Matrix metalloproteinases involvement in rheumatoid arthritis

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CHAPTER

MATRIX METALLOPROTEINASES-3, -8, -9 AS MARKERS OF DISEASE ACTIVITY AND JOINT DAMAGE PROGRESSION IN EARLY RHEUMATOID ARTHRITIS

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Abstract

Objective. The aim of the present study was to analyze the relationship between systemic levels of proMMP-3, -8 and -9, MMP activity in α_2M/MMP complexes and the progression of joint destruction in recent-onset rheumatoid arthritis (RA) patients.

Methode. One hundred and nine patients diagnosed with RA of recent-onset were entered in this study. In this longitudinal study, the patients were followed for 2 years; clinical data, blood samples and radiographs were obtained at baseline, at 1 year and at 2 years. Serum levels of MMPs were quantified using sandwich ELISA and MMP activity assays.

Results. During the 2-year study, joint damage progressed from 0 to 10 (median Sharp score, $P < 0.001$). Stable levels of proMMP-3 and a significant decrease in the levels of proMMP-8 and -9 and α_2M/MMP complexes were observed throughout the 2 years. Regression analysis showed that serum proMMP-3 levels at the disease onset were independently associated with the progression of joint damage (B: 0.37, 95% CI: [0.13-0.61], $P = 0.003$).

Based on the rate of joint destruction, the patients were divided into two subgroups: patients with mild and severe joint damage progression. The proMMP-3 levels were significantly higher in the severe vs. mild group at all time points, a decrease in the levels of proMMP-8 and -9 was found in both groups whereas α_2M/MMP complexes levels decreased in the mild group only.

Conclusion. Whereas serum levels of studied MMPs are associated with the disease activity, serum proMMP-3 levels at the onset of disease are also predictive of joint damage progression.

Introduction

Rheumatoid arthritis (RA)[I] is a chronic and potentially crippling disease characterized 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 play 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 at tissue level, in the synovial fluid (SF) and in the systemic circulation of the RA patients. 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 are also capable of activating other MMPs, thus playing an important role in the MMP cascade.[5-7] ProMMP-3 levels were shown to correlate with systemic inflammatory markers and were suggested to be involved in cartilage degradation.[8-10] Collagenases are capable of degrading the intact collagen molecule, one of the main components of the articular cartilage. SF proMMP-1 (collagenase-1) levels were shown to correlate with the degree of synovial inflammation[11] and serum proMMP-1 levels with development of joint erosions.[12] Systemic levels of proMMP-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 were also shown to be associated with the development of radiographic erosion in RA patients.[14] After MMPs are produced and activated their action is further controlled by specific inhibitors of Matrix Metalloproteinases (TIMP). Normally a tight balance exists between MMPs and their tissue inhibitors. However, in pathological situations such as rheumatoid arthritis, an MMP/TIMP imbalance is present which leads to an excess of activated MMPs and has been implicated to play an important role in the chain of event leading to the excessive cartilage degradation.[15] In the systemic circulation and SF, MMPs are also readily captured by α_2Macroglobulin, a natural protease inhibitor.[16-18] It has been previously shown that in SF and in the systemic circulation of RA patients the levels of MMP/α_2M complex are increased, suggesting that levels of MMP/α_2M complexes represent the MMP/TIMP imbalance present in RA.

The present study was designed to investigate whether levels of matrix metalloproteinases measured in the systemic circulation have predictive value with regard to joint destruction. Since our previous results of MMP measurements in SF and systemic circulation yielded the highest differences between RA/OA and healthy controls for proMMP-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 (proMMP-3), collagenase-2 (proMMP-8) and gelatinase B (proMMP-9) as well as general MMP

[I] Abbreviations: α_2M: alpha$_2$Macroglobulin; CRP: C-reactive protein; DAS: Disease Activity Score; EAC: Early Arthritis Clinic; JDS: Joint Damage Score; MMPs: Matrix Metalloproteinases; proMMPs: pro-Matrix Metalloproteinases; RA: rheumatoid arthritis; SE: Shared Epitope; SF: Synovial Fluid; TIMPs: Tissue Inhibitors of Metalloproteinases.

activity in α_2M/MMP complexes were analyzed in relation to the rate of radiological progression and disease activity in recent-onset RA patients.

