3 only correlated with the mean CRP (r = 0.44, p = 0.012).

Individual changes – expressed in percentages – of the mean values between the two periods (Fig. 2) showed a decrease in both serum MMP-3 and CRP in 19 and an increase in 3 patients, in parallel with other markers of disease activity in the patients (69% of cases). There was a discrepancy between the change in mean serum MMP-3 and CRP in 6 patients (19% of cases). In 5 of these patients serum MMP-3 levels ranged from 22-39 ng/ml and in 2 CRP levels ranged from 2-8 mg/l. Small changes in measured serum MMP-3 levels resulted in great changes in terms of percentage. In the remaining 4 patients (12% of cases) serum MMP-3 decreased but no CRP response could be registered because CRP levels were within the normal range (< 2 mg/l) in

both periods. Spearman correlation showed that   serum MMP-3 correlated significantly with   ESR (r = 0.47, p = 0.01) and   DAS (r = 0.43, p=0.01). The correlation between   serum MMP-3 and   CRP was borderline significant (r = 0.33 p 0.06). A separate analysis excluding the data of the "CRP non-responders" (n = 4) showed a correlation coefficient of 0.40 with a p value of 0.03.

The median change in serum MMP-3 was 61.8% (range: 4-204%), the median change in CRP was 54.7% (range: 13-228%).

The individual change – expressed in percentages – in the mean serum MMP-3 or CRP did not correlate with the difference in radiological progres-
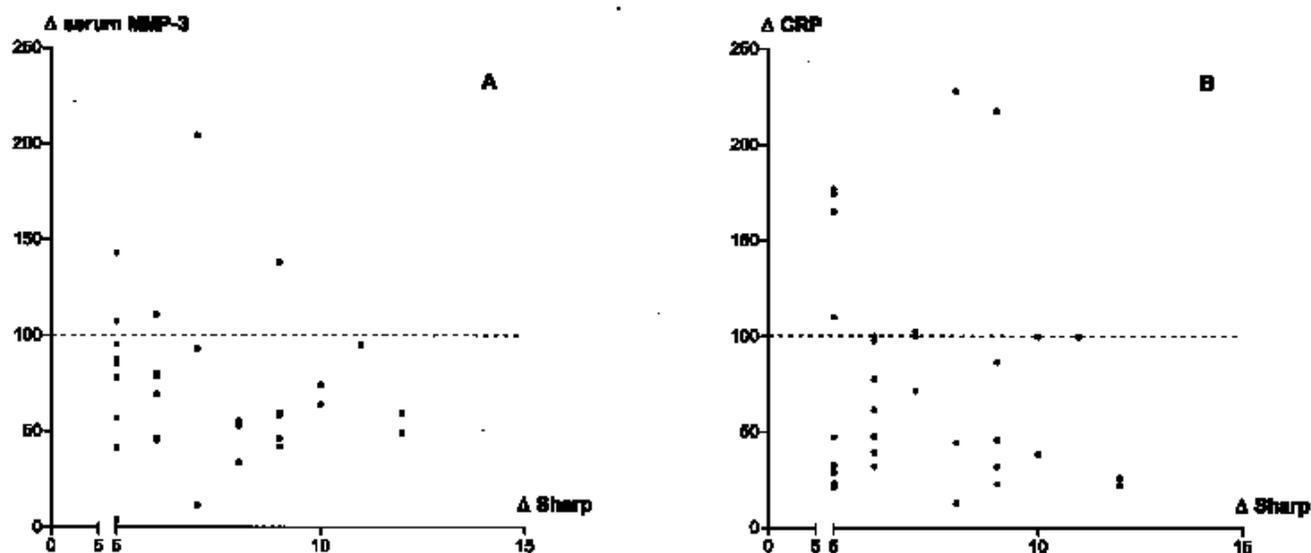
Fig. 3. Individual changes – expressed in percentages ( ) – of the mean values of serum MMP-3 (**A**) and CRP (**B**) in relation to the difference in the Sharp score between the 2 periods.Each dot represents 1 patient (n=32). The individual change (mainly decrease, defined as < 100%) in mean MMP-3 or CRP did not correlated with the difference in radiological progression between the two periods (serum MMP-3 vs. Sharp: r=-0.14, p=0.45 and CRP vs. Sharp: r=-0.12, p=0.50).

sion (Fig. 3) between the 2 periods (serum MMP-3 versus Sharp: r = –0.14, p = 0.45 and CRP versus Sharp: r=-0.12, p=0.50)

In the period without radiological progression, disease activity was high to moderate (DAS > 2.4) in 9 patients, low (DAS 2.4 - 1.6) in 16 patients and 7 patients were in remission (DAS < 1.6), (Fig. 1D). Patients with an increase in serum MMP-3, CRP or ESR in the period without radiological progression (n=3) had DAS scores of > 1.6.

