Serum Matrix Metalloproteinase 3 Levels During Treatment with Sulfasalazine or Combination of Methotrexate and Sulfasalazine in Patients with Early Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine the effects of treatment with sulfasalazine (SSZ) or the combination of methotrexate (MTX) and SSZ on serum matrix metalloproteinase 3 (MMP-3) levels in patients with early rheumatoid arthritis (RA).

Methods. Eighty-two patients with early RA (symptoms < 1 year and DMARD-naive at presentation) were selected who had been treated with SSZ (2000 mg/day) or with the combination of MTX (7.5–15 mg/week) and SSZ. Serum MMP-3 levels, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), swollen joint count (SJC), tender joint count (TJC), Ritchie articular index (RAI), and the Disease Activity Score (DAS) were determined at 4 week intervals during a followup of 28 weeks for each treatment group. Response was based on clinical grounds and CRP at 12, 20, and 28 weeks.

Results. SSZ responders (n = 52) had lower baseline values of serum MMP-3, CRP, and ESR, compared to partial/nonresponders (n = 30), but did not differ in joint scores and DAS. In the SSZ responder group all variables decreased. In the SSZ partial/nonresponders, 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 of the 30 patients who had shown a partial or no response taking SSZ. In the SSZ responders there was a delayed decrease in serum MMP-3 compared to CRP.

Conclusion. Serum MMP-3 levels decrease in patients with early RA 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 indicate a delayed response compared to CRP. (J Rheumatol 2002;29:883–9)

Key Indexing Terms:
SERUM MATRIX METALLOPROTEINASE 3 STROMELYSIN 1
EARLY RHEUMATOID ARTHRITIS DISEASE MODIFYING ANTIRHEUMATIC DRUGS
SULFASALAZINE METHOTREXATE

In patients with rheumatoid arthritis (RA) matrix metalloproteinase 3 (MMP-3, stromelysin 1) is of interest because this proteolytic enzyme is thought to play a prominent role in the pathogenesis of matrix degradation[1-3], even though it is not the only key enzyme[4]. In RA MMP-3 is locally produced and activated within the inflamed joint and released into the peripheral blood. Systemic MMP-3 levels are a reflection of local synthesis. Thus serum MMP-3 can be used as a systemic marker of local joint inflammation[5-9] and/or destruction[10,11].

Studies concerning MMP are important not only for unraveling the pathogenesis of RA but also for analyzing mechanisms of drug therapy. For example, specific MMP inhibitors may uncouple the relation between surrogate markers of joint inflammation (for example, C-reactive protein, CRP) and joint damage. Radiological progression might be stopped by these new agents without inhibiting inflammation and acute phase response. In that case, especially in early disease, new markers like systemic MMP, including its level of activation, are essential[12].

Sulfasalazine (SSZ) and methotrexate (MTX) are disease modifying antirheumatic drugs (DMARD) widely used in early RA to suppress disease activity and thereby to prevent or delay joint destruction[13,14]. Studies investigating the effects of SSZ or MTX on MMP, especially on MMP-3, are rare. We analyzed whether serum MMP-3 levels are influenced by treatment with SSZ or the combination of MTX and SSZ in a prospective followup study of patients with early RA (symptoms < 1 year). In responders to these treatments we also analyzed the influence on serum MMP-3

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levels in relation to the influence of treatment on conventional clinical and other biochemical disease activity measures.

MATERIALS AND METHODS

Patients. Eighty-two patients with early RA were selected from a cohort of patients with RA according to the 1987 American College of Rheumatology criteria[15] with joint symptoms existing less than one year at presentation and who had not previously received DMARD. These patients participated in a prospective followup study at the Department of Rheumatology at Groningen University Hospital.

Clinical and laboratory investigations were performed at monthly intervals during a followup of 28 weeks.

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)[16], tender joint count (TJC), swollen joint count (SJC), and the Disease Activity Score (DAS) according to van der Heijde with 3 variables[17].