Patients and Methods

In 1993 a special Early Arthritis Clinic (EAC) was started at the Department of Rheumatology of the Leiden University Medical Center, the only center 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 1) the arthritis was confirmed by a rheumatologist, 2) the history of symptoms lasted less than 2 years and 3) the patients had not been visiting a rheumatologist elsewhere for the same problem.

In the present study, only patients with the diagnosis RA defined according to the 1987 ACR criteria[19] or probable RA using the 1958 ACR criteria,[20] but without the 6 weeks duration observed by a physician,[21] were analyzed. Sixty six percent 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*

	RA patients (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 shown is median [25th - 75th percentiles]*

*** RA = rheumatoid arthritis according to the 1987 ACR classification criteria*

**** probable RA = probable rheumatoid arthritis according to the 1958 ACRclassification criteria. Sixty six percent 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.*

The study group comprised 109 patients recruited to the EAC. All patients were initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs). After approximately four months those still having active disease received disease modifying anti-rheumatic drugs

(DMARDs) such as chloroquine (CQ) or sulfasalazine, if CQ 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 experienced side effects or required a treatment change due to lack of efficacy.[22] Six patients received prednisone at some point during the study.

For further analysis, the whole study population was divided into two subgroups. The subgroups were defined based on the joint damage score (JDS, represent the Sharp-van der Heijde total damage scores[23]). The mild disease progression group (mild progressors) had JDS lower than or equal to the median of the JDS of the whole study population during the 2 year follow-up (the median Sharp score of the whole group is 10) and comprised of 59 patients (mean ± SD age: 54 ± 16; 73% females, 39% received DMARD). Patients with JDS greater than the median of the whole group were assigned in the severe disease progression group (severe progressors, N = 50; mean ± SD age: 57.2 ± 14; 72% females, 72% received DMARD).

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 disease activity score 3 was as follows: disease activity score 3= 0.54*(√Ritchie score) + 0.065*(number of swollen joints) + 0.33*ln erythrocyte sedimentation rate + 0.224. All joints were assessed as in the Ritchie Articular Index except for the acromioclavicular, subtalar and midtarsal 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, one year and two years. Radiographs were scored randomly by one experienced rheumatologist according to the modified Sharp/ van der Heijde method.[23] The intra-class correlation coefficient for the assessor's scoring was 0.95, as measured in 39 patients. The laboratory variable RF-IgM was determined at study entry and measured by ELISA as described previously.[26] RF-IgM titers of ≥ 5 units were considered positive. Also the HLA phenotypes according to the Shared Epitope (SE) model were analyzed 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. The demographic, clinical and radiological data of the patient group are shown in **Table 1**.

MMP analyses

Serum was prepared after blood collection and all samples were stored at –20 °C prior to analysis. ProMMP-3 was analyzed using the sandwich ELISA for proMMP-3 according to the manufacturer's instructions (Amersham Biosciences, Little Chalfont, UK). MMP-8 and -9 (proMMPs were captured using monoclonal antibody and its activity was measured after APMA 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_2Macroglobulin 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 loose 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™ (1 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 6 hrs at 30°C using a Cytofluor 4000 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Differences between the subgroups of interest were tested with the Mann-Whitney U test. The changes within patients in time were analyzed using linear mixed model per study subgroup (repeated measurements parameter was entered into the model, data is log transformed for JDS and proMMP-3 levels and square-root transformed for promMP-8 and -9 levels for the analysis). Correlations for non-parametric data were evaluated by calculating 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, CRP, RF, SE, log transformed proMMP-3, -8, -9 and activated MMP in α_2M. Before log transformation, 1 point was added to all progression scores (Δ Sharp score = Sharp score$_{endpoint}$ - Sharp score$_{baseline}$) to avoid a zero Δ Sharp score in order to be able to perform log transformation. All tests were two-tailed and $P \leq 0.05$ 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 present study group declined significantly from 3.4 to 2.5 ($P < 0.001$), the median CRP levels 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 2 years ($P < 0.001$). A stable serum level of proMMP-3 throughout the two years follow-up was observed in the study group (median [25th - 75th percentiles]: 25 [11 - 47], 26 [16 - 65] and 25 [14 - 58] ng/ml, at baseline, at 1 and 2 years, respectively; **Figure 1**). A significant decrease in levels of proMMP-8 and -9 was found in the study group: proMMP-8 decreased from 91 [57 - 127] to 54 [33 - 108] U/ml ($P = 0.002$) and proMMP-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 one 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 time points (**Table 2**).

