The significance of serum matrix metalloproteinase 3 in patients with early rheumatoid arthritis

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Chapter 8

Summary, general conclusions and future perspectives

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SUMMARY

Rheumatoid arthritis (RA) is a systemic inflammatory disease, characterized by a chronic inflammation of synovial tissue and in most cases progressive destruction of cartilage and bone [1]. In particular joint destruction is one of the strongest predictors of long term outcome and disability in RA [2].

The matrix metalloproteinases (MMPs) are considered to be key mediators in the destruction of the matrix components of cartilage and bone in inflamed RA joints [3]. In synovial tissue, chondrocytes and synovial fluid of inflamed RA joints many MMPs are upregulated [4,5]. In particular MMP-3 is of interest because it is supposed to play a prominent role in the pathogenesis of matrix degradation in the inflamed joint [6], although it is not the only key enzyme [7]. It is locally produced as a latent pro-enzyme and activated within the affected joint, resulting in elevated levels in local tissue [8-10] and synovial fluid [4,11].

Serum MMP-3 in RA is thought to originate from these inflamed joints because there are significant correlations between MMP-3 levels in synovial fluid and serum [12], as well as between serum MMP-3 levels and the number of clinically active inflamed joints [12,13]. Furthermore local therapies, such as intra-articular corticosteroid injections, cause significant reductions in serum MMP-3 levels in synovial fluid and in the systemic circulation of patients with RA [14,15].

Elevated serum MMP-3 levels are not specific for RA, they are also found in several other rheumatic and non-rheumatic diseases (as described in **chapter 1)**. Nevertheless, in RA the systemic MMP-3 levels are considered to be a direct reflection of local synthesis induced by pro-inflammatory cytokines. As such, serum MMP-3 could be a useful, systemic marker of disease activity [16] and joint destruction [17,18] in RA. In this respect serum MMP-3 may reflect joint inflammation and/or destruction more directly than C-reactive protein (CRP), which is produced by the liver indirecly after cytokine stimulation [19]. In addition it has been suggested that the pathophysiologic mechanisms of joint inflammation, as reflected by CRP, may be partially independent of the mechanisms of destruction [20-22], which are also determined by the prominent local cytokine-, protease-, and inhibitor-environment. This possible uncoupling of inflammation and destruction could be one of the explanations for the wide inter-individual variation in radiological damage despite comparable inflammation as reflected by, for example, CRP [23]. Moreover it might imply re-evaluation of traditional indicators and a search for new, more specific markers of disease activity and joint destruction.

The **aim of the thesis** was to investigate the significance of serum MMP-3 in relation to disease activity, and in particular in relation to radiological progression, in patients with early RA.

The results of the clinical studies are based on data derived from a prospective observational study in patients with early RA (disease symptoms < 1 year and disease modifying anti-rheumatic drugs (DMARDs) naive at study entry). The follow-up protocol included monthly assessments of disease activity and X-rays of hands and feet every 6 months.

In **chapter 2** a general review is provided on matrix metalloproteinases. MMPs are a family of zinc-containing proteases and are thought to be key mediators involved in the remodeling of the extra-cellular matrix. The regulation of MMP production, activation and inhibition is discussed, as well as the role of MMPs in physiological and pathological conditions, in particular in RA. Finally, therapeutic options of MMP-inhibition are set in perspective.

In **chapter 3** the significance of serum MMP-3 levels in relation to the development of radiological damage in early RA patients was evaluated.

Serum MMP-3 levels were measured in 46 healthy controls, 19 osteoarthritis patients and in 78 early RA patients (joint symptoms < 1 year at presentation (T_0)): 48 patients without and 30 with radiological damage at T_0. Serum MMP-3, measured by ELISA, and radiological damage, scored according to Sharp/van der Heijde method were assessed at 0, 6, 12 and 24 months.

MMP-3 levels in controls and osteoarthritis patients were low or undetectable with no differences between the groups. Levels in RA were higher than in controls. Initial MMP-3 levels in the patients with radiological damage at T_0 (n=30) were higher than the levels in patients without any radiological damage during follow-up (n=19), but were not different from patients who developed radiological damage during the study (n=29). In the patients without radiological damage at T_0 there was a significant correlation between MMP-3 at T_0 and the total radiological damage after 6 months and 12 months. This correlation was almost exclusively determined by the item joint space narrowing in the Sharp score.

In conclusion the initial serum MMP-3 level seems to be an indicator for the development of radiological damage in patients with early RA and appears to be particularly indicative of cartilage degradation.

In **chapter 4** the clinical significance of serial measurements of serum MMP-3 levels in relation to markers of disease activity and radiological progression in early RA was analyzed.

