EXTENDED REPORT

Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis

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Objective: To analyse the relation between systemic levels of pro-MMP-3, -8, and -9 matrix metalloproteinase (MMP) activity in α_2 macroglobulin (α_2M)/MMP complexes and the progression of joint destruction in patients with recent onset rheumatoid arthritis (RA).
Methods: 109 patients with RA of recent onset were entered into this longitudinal study. Patients were followed up for two years; clinical data, blood samples, and radiographs were obtained at baseline and at 1 and 2 years. Serum levels of MMPs were measured by sandwich ELISA and MMP activity assays.
Results: During the two years joint damage progressed from 0 to 10 (median Sharp score, $p<0.001$). Stable levels of pro-MMP-3 and a significant decrease in the levels of pro-MMP-8 and -9 and α_2M/MMP complexes were seen throughout the two years. Regression analysis showed that serum pro-MMP-3 levels at disease onset were independently associated with the progression of joint damage (B = 0.7, 95% CI 0.3 to 1.1, p = 0.001). Based on the rate of joint destruction, patients were divided into two subgroups: patients with mild and severe joint damage progression. The pro-MMP-3 levels were significantly higher in the group with severe compared with mild disease at all times. Levels of pro-MMP-8 and -9 were decreased in both groups, whereas α_2M/MMP complex levels decreased in the group with mild disease only.
Conclusion: Serum levels of the MMPs studied are associated with disease activity, but serum pro-MMP-3 levels at the onset of disease are also predictive of joint damage progression.

Rheumatoid arthritis (RA)[1] is a chronic and potentially crippling disease characterised by systemic inflammation and joint tissue degradation. Degradation of articular cartilage is one of the early features of the disease and is mediated by the increased activity of proteolytic systems.[1] Among several enzymes involved in the process, matrix metalloproteinases (MMPs) have been shown to have an important role in the invasion of the synovial tissue in cartilage, cartilage destruction, and bone erosion formation.[2 3] Increased levels of MMPs are found in tissue, in the synovial fluid (SF), and in the systemic circulation of patients with RA. Based on the substrate specificity, the family of MMP enzymes is subdivided into subgroups such as stromelysins (MMP-3, -10, and -11), collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), and membrane-type MMPs (MMP-14, -17, -22, -24, -25).[4]

Stromelysins have a wide range of substrate specificity and can also activate other MMPs, thus playing an important role in the MMP cascade.[5–7] Pro-MMP-3 levels have been shown to correlate with systemic inflammatory markers, and it was suggested that they participate in cartilage degradation.[8–10] Collagenases can degrade the intact collagen molecule, one of the main components of the articular cartilage. SF pro-MMP-1 (collagenase-1) levels have been shown to correlate with the degree of synovial inflammation[11] and serum pro-MMP-1 levels with development of joint erosions.[12] Systemic levels of pro-MMP-8 (collagenase-2) are increased in clinically and serologically active RA and are correlated with markers of the systemic inflammation.[13] Gelatinases degrade the denatured collagen and have also been shown to be associated with the development of radiographic erosion in patients with RA.[14] After MMPs are produced and activated their action is further controlled by specific inhibitors of matrix metalloproteinases (TIMPs). Normally, a tight balance exists between MMPs and their tissue inhibitors. However, in pathological situations such as RA an MMP/TIMP imbalance is present, which leads to an excess of activated MMPs which have an important role in the chain of events leading to excessive cartilage degradation.[15] In the systemic circulation and SF, MMPs are also readily captured by α_2 macroglobulin (α_2M), a natural protease inhibitor.[16–18] It has been previously shown that in SF and in the systemic circulation of patients with RA levels of the MMP/α_2M complex are increased, suggesting that levels of MMP/α_2M complexes represent the MMP/TIMP imbalance present in RA.

Our study was designed to investigate whether levels of MMPs measured in the systemic circulation predict joint destruction. Because our previous results for MMP measurements in SF and the systemic circulation yielded the highest differences between RA/osteoarthritis and healthy controls for pro-MMP-3, -8, and -9 (Tchetverikov, unpublished results), the present study focused on these enzymes.