Table 2. *Correlations (r_s) between proMMP and activated MMP levels and CRP and DAS in the rheumatoid arthritis patients at baseline, one year and two years.*

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

[a]P<0.005, [b]P<0.05 (Spearman's rho). CRP = C-reactive protein, MMP = matrix metalloproteinase, DAS = disease activity score

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

It was previously suggested that both CRP and systemic proMMP-3 levels[9] at disease onset are predictive of joint damage progression. To analyze which baseline parameters were associated with progression of joint damage, a stepwise log linear regression analysis was performed. Next to proMMP-3, -8, -9 and MMP activity in α_2M/MMP complexes other potential predictors such as Rheumatoid Factor (RF), Shared Epitope (SE), DAS and CRP levels at baseline were included into the model. The strongest association between the input factors and joint damage progression was found for proMMP-3 levels at onset of the disease. The association between joint damage progression and proMMP-3 levels (B: 0.7, 95% C.I. [0.3 - 1.01], $P < 0.001$) was independent from all other parameters. From the others potential predictors included in the analysis, only SE was also independently associated with progression of joint damage (B: 1.02, 95% C.I. [0.3 - 1.74], $P = 0.006$). The baseline parameters RF was also associated with joint damage progression in this model, however not independently. Since MMPs are likely to be mainly involved in cartilage degradation in RA, similar stepwise log linear regression analysis 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 represent loss of the articular cartilage. From the input parameters, the strongest association with joint space narrowing progression was

found for baseline levels of proMMP-3 (B: 0.58, 95% C.I. [0.23 - 0.92], $P = 0.001$). Again, the association was independent from all other input parameters and only SE was also independently associated with joint space narrowing (B: 0.7, 95% C.I. [0.09 - 1.31], $P = 0.026$). To complete the analysis, the erosion sub-component of the Sharp-van der Heijde joint damage score was used as outcome measure. The results showed that in this model proMMP-3 levels at the onset of the disease and RF were the strongest predictor of the erosion formation (B: 0.49, 95% C.I. [0.1 - 0.88], $P = 0.015$ and B: 0.98, 95% C.I. [0.26 - 1.7], $P = 0.009$, respectively).

