Serum MMP-3 Level as a Biomarker for Monitoring and Predicting Response to Etanercept Treatment in Ankylosing Spondylitis

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ABSTRACT. ***Objective.*** To investigate whether level of serum matrix metalloproteinase-3 (MMP-3) can serve as a biomarker for monitoring and predicting response to etanercept treatment in patients with ankylosing spondylitis (AS) in daily clinical practice.

Methods. Ninety-two consecutive AS outpatients with active disease who started etanercept treatment were included in this longitudinal observational study. Clinical data were collected prospectively at baseline and after 3 and 12 months of treatment. At the same timepoints, serum MMP-3 levels were measured retrospectively by ELISA.

Results. Since baseline serum MMP-3 levels were significantly higher in male compared to female patients with AS, data analysis was split for gender. Changes in serum MMP-3 levels after etanercept treatment correlated positively with changes in clinical assessments of disease activity and physical function in both male and female patients. Receiver operating characteristic analysis in male patients showed that baseline serum MMP-3 levels had poor accuracy (AUC < 0.7) to discriminate between Assessments in Ankylosing Spondylitis 20 (ASAS20) or ASAS40 responders and nonresponders after 3 or 12 months of treatment. The accuracy of change in serum MMP-3 levels from baseline to 3 months in predicting response after 3 or 12 months of treatment was poor for ASAS40 (AUC < 0.7) or moderate for ASAS20 (AUC = 0.752 and 0.744, respectively), and was not superior to the accuracy of change in the currently used objective biomarkers, erythrocyte sedimentation rate and C-reactive protein.

Conclusion. Although significant changes in serum MMP-3 levels were found after etanercept treatment, data analysis indicates that serum MMP-3 levels are not very useful for monitoring and predicting response to etanercept treatment in patients with AS in daily clinical practice. (J Rheumatol First Release June 1 2011; doi:10.3899/jrheum.101128)

Key Indexing Terms:
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Ankylosing spondylitis (AS) is a chronic inflammatory disease that primarily affects the axial skeleton. New bone formation can lead to the formation of syndesmophytes and ankylosing of the spine and sacroiliac joints. Besides this ossification, osteoporosis is also a well recognized complication of AS[1,2].

The availability of tumor necrosis factor-α (TNF-α)-blocking agents has significantly improved clinical outcome in AS[3,4,5]. In daily clinical practice, starting and continuation of TNF-α-blocking therapy in patients with AS is based mainly on subjective measures of disease activity such as the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the global opinion of the physician, since more objective measures like erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are, in contrast to patients with rheumatoid arthritis (RA), elevated only in a minority of patients with AS[6,7,8]. Thus, better objective measures for evaluating response to TNF-α-blocking therapy in AS are needed.

Recently, Woo, *et al* suggested the potential usefulness of

matrix metalloproteinase-3 (MMP-3; stromelysin-1) as a biomarker for monitoring response to TNF-α-blocking therapy in AS[9]. MMP are produced in response to proinflammatory cytokines such as TNF-α and interleukin 1 (IL-1)[10,11] and play an important role in degradation of extracellular matrix components[12]. Studies in AS have shown that serum levels of MMP-3 are related to clinical and laboratory measures of disease activity[9,13,14,15,16], and that baseline serum MMP-3 is an independent predictor of 2-year radiographic progression of the spine[17]. Various studies have reported that TNF-α-blocking therapy significantly reduces serum MMP-3 levels in patients with AS[9,15,16,18,19,20]. To date, knowledge of the predictive value of serum MMP-3 levels for response to TNF-α-blocking therapy is limited. Identification of objective predictors of response to TNF-α-blocking therapy seems important, especially in view of the costs and potential side effects of these agents.

The aim of our study was to investigate whether serum MMP-3 levels can serve as a biomarker for monitoring and predicting response to etanercept treatment in patients with AS in daily clinical practice.