Thus, in the present large longitudinal study the serum levels of stromelysin-1 (pro-MMP-3), collagenase-2 (pro-MMP-8), and gelatinase B (pro-MMP-9) as well as general

Abbreviations: ACR, American College of Rheumatology; α_2M, α_2 macroglobulin; CI, confidence interval; CRP, C reactive protein; DAS, disease activity score; DMARD, disease modifying antirheumatic drug; EAC, early arthritis clinic; ELISA, enzyme linked immunosorbent assay; JDS, joint damage score; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope; SF, synovial fluid; TIMP, tissue inhibitor of matrix metalloproteinase

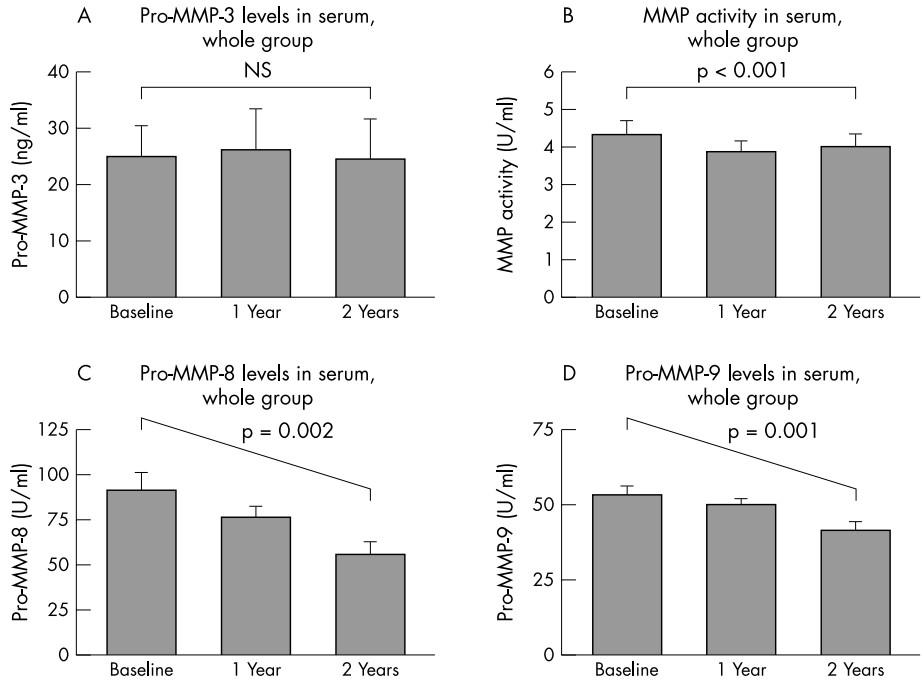

Figure 1 Serum pro-MMP-3 levels (A), MMP activity (B), pro-MMP-8 levels (C), pro-MMP-9 levels (D) at baseline and at 1 and 2 years in the whole group.

MMP activity in α_2M/MMP complexes were analysed and related to the rate of radiological progression and disease activity in patients with recent onset RA.

PATIENTS AND METHODS

In 1993 a special early arthritis clinic (EAC) was started at the department of rheumatology of the Leiden University Medical Centre, the only centre for rheumatic patients in an area with 300 000 inhabitants. The general practitioners were encouraged to refer patients to the EAC if at least two of the following features were present: joint pain, joint swelling, or reduction of joint mobility. All patients referred to the special EAC were seen within two weeks. The patients were included in the EAC if (*a*) the arthritis was confirmed by a rheumatologist; (*b*) the history of symptoms was <2 years; and (*c*) the patients had not been visiting a rheumatologist elsewhere for the same problem.

In this study only patients with the diagnosis RA defined according to the 1987 American College of Rheumatology (ACR) criteria[19] or probable RA using the 1958 ACR criteria,[20] but without the six weeks duration observed by a doctor,[21] were analysed. Sixty six per cent of the patients who were initially diagnosed as having probable RA according to the 1958 ACR criteria fulfilled the 1987 ACR classification criteria for rheumatoid arthritis during the follow up period (table 1).