Laboratory analysis. Serum MMP-3 levels were determined by a MMP-3 ELISA developed at our laboratory[10]. Briefly, 96 well plates were precoated with $F(ab)_2$ fragment of goat-antimouse IgG, 1 μg/ml (Jackson Immunoresearch Labs, West Grove, PA, USA). Next, a mouse Mab against human MMP-3, clone 10D6 (R&D Systems, Abingdon, UK) was coated at 0.1 μg/ml. Serum samples were analyzed in 2-fold serial dilutions in high performance ELISA buffer (CLB, Amsterdam, The Netherlands) and incubated 1 h. 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 labeled $F(ab)_2$ fragment of goat-antirabbit IgG (Zymed, San Francisco, CA, USA). Peroxidase activity was determined using tetramethylbenzidine 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 interleukin 1ß (IL-1ß) stimulated RA synovial fibroblasts[18]. The intraassay coefficient of variation (CV) was 6.8%, the interassay CV 8.8%. With an immunoblot we demonstrated that both the monoclonal and the polyclonal antibody reacted with active MMP-3 and pro-MMP-3 as well as with MMP-3 bound to tissue inhibitor of matrix metalloproteinases (TIMP) (data not shown). Further, we found 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 we used the 95th percentile of healthy blood donors (n = 80) (female < 20 ng/ml, male < 60 ng/ml).

CRP was measured by ELISA[19], erythrocyte sedimentation rate (ESR) according to Westergren. IgM rheumatoid factor (RF) was measured using a Dade/Behring BN-2 nephelometer (normal value < 15 IU/ml).

DMARD treatment and definition of response. During followup, patients were treated with nonsteroidal antiinflammatory drugs (NSAID) as indicated clinically. It is our policy to use an intensive treatment strategy in patients with early RA consisting of an immediate start of DMARD and rapid adjustment of dosage and/or drugs (the step-up method) in case of an insufficient response[20]. DMARD treatment was instituted according to the following guidelines: at study entry all patients with active disease started with SSZ 500 mg/day, increased to 2000 mg/day in weekly increments of 500 mg. In case of an insufficient response at Week 12, MTX 7.5 mg/week could be added to SSZ 2000 mg/day. At Week 20 the MTX dose could be increased to 15 mg/week. Corticosteroids were allowed as adjuvant therapy. Decisions about intensifying the treatment with DMARD at 12 and 20 weeks were discussed by an independent observer and the patient's rheumatologist. These decisions were based on clinical markers of disease activity in combination with the CRP level as effect measures.

Patients with a sufficient response, ≥ 50% reduction in joint scores or CRP [or normalization of CRP (< 5 mg/l)], were assumed to be SSZ responders. A SSZ partial responder showed some improvement, but < 50% in joint scores or CRP. SSZ nonresponders showed no response or had deteriorated.

Statistical analysis. Chi-square and Mann-Whitney U tests for differences between groups. The Friedman test with Dunnett's post test was used to analyze differences within groups.

RESULTS

All patients started with SSZ. After 12 weeks of treatment there were 2 groups, SSZ responders (n = 52) and SSZ partial/nonresponders (n = 30). Of these 30 SSZ partial/nonresponders, 24 patients had a sufficient response on subsequent combination therapy with MTX/SSZ (MTX/SSZ responders, n = 24). The 6 MTX/SSZ nonresponders were subsequently treated with the combination of MTX/cyclosporine.

SSZ responders. After 12 weeks of SSZ treatment the response was determined as described above. Sixteen patients (31%) had ≥ 50% reduction in CRP, 13 (25%) had ≥ 50% reduction in joint scores, and 23 patients (44%) had ≥ 50% in both variables.

The baseline characteristics of the SSZ responders (n = 52) and the SSZ partial/nonresponders (n = 30) are shown in Table 1. The SSZ responders had lower baseline values of serum MMP-3, CRP, and ESR levels, but did not differ in joint scores or DAS.