In a 3 year prospective follow-up study of 33 patients with early RA monthly measurements of serum MMP-3 were transformed into time-integrated values for 6 months periods for comparison with other markers of disease activity such as swollen joint count (SJC), tender joint count (TJC), Ritchie articular index (RAI), the disease activity score (DAS), erythrocyte sedimentation rate (ESR), CRP, and radiological progression, assessed according to the Sharp/van der Heijde method.

Significant correlations were found between serum MMP-3 and SJC, ESR, and CRP during all periods and for most 6-months period with the DAS. There were no correlations between serum MMP-3 and TJC or the RAI. During the first 12 months serum MMP-3 only correlated with the item joint space narrowing of the Sharp score. After 12 months of follow-up it 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 was rather constant.

In summary, in this study the time-integrated values of serum MMP-3 correlated with time-integrated values of other markers of disease activity. Of the radiological scores, as outcome measures, especially joint space narrowing was closely correlated with cumulative serum MMP-3.

In **chapter 5** the effects of treatment with sulphasalazine (SSZ) or the combination of methotrexate (MTX) and SSZ on serum MMP-3 levels in patients with early RA were investigated

Eighty-two patients with early RA and DMARD-naive at presentation were selected who were treated with SSZ (2000 mg/day) or with the combination of MTX (7.5-15 mg/week) and SSZ. Serum MMP-3 levels, CRP, ESR, SJC, TJC, RAI, and the DAS were determined at 4-week intervals during a follow-up of 28 weeks for each treatment group. Response or non-response was based on clinical grounds in combination with the influence on CRP levels ($\geq$ 50% reduction in joint scores or CRP (or normalization of CRP)) at 12, 20, and 28 weeks.

SSZ responders (n=52) had lower baseline values of serum MMP-3, CRP, and ESR, compared to partial/non-responders (n=30), but did not differ in joint scores and DAS at entrance. In the SSZ responder group all variables decreased. In the SSZ partial/non-responders CRP, ESR, and SJC decreased in contrast to serum MMP-3, TJC, RAI, and DAS-3. After addition of MTX all variables decreased in

24 out of the 30 patients who had shown a partial or no response on SSZ previously. In the SSZ responders there was a delayed decrease in serum MMP-3 compared to CRP.

In conclusion the serum MMP-3 levels decrease in early RA patients who respond to SSZ or to the combination of MTX and SSZ. In patients who respond to SSZ the changes in serum MMP-3 levels show a delayed response compared to CRP.

In **chapter 6** serum MMP-3 levels in comparison to CRP in periods with and without progression of radiological damage in patients with early RA were evaluated.

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 months period without radiological progression (< 1 point) were selected from a prospective follow-up study of early RA patients. Serum MMP-3 levels, CRP, ESR, DAS, SJC, TJC and, RAI were measured monthly and results were transformed into mean values for the 6 months periods.

During the period with radiological progression the mean serum MMP-3 correlated significantly with the mean CRP, ESR and swollen joint count. In the period without radiological progression the mean serum MMP-3 only correlated with the mean CRP. Individual changes - in terms of percentage (%) - between the 2 periods showed a decrease in both mean serum MMP-3 and CRP in most patients, in parallel with other markers of disease activity. However this individual change (%) in the mean serum MMP-3 or CRP was not correlated with the difference in radiological progression between the two consecutive periods.

So serum MMP-3 and CRP were closely related and there seemed to be no difference between serum MMP-3 and CRP with regard to the monitoring of the progression of radiological damage.

In **chapter 7** the significance of MMP-1 and MMP-3 promoter polymorphisms in relation to disease activity and radiological damage in patients with early RA was analyzed. Functional MMP promoter polymorphisms have been reported for MMP-1 and MMP-3 [24] resulting in different amounts of gene product [25-27]. Consequently this might lead to different levels of MMPs and more or less joint destruction after the same stimulus.

MMP-1 (1G-1607/2G) and MMP-3 (5A-1171/6A) promoter polymorphisms were determined in a cohort of 448 early RA patients. Clinical and laboratory

markers of disease activity and severity at presentation (T_0) were related to these polymorphisms. In addition the relations with total radiological damage and radiological progression (Sharp/van der Heijde method) after 2 years were evaluated. In a subgroup initial serum MMP-3, cumulative serum MMP-3 (serum MMP-3 area under the curve; sMMP-3^{AUC}) over 0.5 and 1 year and serum MMP-3^{AUC}/CRP^{AUC} ratio in relation to the MMP-3 promoter polymorphism were studied.