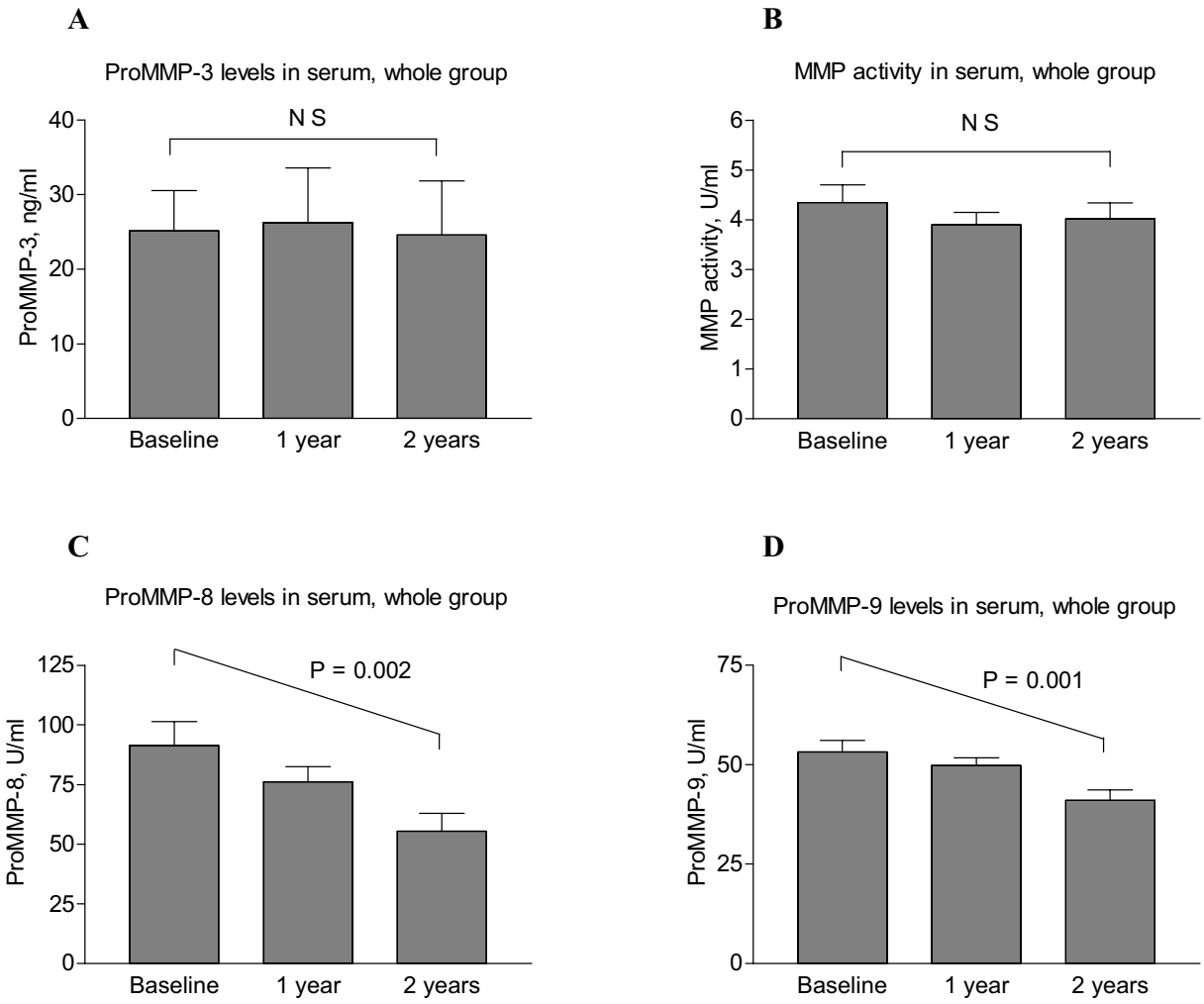

Figure 1. *Group median (+ SE) values of Matrix Metalloproteinases (MMPs) measured in serum of 109 patients with recent-onset RA. Levels of proMMP-3, proMMP-8, proMMP-9 and MMP activity in α_2Macroglobulin/MMP (α_2M/MMP) complexes were measured at baseline and at 1 and 2 years of follow-up.*

A. ProMMP-3 levels remain stable during the follow-up period.

B. MMP activity in α_2M/MMP levels showed slight but significant decrease.

C. ProMMP-8 levels decreased significantly during the follow-up period.

D. ProMMP-9 levels decreased significantly during the follow-up period.

P value indicates the significance of changes within the group in time (Linear mixed model).

Patients with severe vs. mild disease progression

Inasmuch as proMMP-3 levels were found to predict joint damage progression and proMMP-8 and -9 were not, the study population was divided to elucidate the involvement of the MMP subclasses in the disease process. Two subgroups were defined based on the progression of the joint damage score (JDS, represent the Sharp-van der Heijde total damage scores[23]). The mild disease progression group (mild progressors) had JDS of ≤ 10 during the 2-year follow-up (the median of the whole group), whereas the severe disease progression group (severe progressors) included patients with JDS greater than the median JDS of the whole group.The results are shown in **Table 3**.