After 12 weeks of SSZ all variables were significantly decreased in the SSZ responders (Table 2). In the SSZ partial/nonresponders, CRP, ESR, and SJC decreased, in contrast to serum MMP-3, TJC, RAI, and DAS-3 (Table 3).

Effects of MTX in SSZ partial/nonresponders. Study variables at the moment of the addition of MTX were taken as a new baseline (Week 0 MTX). In most patients MTX was added at Week 12 or 16. Due to this variation the values at Week 12 in SSZ partial/nonresponders (Table 3) were not exactly the same as the values at Week 0 MTX (Table 4).

Ten patients (42%) had a ≥ 50% reduction in CRP, 4 patients (16%) ≥ 50% reduction in joint scores, and 10 patients (42%) ≥ 50% reduction in both variables. Because

Table 1. Baseline characteristics of 82 patients with early RA; sulfasalazine (SSZ) responders (n = 52) and SASP partial/nonresponders (n = 30)

	Responders, N = 52	Partial/nonresponders, N = 30
Age, yrs range	49.8 (20.3–78.6)	51.4 (21.5–66.2)
Sex, female/male (% female)	37/15 (71)	16/14 (53)
IgM RF positive (%)	45 (87)	26 (87)
Tender joint count	12 (0–35)	12 (0–38)
Swollen joint count	11 (3–29)	12 (4–28)
Ritchie Articular index	6 (0–34)	6 (0–24)
Disease Activity Score	3.52 (1.73–5.69)	3.76 (1.88–5.55)
Serum MMP–3, ng/ml	64 (14–495)	112 (17–1290)*
CRP, mg/l	14.5 (2–110)	33 (2–139)*
ESR, mm/h	27 (5–114)	40 (9–114)*
Sharp Score > 0 (%)	32 (63)	16(53)

Values are the median and range. Chi-square test for sex, IgM RF positivity, and Sharp > 0. Mann-Whitney U test for the other variables. *SSZ responders vs SSZ partial/non responders p < 0.01.

Table 2. Median values of clinical and biochemical variables in SSZ responders (n = 52) during 28 weeks of followup.

Week	MMP–3, ng/ml	CRP, mg/l	ESR, mm/h	SJC	TJC	RAI	DAS–3
0	64	14	27	11	12	6	3.52
4	68	13	22	9	7	5	2.97
8	56	9	15	7	6	4	2.54
12*	55	8	13	5	4	2	2.32
16	44	6	10	3	2	2	1.89
20	38	4	8	3	2	2	1.96
24	39	4	8	1	1	1	1.72
28*	38	4	9	2	1	1	1.56

*After 12 weeks of SSZ the response was determined. In the SSZ responders all variables were significantly decreased at Week 12 and at the end of the followup, at 28 weeks ($p < 0.05$, Friedman test).

Table 3. Median values of clinical and biochemical variables in SSZ partial/nonresponders (n = 30) at study entry and at 4, 8, and 12 weeks.

Week	MMP–3, (ng/ml)	CRP, (mg/l)	ESR, (mm/h)	SJC	TJC	RAI	DAS–3
0	112	33	40	12	12	6	3.76
4	136	35	36	13	14	7	3.67
8	121	30	35	12	9	7	3.40
12*	132	17	30	9	10	6	3.27
p	NS	< 0.01	< 0.01	0.03	NS	NS	NS

* After 12 weeks of SSZ the response was determined. In the SSZ partial/nonresponders CRP, ESR, and swollen joint count were significantly decreased ($p < 0.05$, Friedman test) in contrast to serum MMP–3, TJC, RAI, and DAS–3.

Table 4. Median values of clinical and biochemical variables in MTX/SSZ responders (n = 24) during 28 weeks after the addition of MTX.