No association was found between MMP-1 and MMP-3 promoter polymorphisms and disease activity, severity or radiological damage at T_0. MMP-1 and MMP-3 promoter polymorphisms showed strong linkage disequilibrium, but no associations were found with "risk factors" such as rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) and HLA-DR4 status. The MMP-1 and MMP-3 promoter polymorphisms were not associated with radiological damage or radiological progression after a follow-up of 2 years.

Serum MMP-3^{AUC} correlated significantly with radiological progression after 6 and 12 months. However the serum MMP-3^{AUC} after 6 and 12 months did not differ across the three MMP-3 promoter polymorphism groups. On the other hand the MMP-3 promoter polymorphisms 5A/6A and 6A/6A were associated with higher serum MMP-3 levels relative to CRP (serum MMP-3^{AUC}/CRP^{AUC} ratios), as analyzed over a 12 months period.

These last results are in contrast with *in vitro* experiments in which the 6A/6A genotype seems to be a "low producer" of MMP-3 transcripts [27]. On the other hand, our results might be in line with the data of Constantin et al who found more radiological damage in the 5A/6A and 6A/6A genotypes.

So, although cumulative MMP-3 levels are associated with radiological progression in RA, the influence of the MMP-3 promoter polymorphism is apparently not of great clinical relevance.

CONCLUSIONS

The presented studies reveal several interesting conclusions with respect to the significance of serum MMP-3 in early RA:

1. The initial serum MMP-3 level is an prognostic factor for the development of radiological damage (chapter 3)
2. The initial serum MMP-3 and cumulative serum MMP-3 (time integrated values) levels are correlated with radiological progression, in particular with joint space narrowing (chapter 3 and 4)
3. Serum MMP-3 is correlated with other markers of disease activity, such as joint swelling, ESR, CRP and the DAS (chapter 4)
4. Serum MMP-3 levels decrease in patients who respond to treatment with the DMARDs sulphasalazine or to the combination methotrexate/sulphasalazine. In the patients who respond to sulphasalazine the changes in serum MMP-3 show a delayed response compared to CRP (chapter 5)
5. Serum MMP-3 and CRP are closely correlated (chapter 4 and 6) and there seems to be no difference between serum MMP-3 and CRP with regard to the monitoring of the progression of radiological damage (chapter 6)
6. MMP-1 and MMP-3 promoter polymorphisms were not associated with disease activity or severity at presentation. In addition the MMP-1 and MMP-3 promoter polymorphisms were not associated with radiological damage or radiological progression after a follow-up of 2 years. However the MMP-3 promoter polymorphisms 5A/6A and 6A/6A were associated with increased serum MMP-3 levels relative to CRP, as analyzed over a 12 months period. (chapter 7).

In summary:

I. Serum MMP-3 is an indicator (marker) of disease activity and radiological damage/progression
II. Serum MMP-3 is not superior to CRP with respect to the monitoring of disease activity and radiological progression, probably due to the close correlation between serum MMP-3 and CRP
III. MMP-3 promoter polymorphisms are apparently not of clinical significance in patients with early RA.

FUTURE PERSPECTIVES

Despite optimal care and recent new therapeutic advances in RA, such as tumor necrosis factor α (TNF-α) blocking agents and Interleukin 1 (IL-1) receptor antagonist, it is often not possible to completely stop joint destruction [28]. This progressive joint destruction is considered to be a consequence of a dysbalance between an excess of activated MMPs and inadequate levels of their inhibitors.

Over the last 10-15 years a number of "specific" MMP-inhibitors, mainly focused on inhibition of MMP activity, have been developed, with disappointing results (see chapter 1) [29].

Recent studies are focused at the signal transduction pathways. Blockade of the MAPK, NFκB and JAK/STAT pathways lead to inhibition of MMP expression in tissue culture experiments and in animal models of arthritis [30-32]. This implicates that this type of intervention leads to both a direct inhibition of MMP production as well as an indirect inhibition of MMP production by inhibition of the pro-inflammatory cytokines. Clinical studies targeted at these signal transduction pathways are currently evolving [33].

In this context it is essential to realize that further research with respect to markers of disease activity and destruction is of great importance. Not only because more specific MMP inhibition might result in an uncoupling of inflammation and destruction but also because currently used parameters of inflammation like the acute phase proteins such as CRP are mediated by similar signal transduction pathways [34]. This means that the use of CRP and possibly other acute phase proteins to monitor inflammatory activity during treatment with signal transduction inhibitors, such as p38 MAPK inhibitors, may not be valid. The production may be influenced both at the level of cytokine production (inhibition of TNF-α, IL-1, IL-6) and at the protein synthesis level of the liver-response itself.

Future studies will be focused on these signal transduction pathways. Not only with respect to effects on disease activity and joint destruction but also regarding the effects on markers of inflammation and destruction.

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