Table 3. *Differences within the patient groups with mild or severe disease groups in disease activity score, levels of proMMP-3, -8, and -9 and activated MMP levels at baseline, one year and two year follow-up.*

	Mild Group (N = 59)	***P* value**	**Severe Group** (N = 50)	***P* value**
JDS				
Baseline	0 [0-0]	<0.001	2 [0-11]	<0.001
Year 1	0 [0-3]		18 [13-41]	
Year 2	2 [0-6]		26 [17-52]	
DAS				
Baseline	3.2 [2.6-4.0]	<0.001	3.8 [3.1-4.6]	<0.001
Year 1	2.7 [1.3-3.5]		3.4 [2.6-4.2]	
Year 2	1.6 [1.1-3.0]		3.0 [1.9-3.4]	
ProMMP-3, ng/ml				
Baseline	17 [9-46]	NS	31 [19-50]	NS
Year 1	18 [12-27]		42 [26-77]	
Year 2	20 [11-28]		46 [22-76]	
ProMMP-8, U/ml				
Baseline	91 [54-121]	<0.001	93 [59-130]	<0.001
Year 1	78 [43-102]		65 [45-111]	
Year 2	51 [23-108]		65 [49-102]	
ProMMP-9, U/ml				
Baseline	54 [44-66]	<0.001	53 [42-74]	<0.001
Year 1	51 [34-57]		50 [40-60]	
Year 2	39 [31-48]		49 [30-59]	
MMP/α_2M, U/ml				
Baseline	4.2 [3.5-5.7]	<0.001	4.6 [3.6-7.4]	NS
Year 1	3.4 [2.7-4.6]		4.0 [3.5-6.1]	
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 the joint damage score (JDS) progression. Patients who had JDS progression lower than or equal to the median JDS progression of the whole study population comprised the mild disease group, whereas patients with JDS progression greater than the median of the whole study population were included in the severe group. Data shown are median [25th and 75th percentiles]. DAS = Disease Activity Score, (pro)MMP = (pro)Matrix Metalloproteinase, MMP/α_2M = MMP/α_2Macroglobulin complexes. P values show the significance of the changes per group in time (Linear mixed model).

The RA patient group with mild disease progression showed a 50 % improvement in DAS (3.2 to 1.6, $P < 0.001$, Table 3). In the patient group with severe disease progression, the DAS decreased less, but statistically significant over two years (3.8 to 3.0, $P = 0.001$, Table 3). Similar results were seen with regard to the CRP levels. CRP (mg/L, mean ± SEM) was significantly decreased both in the patient group with mild disease (from 25 ± 4 to 11 ± 2, $P = 0.008$, baseline vs. 2 year) and in the patients group with severe disease (from 38 ± 5 to 28 ± 6, $P = 0.03$).

ProMMP-3 levels

Comparison of serum proMMP-3 levels between the 2 subgroups (severe vs. mild) revealed higher proMMP-3 levels in patients with severe progressive disease at each time-point (31 vs. 17 ng/ml at baseline, $P = 0.02$; 42 vs. 18 at one year, $P < 0.001$, and 46 vs. 20 ng/ml at two years, $P = 0.004$, Table 3). Overall, proMMP-3 levels were correlated with CRP at different time points for both groups (at baseline and one year in mild progressors and at one and two years in severe progressors, data not shown). Although proMMP-3 levels and CRP were correlated, the proMMP-3 levels did not decrease in either mild or progressive patients group during the 2-year follow-up, whereas a significant decrease in CRP levels for both groups was observed, implying that proMMP-3 and CRP may not reveal the same information.

ProMMP-8 and -9 levels

No differences in proMMP-8 and -9 levels between mild and severe disease progression groups were observed at baseline or at later time points (Table 3, ProMMP-8: $P = 0.802$, $P = 0.581$, $P = 0.226$ and proMMP-9: $P = 0.993$, $P = 0.580$, $P = 0.159$ for T = 0, T = 1, T = 2, respectively). ProMMP-8 and -9 levels decreased during the 2-year follow-up period 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 mild progression group ($P < 0.001$; Table 3), while a slight decrease in the MMP activity levels in the severe group did not reach the level of statistical significance. Levels in the severe group were significantly higher at one and two years (at one year: 4.0 U/ml vs. 3.4 U/ml, $P = 0.01$, and at two years: 4.4 U/ml vs. 3.4 U/ml, $P = 0.04$; Table 3).