Table 1 Demographic and clinical data from the patient group at study entry

	Patients with RA (n = 109)
Age (years)*	58 (44–67)
Female (%)	73
Duration of symptoms (days)*	162 (89–311)
Time from symptom onset to initiation of DMARD (days)	331 (199–545)
Disease duration before treatment (days)	123 (50–273)
RA (%)* †	68
Probable RA (%)* ‡	32
Rheumatoid factor positivity (%)	58
Shared epitope positivity (%)	69
Baseline joint damage*	0 (0–2)

*Data are shown as median (25th–75th centiles); †RA according to the 1987 ACR classification criteria; ‡probable RA according to the 1958 ACR classification criteria.

The study group comprised 109 patients recruited to the EAC. All patients were initially treated with non-steroidal anti-inflammatory drugs. After about four months those who still had active disease received disease modifying antirheumatic drugs (DMARDs) such as chloroquine, or sulfasalazine if chloroquine was contraindicated. This was the treatment strategy for RA at that time in the Netherlands. After the initial treatment, the rheumatologists were free to choose another DMARD for patients who had side effects or required a treatment change because of lack of efficacy.[22] Six patients received prednisone at some point during the study.

For further analysis the whole study group was divided into two subgroups according to joint damage score (JDS, represents the Sharp-van der Heijde total damage scores[23]). The group with mild disease progression (mild progressors) comprised 59 patients (mean (SD) age 54 (16); 43 (73%) women, 23 (39%) received a DMARD) who had a JDS lower than or equal to the median JDS of the whole study group during the two year follow up (the median Sharp score of the whole group was 10). The group with severe disease progression (severe progressors) comprised 50 patients (mean (SD) age 57.2 (14); 36 (72%) women, 36 (72%) received a DMARD) who had a JDS greater than the median JDS of the whole study group.

Clinical examinations

In the study group clinical variables of disease activity were assessed at study entry and thereafter at one and two years. We used a modified disease activity score 3 (DAS)[24 25] to measure disease activity. The formula for the DAS 3 was as follows: DAS $3 = 0.54 \times (\sqrt{\text{Ritchie score}}) + 0.065 \times (\text{number of swollen joints}) + 0.33 \times \ln \text{erythrocyte sedimentation rate} + 0.224$. All joints were assessed as in the Ritchie articular index except for the acromioclavicular, subtalar, and mid-tarsal joints. For the swollen joint index the metacarpophalangeal, proximal interphalangeal, and metatarsophalangeal

joints were scored as one unit. These ''modified'' DAS will be further referred to as DAS.

Radiographs of the hands and feet were obtained at study entry, 1 and 2 years. Radiographs were scored randomly by one experienced rheumatologist according to the modified Sharp/van der Heijde method.[23] The intraclass correlation coefficient for the assessor's scoring was 0.95, as measured in 39 patients. The laboratory variable, IgM rheumatoid factor (RF), was determined at study entry and measured by enzyme linked immunosorbent assay (ELISA) as described previously.[26] IgM RF titres of ⩾5 units were considered positive. Also, the HLA phenotypes according to the shared epitope (SE) model were analysed at study entrance.[27 28] DNA isolation, DRB1 typing and subtyping were performed as described previously.[27] SE positive DRB1 alleles were *0101, *0102, *0401, *0404, *0405, *0408 and *1001. Table 1 shows the demographic, clinical, and radiological data of the patient group.

MMP analyses

Serum was prepared after blood collection and all samples were stored at −20°C before analysis. Pro-MMP-3 was analysed using the sandwich ELISA for pro-MMP-3 according to the manufacturer's instructions (Amersham Biosciences, Little Chalfont, UK). MMP-8 and -9 (pro-MMPs were captured using monoclonal antibody and its activity was measured after *p*-aminophenylmercuric acetate activation) were detected using respective MMP activity assays according to the manufacturer's instructions (Biotrak activity assay, Amersham Biosciences, Little Chalfont, UK). General MMP activity in α_2M complexes was measured using 10 μM (all concentrations are final) fluorogenic substrate TNO211-F[17] in the presence or absence of 10 μM BB94 (a general MMP inhibitor). It has been shown previously that activated MMPs form complexes with α_2M in biological fluids such as SF, serum, or wound fluid.[17 18] After the complexes are formed MMPs lose the ability to degrade their natural substrates such as collagen type II, but can still be detected using small molecular weight substrates,[16] such as TNO211-F. TNO211-F is mainly converted by MMP-2, -8, -9, and -13 and at lower rate by MMP-3 and -1.[17] Serum samples were diluted (final dilution 1/50) in MMP buffer[17] containing EDTA-free Complete (one tablet in 10 ml). The MMP activity in each sample was calculated as the difference in the initial rate of substrate conversion (linear increase in fluorescence in time) between samples with and without BB94 addition. Fluorescence was measured for six hours at 30°C using a Cytofluor 4000 (Applied Biosystems, Foster City, CA, USA).