Weeks MTX	MMP–3, ng/ml	CRP, mg/l	ESR, mm/h	SJC	TJC	RAI	DAS–3
0	145	29	26	11	11	6	3.31
4	102	15	18	7	6	5	2.72
8	79	13	13	7	3	3	2.61
12	73	12	8	5	7	4	2.48
16	64	10	10	5	4	3	2.16
20	50	7	10	2	2	2	1.89
24	45	6	7	3	2	2	1.93
28	46	6	10	2	1	1	1.68
p*	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

*During followup all variables decreased significantly ($p < 0.05$, Friedman test).

of the small size of the nonresponder group (n = 6), only the responders taking MTX/SSZ (n = 24) were evaluated.

The effects of adding MTX to SSZ in partial/nonresponders during 28 weeks' followup are shown in Table 4. All variables decreased significantly.

Serum MMP-3 levels compared to other disease activity variables. SSZ responders (n = 52) and MTX/SSZ responders (n = 24) were eventually followed for 28 weeks. In both groups, serum MMP-3 and CRP decreased during followup (Tables 2 and 4).

To evaluate differences between serum MMP-3 and CRP, changes of each variable were expressed as a percentage of the initial level. Serum MMP-3 was significantly decreased after 16 weeks in both groups (Figure 1). A significant reduction in CRP was reached after 8 weeks of treatment in both groups.

In both responder groups there were some patients with serum MMP-3 levels in the normal range (female < 20 ng/ml, male < 60 ng/ml). To analyze the influence of these "normal levels" on the overall results, we separately evaluated the patients with an elevated serum MMP-3 level at study entry. The results were the same in the SSZ respon-