Discussion

In the present longitudinal study, we observed that high levels of proMMP-3 at 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 proMMP-8 and -9 levels in serum of RA patients. Moreover, a detectable surplus of activated MMPs, captured by α_2Macroglobulin, was found in serum of RA patients. This surplus was higher in the

systemic circulation of RA patients with high rate of joint damage progression compared to the patients with low rate of joint damage progression.

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

However, other studies suggest that MMP-3 mainly reflects the inflammatory component of RA. Indeed, ample evidence have been generated that proMMP-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 since both CRP and proMMP-3 are regulated through pro-inflammatory cytokines. However, our results also show that proMMP-3 and CRP do not necessarily reveal the same information. Firstly, data analysis showed that proMMP-3 levels at onset of disease were associated with joint damage progression, whereas CRP levels were not. Secondly, a significant decrease in CRP levels was observed in the study group during the 2-years follow up period, whereas proMMP-3 levels remained unchanged in the whole group. These differences could be explained by the nature of CRP and proMMP-3. CRP is an acute phase protein produced by the liver in response to circulating pro-inflammatory cytokines and is therefore regarded as a marker of systemic inflammation, including that originating in the joint. ProMMP-3 measured in the systemic circulation of RA patients is likely to be produced in the affected joints, where it is directly involved in tissue degradation and therefore, serum levels of proMMP-3 may more specifically reflect the destructive disease process. Further, proMMP-3 levels were found to be significantly higher in the patients group with severe disease when compared to the mild disease group not only at baseline but also during the 2 years of follow up. Moreover, we observed that a decrease in proMMP-8 and -9 levels, and not proMMP-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 underling joint tissue degradation in RA.

It could be questioned whether the treatment, which patients in this study received, could be a confounding factor in the relationship between MMP levels and joint destruction. The results, however, showed that patients in the severe group had higher MMP levels and higher increase in joint damage during the study than patients in the mild group while they also received more treatment than patients in the mild group did. Given these observations, it seems unlikely that the treatment regiment as such could disturb the interpretation of the results or undermine the conclusions of this study.

ProMMP-8 and -9 levels measured in the systemic circulation of RA patients were not predictive of progression of joint damage in the whole group during the study period. The absence of this correlation between the baseline proMMP-8 and -9 levels and radiological joint damage progression is not entirely surprising. ProMMP-8 and -9 are produced mainly by leukocytes: neutrophils (proMMP-8 and -9)[33] and macrophages (proMMP-9).[34] In case of RA, leukocytes are recruited into the joints during inflammation[35] and are highly increased in SF. According to our previous results (Tchetverikov, unpublished), systemic circulation and synovial fluid levels of proMMP-8 and -9 in RA patients are highly correlated, suggesting that proMMP-8 and -9 levels found in the systemic circulation may represent the situation in the affected joints. Since proMMP-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 population, we observed a 33% reduction in DAS, decreased CRP levels and also significant decreases in the proMMP-8 and -9 levels. Altogether, these results imply that MMP-8 and MMP-9 are involved 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_2Macroglobulin/MMP complexes was detected in both the mild and severe disease groups. However, a significant decrease of approximately 20%, which was detected in the mild disease group over the 2-year follow-up period, was not observed in the severe disease group indicating insufficient reduction of the disease activity as suggested by DAS and CRP. The presence of activated MMPs captured by α_2M in the systemic circulation of RA patients supports the theory of the 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 previously shown 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 RA patients with severe disease activity indicate a significant imbalance between MMPs and TIMPs in favor of MMPs, which are involved in the disease process of early RA.

In conclusion, this study shows that MMP-3, -8, -9 are involved in the pathophysiology of recent-onset RA regarding both joint inflammation and joint damage. ProMMP-8 and -9 were decreased during the study period as well as the disease activity; proMMP-3 and -8 were correlated with levels of C-reactive protein in the systemic circulation, whereas serum proMMP-3 levels predicted the progression of joint damage in the study group.

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