Table 2 Correlations (r_s) between pro-MMP and activated MMP levels and CRP and DAS in the patients with RA at baseline, 1 and 2 years

	CRP	DAS
Baseline		
Pro-MMP-3	0.33†	0.28*
Pro-MMP-8	0.32†	−0.03
Pro-MMP-9	0.04	−0.18
MMP activity in α_2M	0.36†	0.28*
One year		
Pro-MMP-3	0.70†	0.38*
Pro-MMP-8	0.28*	−0.04
Pro-MMP-9	0.12	−0.03
MMP activity in α_2M	0.54†	0.41†
Two years		
Pro-MMP-3	0.52†	0.22
Pro-MMP-8	0.14	−0.12
Pro-MMP-9	−0.03	−0.13
MMP activity in α_2M	0.46†	0.33*

*p<0.05; †p<0.005 (Spearman's r_s).
CRP, C reactive protein; MMP, matrix metalloproteinase, DAS, disease activity score; α_2M, α_2 macroglobulin.

Statistical analysis

Differences between the subgroups of interest were tested with the Mann-Whitney U test. The changes within patients over time were analysed using a linear mixed model for each study subgroup (repeated measurements parameter was entered into the model, data were log transformed for JDS and pro-MMP-3 levels and square root transformed for pro-MMP-8 and -9 levels for the analysis). Correlations for non-parametric data were evaluated by the Spearman rank correlation coefficients. Within the study group the log transformed Sharp progression score was the outcome parameter in a stepwise linear regression analysis that included the following possible baseline predictors: DAS, C reactive protein (CRP), RF, SE, log transformed pro-MMP-3, -8, -9, and activated MMP in α_2M. Before log transformation, one point was added to all progression scores (Δ Sharp score = Sharp score$_{\text{end point}}$ − Sharp score$_{\text{baseline}}$) to avoid a zero Δ Sharp score in order to be able to perform log transformation. All tests were two tailed and $p \leqslant 0.05$ was considered significant. The statistical analysis was performed using S-Plus (MathSoft, Seattle, WA) and SPSS (Chicago, IL) statistical software.

RESULTS

Total group

During the two year follow up, the median DAS score in the study group fell significantly from 3.4 to 2.5 ($p<0.001$), the median CRP levels fell from 20 to 10 mg/l ($p<0.001$), and the joint damage progressed from a median Sharp score of 0 at baseline to 10 after two years ($p<0.001$).

A stable serum level of pro-MMP-3 throughout the two year follow up was seen in the study group (median (25th–75th centiles): 25 (11–47), 26 (16–65), and 25 (14–58) ng/ml, at baseline, and at 1 and 2 years, respectively; fig 1). A significant decrease in levels of pro-MMP-8 and -9 was found in the study group: pro-MMP-8 decreased from 91 (57–127) to 54 (33–108) U/ml ($p = 0.002$) and pro-MMP-9 from 53 (44–71) to 41 (31–56) U/ml ($p = 0.001$). Levels of MMP activity in α_2M complexes decreased slightly, but significantly during the 2 year follow up period (4.4 (3.5–6.0) U/ml at baseline, 3.9 (3.0–5.3) U/ml at 1 year and 4.0 (2.5–5.4) U/ml at 2 years, $p<0.001$). MMPs correlated significantly with the CRP levels and DAS at several times (table 2).