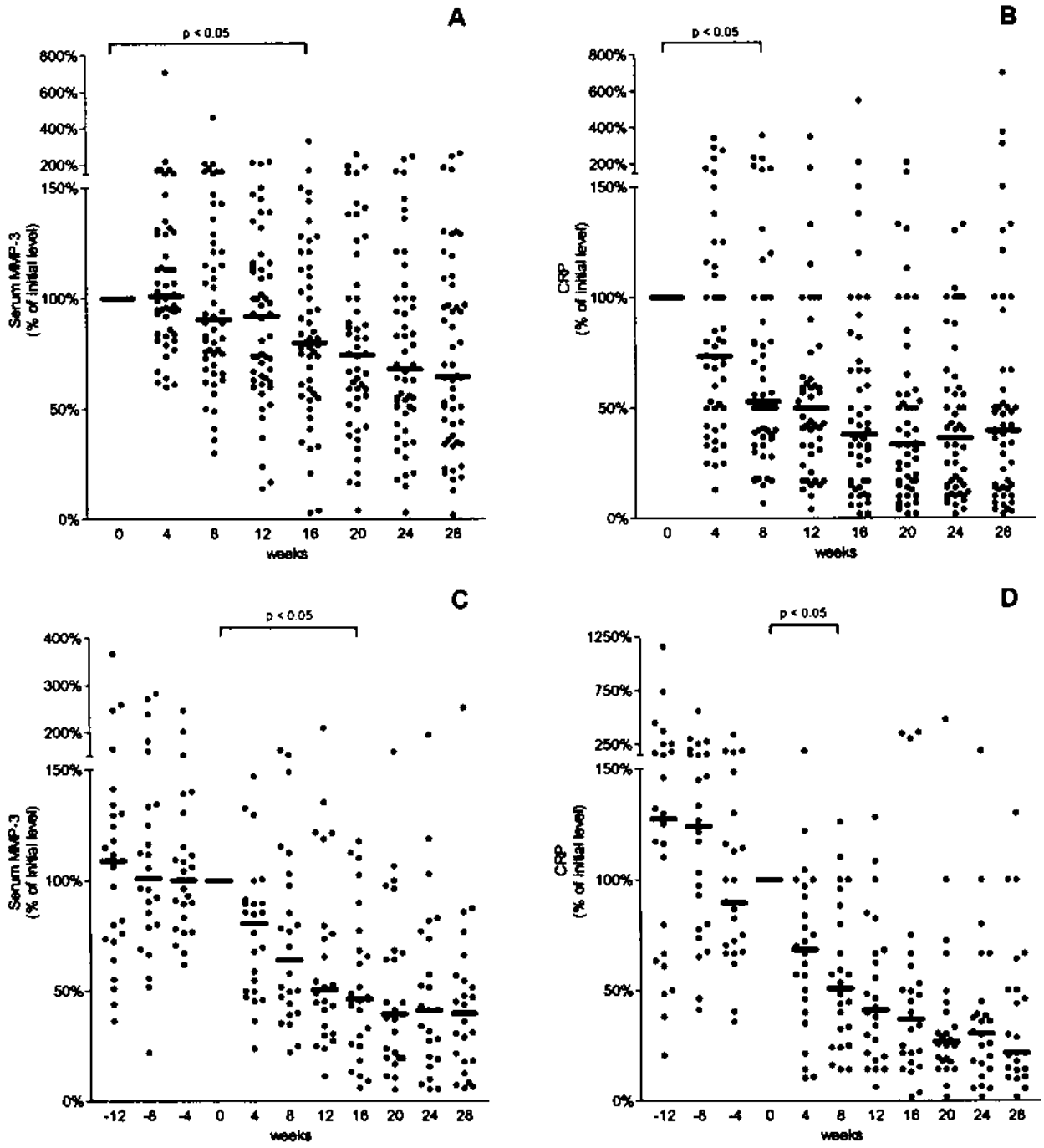

Figure 1. Serum MMP-3 and CRP as percentage of the initial level in SSZ responders (panels A and B; n = 52) and MTX/SSZ responders (panels C and D; n = 24). All variables decreased during 28 weeks' followup. $p < 0.05$ was reached after 16 weeks for serum MMP-3 and after 8 weeks for CRP (Friedman test with Dunnett post-test on the original crude data).

ders (n = 42 out of 52). In the MTX/SSZ responders (n = 22 out of 24), serum MMP-3 and CRP were both significantly decreased after 12 weeks (data not shown).

We evaluated differences between serum MMP-3 and CRP by changes expressed as a percentage of the initial level. Small variations within or close to the normal range could result in exceptional relative (in terms of percentage) elevations. In some instances serum MMP-3 and CRP were, for example, > 400% from baseline (Figure 1). Further evaluation of these data showed that these exceptional, relative elevations were indeed mainly caused by variations within or close to the normal range.

Interindividual differences in the course of serum MMP-3 and CRP levels. Data from 4 randomly selected patients are shown in Figure 2. These data illustrate the wide variations in absolute values of serum MMP-3 and CRP, the close relation between MMP-3 and CRP, and the individual response on treatment.

DISCUSSION

Our data show that serum MMP-3 levels decrease in patients with early RA who respond to SSZ or to the combination of MTX and SSZ. Actually all variables, including CRP, ESR, and clinical variables, decreased, confirming the close relation between serum MMP-3 and markers of disease activity.

MMP are thought to play an important role in the pathogenesis of RA based on their capacity to degrade many matrix components, their local expression in synovial tissue, and their increased levels in synovial fluid and serum[2,3]. Inhibition of the production and/or activation of these MMP could be an explanation for the restraining effects of DMARD on radiological progression. Therefore it is of interest to investigate the influence of DMARD like SSZ and MTX on MMP.

In RA MMP-3 is locally produced and activated in the inflamed joints, and systemic levels are a direct reflection of this local synthesis. This is in contrast to CRP, which is an indicator of inflammation in general that may be influenced by other stimuli of the acute phase response, like infections[21,22]. Especially in early disease, the use of markers of joint inflammation and destruction are of importance for prognostic and therapeutic reasons[23]. Thus serum MMP-3 is an interesting marker to investigate the influence of SSZ or the combination of MTX/SSZ on MMP production.