Discussion

In RA the markers of disease activity and destruction are important, not only for prognostic but also for therapeutical reasons. Acute phase proteins such as CRP are indirectly produced by the liver after stimulation by cytokines produced at the inflammatory site. Serum MMP-3 is locally produced and activated in the inflamed joint and systemic levels are supposed to be a direct reflection of local synthesis. As such, serum MMP-3 could be used as a systemic marker of local inflammation and destruction.

In RA serum MMP-3 is closely correlated to other markers of disease activity like swollen joint count, CRP and ESR (16, 20, 22, 24, 25, 27, 27-29, 34, 44, 45). Cross-sectional and long-term

follow-up studies also show a correlation between serum MMP-3 and radiological detectable damage (16, 29).

Recent studies suggest that the pathophysiologic mechanisms of joint inflammation, as reflected by CRP and destruction, may be partially independent (32-34). Because of the key role of MMP-3 in matrix degradation and the relationship between serum MMP-3 levels and radiological damage, we evaluated the serum MMP-3 levels in periods with and without progression of radiological damage in patients with early RA. We hypothesised that serum MMP-3 is more closely related to the progression of radiological damage in comparison with CRP.

In rheumatoid arthritis, a distinction must be made between process variables such as CRP 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 (46). For equally spaced observations, as is the case in this study, the area under the curve is essentially the same as the

mean of all measurements (47). Such a transformation of data enables the comparison of process variables with outcome measures.

In this select group of RA patients we found in the first place a close correlation between mean serum MMP-3 and CRP in both periods. Secondly, evaluation of individual changes (in percentages) between the two periods showed a discrepancy between the change in mean serum MMP-3 and CRP in only a minority of patients, especially in patients with low or close to normal values. In the third place, individual changes (expressed in percentages) in the mean serum MMP-3 or CRP did not correlate with the differences in radiological progression between the two periods. In this study we did not observe a difference between serum MMP-3 and CRP in relation to the progression of radiological damage. In other words, in this select group of RA patients we could not find arguments for an uncoupling of inflammation as reflected by CRP and of destruction as reflected by serum MMP-3.

An influence of differences in therapy was considered but could not, unfortunately, be evaluated due to the diversity in the DMARDs used in this relatively small number of RA patients.

The lack of a difference between serum

MMP-3 and CRP in relation to the progression of radiological damage could have several reasons. In the first place patient selection could be of influence. In our attempt to look for differences between serum MMP-3 and CRP in relation to the progression of radiological damage we have chosen a radiological criterium. Patients with radiological progression (5 points according to Sharp's method) during 6 months followed by a 6-month period with no radiological progression (1 point) were selected from a large follow-up cohort of RA patients. The cut-off point of 5 Sharp points was based on the publications concerning the minimal clinically important difference and the smallest detectable difference in the Sharp score which scores damage in the hands and feet (48). With these criteria, even in the non-progressive period, 9 patients had a high to moderate disease activity score (see Fig. 1D) probably due to disease activity in other joints. This persistent disease activity makes it difficult to evaluate the uncoupling of disease activity as assessed by the CRP, joint counts, DAS and radiological damage scored by X-rays of the hands and feet.

In addition, it is of importance to notice that there were some patients with radiological progression despite a relatively low disease activity based on their DAS score. This emphasises the inter-individual difference in the relationship between parameters of disease activity and radiological damage.

Secondly it is conceivable that a lag time could play a role (35, 49). Radiologically detectable damage could lag behind disease activity as measured by CRP or serum MMP-3. It remains debatable if all the 32 patients were actually in "radiological remission".

In the third place sample size could be of importance. With the criteria mentioned we could select 32 patients out of a cohort of 405 RA patients. This number of patients could have been too small because serum MMP-3 and CRP are closely related, which implies a relatively great sample size.

Finally, in RA MMP-3 is produced in the inflamed joints as an inactive pro-enzyme. Upon activation it is able to degrade many components of the matrix. Inactivation occurs by inhibitors such as TIMPs (tissue inhibitors of matrix metalloproteinases). It is conceivable that an imbalance between production, activation and inhibition results in joint destruction (radiological damage). In our hypothesis we assume that serum MMP-3, consisting mainly of pro-MMP-3, is a direct reflection of local pro-MMP-3 production as well as of its local activation and subsequent joint destruction. In this respect serum MMP-3 could reflect joint destruction more directly compared to CRP which is produced indirectly by the liver after cytokine stimulation. The fact that we did not find a difference between serum MMP-3 and CRP with regard to radiological progression implies that not only production, but rather the balance between activation and inhibition is of major importance. Unfortunately these mechanisms are difficult to evaluate.

The optimal biochemical marker for monitoring destruction still remains to be defined (50). Therefore, although serum MMP-3 remains an interesting marker in patients with rheumatoid arthritis, it is not superior to CRP. CRP is still an excellent parameter not only for monitoring disease activity but also for monitoring the progression of radiological detectable damage.

In conclusion, serum MMP-3 and CRP are closely related and there seems to be no difference between serum MMP-3 and CRP with regard to the monitoring of the progression of radiological damage.

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