Pro-MMP-3 is an independent predictor of joint damage progression in RA

It was previously suggested that both CRP and systemic pro-MMP-3 levels[9] at disease onset are predictive of joint damage progression. To analyse which baseline parameters were associated with progression of joint damage a stepwise log linear regression analysis was performed. Next to pro-MMP-3, -8, -9 and MMP activity in α_2M/MMP complexes other potential predictors such as RF, SE, DAS, and CRP levels at baseline were included in the model. The strongest association between the input factors and joint damage progression was found for pro-MMP-3 levels at onset of the disease. The association between joint damage progression and pro-MMP-3 levels ($B = 0.7$, 95% confidence interval (CI) 0.3 to 1.1, $p = 0.001$) was independent of all other parameters. From the other potential predictors included in the analysis, only SE was also independently associated with progression of joint damage ($B = 1.02$, 95% CI (0.3 to 1.74, $p = 0.006$). The baseline parameter RF was also associated with joint damage progression in this model, but not independently. Because MMPs are likely to be mainly involved in cartilage degradation in RA, a similar stepwise log linear regression analysis

Table 3 Differences within the patient groups with mild or severe disease in disease activity score, levels of pro-MMP-3, -8, and -9, and activated MMP levels at baseline, one and two years

	Group with mild disease (n = 59)	p Value	Group with severe disease (n = 50)	p Value
JDS				
Baseline	0 (0–0)		2 (0–11)	
Year 1	0 (0–3)	<0.001	18 (13–41)	<0.001
Year 2	2 (0–6)		26 (17–52)	
DAS				
Baseline	3.2 (2.6–4.0)		3.8 (3.1–4.6)	
Year 1	2.7 (1.3–3.5)	<0.001	3.4 (2.6–4.2)	<0.001
Year 2	1.6 (1.1–3.0)		3.0 (1.9–3.4)	
Pro-MMP-3 (ng/ml)				
Baseline	17 (9–46)		31 (19–50)	
Year 1	18 (12–27)	NS	42 (26–77)	NS
Year 2	20 (11–28)		46 (22–76)	
Pro-MMP-8 (U/ml)				
Baseline	91 (54–121)		93 (59–130)	
Year 1	78 (43–102)	<0.001	65 (45–111)	<0.001
Year 2	51 (23–108)		65 (49–102)	
Pro-MMP-9 (U/ml)				
Baseline	54 (44–66)		53 (42–74)	
Year 1	51 (34–57)	<0.001	50 (40–60)	<0.001
Year 2	39 (31–48)		49 (30–59)	
MMP/α_2M (U/ml)				
Baseline	4.2 (3.5–5.7)		4.6 (3.6–7.4)	
Year 1	3.4 (2.7–4.6)	<0.001	4.0 (3.5–6.1)	NS
Year 2	3.4 (2.3–4.8)		4.4 (3.7–5.5)	

For detailed analysis of the MMPs during the study follow up, the study group was divided into two subgroups according to progression of the JDS. Patients who had JDS progression lower than or equal to the median JDS progression of the whole study group comprised the group with mild disease, whereas patients with JDS progression greater than the median of the whole study group were included in the group with severe disease. Data shown are median (25th–75th centiles).
DAS, disease activity score; (pro)-MMP, (pro)-matrix metalloproteinase; MMP/α_2M, MMP/α_2 macroglobulin complexes.
p Values show the significance of the changes in each group between baseline and year 2 (linear mixed model).

was performed for joint space narrowing as outcome measure. Joint space narrowing is a part of the total Sharp-van der Heijde joint damage score and represents loss of the articular cartilage. From the input parameters, the strongest association with joint space narrowing progression was found for baseline levels of pro-MMP-3 (B = 0.58, 95% CI 0.23 to 0.92, p = 0.001). Again, the association was independent of all other input parameters, and only the SE was also independently associated with joint space narrowing (B = 0.7, 95% CI 0.09 to 1.31, p = 0.026). To complete the analysis the erosion subcomponent of the Sharp-van der Heijde joint damage score was used as outcome measure. The results showed that in this model pro-MMP-3 levels at the onset of the disease and RF were the strongest predictors of the erosion formation (B = 0.49, 95% CI 0.1 to 0.88, p = 0.015 and B = 0.98, 95% CI 0.26−1.7, p = 0.009, respectively).

Patients with severe *v* mild disease progression

Because pro-MMP-3 levels were found to predict joint damage progression and pro-MMP-8 and -9 were not, the study group was divided to elucidate the involvement of the MMP subclasses in the disease process. Two subgroups were defined based on the progression of the JDS, which represents the Sharp-van der Heijde total damage scores.[23] The group with mild disease progression (mild progressors) had a JDS of ⩽10 during the two year follow up (the median of the whole group), whereas the group with severe disease progression (severe progressors) included patients with JDS >10.