Studies of the effects of DMARD such as sulfasalazine and methotrexate on MMP are scarce. There is growing evidence that nuclear factor κB (NF-κB) is involved in MMP induction. NF-κB is an important transcription factor for inflammatory cytokine genes such as tumor necrosis factor-α (TNF-α), IL-1, and IL-6[24] as well as MMP such as MMP-3[25,26].

SSZ is a potent and specific inhibitor of NF-κB in *in vitro* cell cultures by interfering with IκBα phosphorylation[27]. By this path the effects of SSZ on MMP-3 and thereby on serum MMP-3 levels could be explained.

MTX in combination with steroids was reported to be effective in reducing neutral protease activity in RA

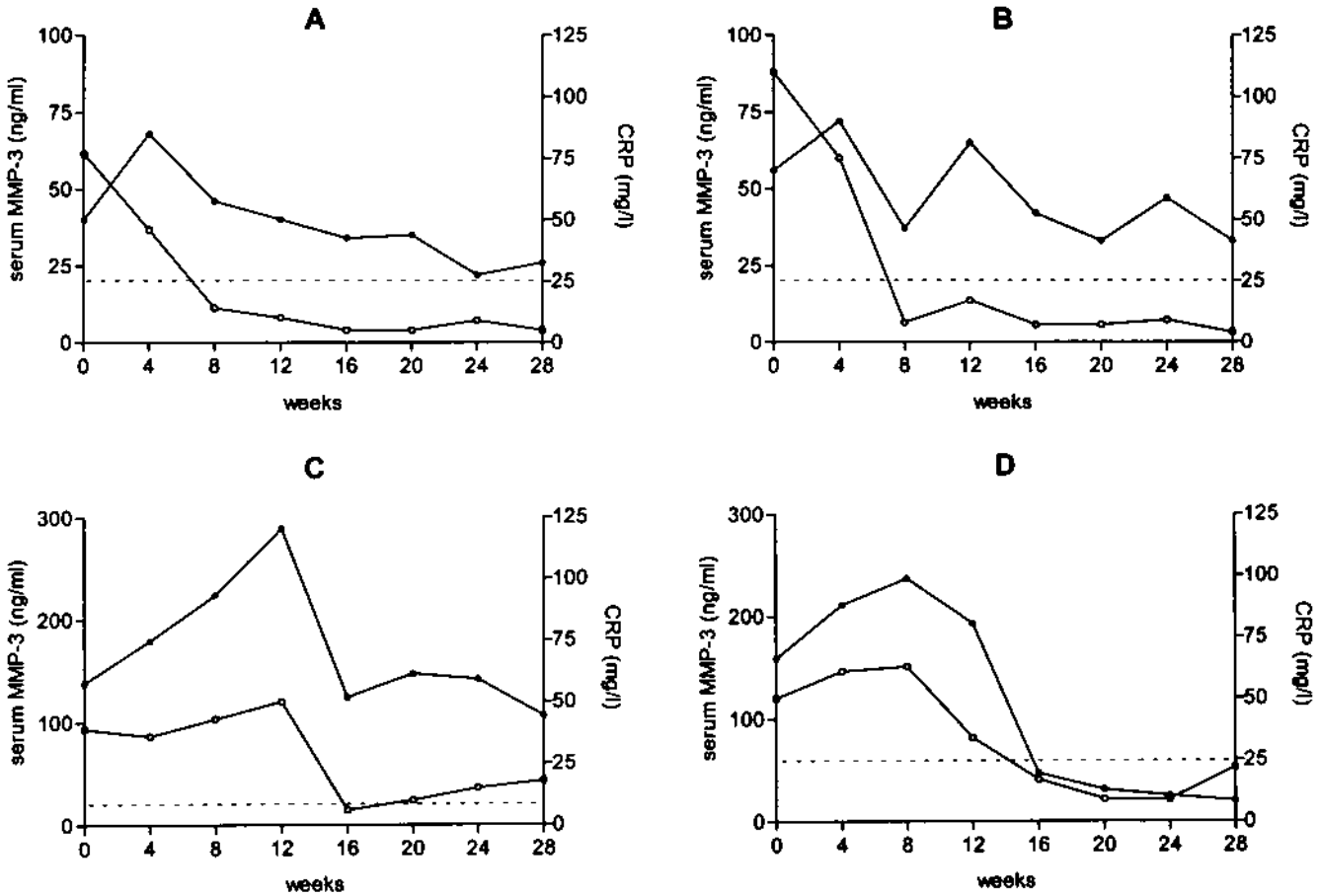

Figure 2. Individual serum MMP-3 (●) and CRP (○) levels in SSZ responders (patients A and B) and MTX/SSZ responders (patients C and D). Broken line represents the normal value for serum MMP-3: for women < 20 (patients A, B, C) and for men < 60 ng/ml (patient D).

synovium and cartilage[28]. In a more recent study MTX therapy decreased collagenase (MMP-1) but not stromelysin-1 (MMP-3) gene expression in RA synovium[29]. More detailed studies concerning the effects of MTX on, for example, transcription factors have to our knowledge not been published[30]. Nevertheless, MTX interferes with proinflammatory cytokines like IL-1, a potent inducer of MMP, by activation of transcription factors such as activator protein 1 (AP-1) and NF-κB[31].

With regard to serum MMP-3 levels, treatment with anti-TNF-α (chimeric monoclonal antibody) resulted in a significant and rapid fall of serum MMP-3[32]. Studies concerning corticosteroids showed conflicting results: intraarticular steroids resulted in a decrease[5], but systemic corticosteroids an increase in serum MMP-3[33]. A prospective open label trial of MTX and tenidap showed close correlations between the DAS, ESR, and CRP and serum MMP-3, but effects on the absolute values were not given[6].