The patients with RA with mild disease progression showed a 50% improvement in DAS (3.2 to 1.6, p<0.001, table 3). In the group with severe disease progression the DAS decreased less, but the change was significant over two years (3.8 to 3.0, p<0.001, table 3). Similar results were seen for CRP levels. CRP (mg/l, mean (SEM)) was significantly decreased both in the group with mild disease (from 25 (4) to 11 (2), p = 0.008, baseline *v* 2 years) and in the group with severe disease (from 38 (5) to 28 (6), p = 0.03).

Pro-MMP-3 levels

Comparison of serum pro-MMP-3 levels between the two subgroups (severe *v* mild) showed higher pro-MMP-3 levels in patients with severe progressive disease at each time (31 *v* 17 ng/ml at baseline, p = 0.02; 42 *v* 18 at one year, p<0.001, and 46 *v* 20 ng/ml at two years, p = 0.004, table 3). Overall, pro-MMP-3 levels were correlated with CRP at different times for both groups (at baseline and 1 year in mild progressors and at 1 and 2 years in severe progressors, data not shown). Although pro-MMP-3 levels and CRP were correlated, the pro-MMP-3 levels did not decrease in either patients with mild or severe progressive disease during the two year follow up, whereas a significant decrease in CRP levels for both groups was seen, suggesting that pro-MMP-3 and CRP may not provide the same information.

Pro-MMP-8 and -9 levels

No differences in pro-MMP-8 and -9 levels between the groups with mild and severe disease progression were seen at baseline or at later times (table 3, pro-MMP-8: p = 0.802, p = 0.581, p = 0.226 and pro-MMP-9: p = 0.993, p = 0.580, p = 0.159 for baseline, 1 year and 2 years, respectively). Pro-MMP-8 and -9 levels decreased during the two year follow up in both disease groups (all p<0.001, table 3).

MMP activity in α_2M complexes

At baseline, no differences were seen between the patients with mild and severe disease. A significant decrease in MMP activity was found in the group with mild progression

($p<0.001$; table 3), but a slight decrease in the MMP activity levels in the severe group did not reach significance. Levels in the group with severe disease were significantly higher at 1 and 2 years (at 1 year: 4.0 U/ml *v* 3.4 U/ml, $p = 0.01$, and at 2 years: 4.4 U/ml *v* 3.4 U/ml, $p = 0.04$; table 3).

DISCUSSION

In the present longitudinal study we found that high levels of pro-MMP-3 at the onset of RA are associated with severe joint damage progression. This association was independent of known risk factors such as SE, RF, and CRP. Further, we found that a decrease in the disease activity was accompanied by a decrease in the pro-MMP-8 and -9 levels in the serum of patients with RA. Moreover, a detectable surplus of activated MMPs, captured by α_2M, was found in the serum of patients with RA. This surplus was higher in the systemic circulation of patients with RA with a high rate of joint damage progression than in patients with a low rate of joint damage progression.

Contradictory reports have been published about the role of MMP-3 in joint tissue degradation or joint inflammation, or both. Cumulative serum pro-MMP-3 levels have been shown to correlate with joint damage progression,[10 29] suggesting that pro-MMP-3 plays a part in joint tissue degradation. Yamanaka *et al* indicated in a small group that serum pro-MMP-3 levels at the onset of disease may predict radiological joint damage.[8] In line with this we show in a longitudinal study of 109 patients with recent onset RA that baseline levels of pro-MMP-3 predict the loss of articular cartilage and total joint damage progression. Moreover, regression analysis showed that the association between pro-MMP-3 levels and joint damage progression was independent of other known predictive factors such as SE, RF, and CRP, thereby suggesting that MMP-3 has a crucial role in joint destruction.