In our study all variables decreased in the SSZ and MTX/SSZ responders. This was to be expected considering the clinical definition of response and the close relation between serum MMP-3 and markers of disease activity like swollen joint count, CRP, and ESR[5,7-9,11,32,34-36]. In the SSZ partial/nonresponders, ESR, CRP, and SJC decreased, but obviously not sufficiently in the opinion of the patients' rheumatologist (Table 3). After the addition of MTX, 24 out of 30 patients showed a sufficient response and in these patients all variables decreased significantly (Table 4).

The serum MMP-3 levels decreased in both responder groups; however, there were differences in comparison with CRP. Especially in the SSZ responders, serum MMP-3 showed a delayed response in comparison with CRP (Figure 1). This difference did not change when only patients with an initially elevated serum MMP-3 (n = 42) were evaluated. In the MTX/SSZ responders, serum MMP-3 showed only a delayed response when the complete group was evaluated. Analysis of only the patients with an initially elevated serum MMP-3 (n = 22) showed a significant response of serum MMP-3 comparable to the CRP response.

This delayed response of serum MMP-3 in comparison with CRP could be explained by differences in metabolism of MMP-3, but as far as we know there are no data concerning the half-life and clearance of MMP-3 or MMP-3/TIMP complexes. Furthermore there is the possibility of an uncoupled relationship between joint inflammation and joint damage. The differences between CRP and serum MMP-3 may indicate that inhibition of the inflammatory response, resulting in decreased production of IL-6 and subsequently decline in serum CRP, does not immediately result in decline in MMP production. This may implicate a temporary cytokine-independent production ("autonomous production") of MMP by synovial cells, reflecting continuing matrix degradation.

Data from individual patients are shown in Figure 2. Although there was wide inter-individual variation in absolute values, intra-individually there was a close relation between serum MMP-3 and CRP that is consistent with our previous study[11].

Serum MMP-3 levels decrease in patients with early RA who respond to sulfasalazine or to the combination of methotrexate and sulfasalazine. In patients who respond to sulfasalazine the changes in serum MMP-3 levels indicate a delayed response compared to CRP.

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