However, other studies suggest that MMP-3 mainly reflects the inflammatory component of RA. Indeed, ample evidence has been generated to show that pro-MMP-3 levels are correlated with systemic CRP,[12] which is generally regarded as an inflammatory marker.[10 30–32] This interrelationship between MMP-3 levels and CRP, which is also found in this study, is not unexpected because both CRP and pro-MMP-3 are regulated through proinflammatory cytokines. However, our results also show that pro-MMP-3 and CRP do not necessarily provide the same information. Firstly, data analysis showed that pro-MMP-3 levels at the onset of disease were associated with joint damage progression, whereas CRP levels were not. Secondly, a significant decrease in CRP levels was seen in the study group during the two year follow up period, whereas pro-MMP-3 levels remained unchanged in the whole group. These differences may be explained by the nature of CRP and pro-MMP-3. CRP is an acute phase protein produced by the liver in response to circulating proinflammatory cytokines and is therefore regarded as a marker of systemic inflammation, including that originating in the joint. Pro-MMP-3 measured in the systemic circulation of patients with RA is likely to be produced in the affected joints, where it is directly involved in tissue degradation, and therefore, serum levels of pro-MMP-3 may more specifically reflect the destructive disease process. Furthermore, pro-MMP-3 levels were found to be significantly higher in the group with severe disease than in the group with mild disease not only at baseline but also during the two year follow up. Moreover, we observed that a decrease in pro-MMP-8 and -9 levels, and not pro-MMP-3, was accompanied by a decrease in disease activity. Taken together, these results indicate that MMP-3 may be seen as a constitutive marker of the pathological process underlying joint tissue degradation in RA.

It might be questioned whether the treatment which patients in this study received may be a confounding factor in the relationship between MMP levels and joint destruction. The results, however, showed that patients in the group with severe disease had higher MMP levels and a greater increase in joint damage during the study than those in the group with mild disease and they also received more treatment than the patients with milder disease. Given these observations, it seems unlikely that the treatment regimen would alter the interpretation of the results or undermine the conclusions of this study.

Pro-MMP-8 and -9 levels measured in the systemic circulation of patients with RA were not predictive of progression of joint damage in the whole group during the study period. The absence of this correlation between the baseline pro-MMP-8 and -9 levels and radiological joint damage progression is not entirely surprising. Pro-MMP-8 and -9 are produced mainly by leucocytes: neutrophils (pro-MMP-8 and -9)[33] and macrophages (pro-MMP-9).[34] In RA, leucocytes are recruited into the joints during inflammation[35] and are greatly increased in the SF. According to our previous results (Tchetverikov, unpublished data), systemic circulation and synovial fluid levels of pro-MMP-8 and -9 in patients with RA are highly correlated, suggesting that pro-MMP-8 and -9 levels found in the systemic circulation may represent the situation in the affected joints. Because pro-MMP-8 and -9 are produced by inflammatory cells and are subject to fluctuations of inflammation in RA, their levels are likely to change during the course of RA according to the disease activity. Indeed, in our study group we noted a 33% reduction in DAS, decreased CRP levels, and also significant decreases in the pro-MMP-8 and -9 levels. Altogether, these results suggest that MMP-8 and MMP-9 play a part in the disease process in RA and may indicate the current status of this proteolytic system, involved in joint inflammation.

In our study group MMP activity in α_2M/MMP complexes was detected in the groups with both mild and severe disease. However, a significant decrease of about 20%, which was detected in the group with mild disease over the two years' follow up, was not seen in the group with severe disease, indicating insufficient reduction of the disease activity as suggested by the DAS and CRP. The presence of activated MMPs captured by α_2M in the systemic circulation of patients with RA supports the theory of an imbalance between MMP and their natural inhibitors, TIMPs. In pathological situations, such as RA, an imbalance exists between levels of MMPs and TIMPs.[15] It has been shown previously that when a surplus of active MMPs over TIMPs exists[16 17] activated MMPs can be captured by the natural proteinase inhibitor α_2M.[36] Thus, our findings of higher MMP activity in α_2M complexes in patients with RA with severe disease activity indicate a significant imbalance between MMPs and TIMPs in favour of MMPs, which are involved in the disease process of early RA.

In conclusion, this study shows that MMP-3, -8, -9 have a role in the joint inflammation and joint damage of recent onset RA. Pro-MMP-8 and -9 decreased during the study period as did the disease activity; pro-MMP-3 and -8 were correlated with levels of CRP in the systemic circulation, whereas serum pro-MMP-3 levels predicted the progression of joint damage in the study group.

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