MATERIALS AND METHODS

Patients. Ninety-two consecutive AS outpatients with active disease who started treatment with etanercept at the Medical Center Leeuwarden (n = 59) or the University Medical Center Groningen (n = 33) were included in this longitudinal observational study. All patients were age ≥ 18 years, fulfilled the modified New York criteria for AS[21], and started etanercept treatment because of active disease according to the Assessments in Ankylosing Spondylitis (ASAS) consensus statement[22]. Patients were excluded if they had previously received TNF-α-blocking therapy. Etanercept was administered as subcutaneous injection once (50 mg) or twice (25 mg) a week. Patients were allowed to receive concomitant medication as usual in daily clinical practice.

The study was approved by the local ethics committees of the Medical Center Leeuwarden and University Medical Center Groningen and all patients provided written informed consent according to the Declaration of Helsinki.

Clinical assessments. Clinical data were collected prospectively at baseline and after 3 months (mean 3.4 mo, SD ± 0.7) and 12 months (mean 12.5 mo, SD ± 1.8) of etanercept treatment. Disease activity was assessed using BASDAI (scale of 0–10)[23], physician and patient global assessment of disease activity (GDA; scale of 0–10), ESR, CRP, and the Ankylosing Spondylitis Disease Activity Score (ASDAS), a composite score calculated from BASDAI questions 2, 3 and 6, patient's GDA, and CRP[24,25]. Physical function was assessed using the Bath Ankylosing Spondylitis Functional Index (BASFI; scale of 0–10)[26].

Continuation of etanercept treatment was based on decrease in BASDAI of at least 50% or 2 units compared with baseline, and/or expert opinion in favor of continuation of treatment. Response to etanercept treatment was defined using ASAS20 and ASAS40 response criteria. ASAS20 response was defined as an improvement of at least 20% and absolute improvement of at least 1 unit (scale of 0–10) compared with baseline in 3 or more of the 4 domains, physical function (BASFI), pain, patient's GDA, and inflammation (mean from BASDAI questions 5 and 6), with no worsening by more than 20% in the remaining domain. ASAS40 response was defined as improvement of at least 40% and absolute improvement of at least 2 units compared with baseline in 3 or more of the 4 domains, with no worsening at all in the remaining domain[27].

Laboratory assessments. Serum MMP-3 levels were measured retrospectively at baseline and after 3 months (mean 3.4 mo, SD ± 0.7) and 12 months (mean 12.5 mo, SD ± 1.8) of etanercept treatment. Samples were stored at –20°C until analysis. Serum MMP-3 levels were measured by enzyme-linked immunosorbent assay (ELISA; Invitrogen, Breda, The Netherlands) according to the manufacturer's instructions. The assay measures total human MMP-3 including pro-MMP-3, active MMP-3, and MMP-3 in complex with tissue inhibitor of metalloproteinase (TIMP).

Statistical analysis. All data were analyzed on an intention-to-treat basis using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and Analyse-It version 2.20 (Analyse-It Software, Ltd., Leeds, UK). Results were expressed as mean ± SD or median (range) for normally distributed and non-normally distributed data, respectively. The independent samples t test and Mann-Whitney U test were used to compare differences between groups. Chi-square test and Fisher exact test were used to compare differences in percentages between groups. Paired samples t test and the Wilcoxon signed-rank test were used to compare differences within groups. Spearman's correlation coefficients were used to analyze the relationship between serum MMP-3 levels and clinical measures of disease activity and physical function. Receiver operating characteristic (ROC) analysis was performed to determine the accuracy of baseline or change in serum MMP-3 levels to predict ASAS20 or ASAS40 response after 3 or 12 months of etanercept treatment. Area under the curve (AUC) < 0.70 was interpreted as poor accuracy, 0.70 < AUC < 0.90 as moderate accuracy, and AUC > 0.90 as high accuracy[28]. A sample size of 29 responders and 29 nonresponders achieved 80% power to detect an AUC of 0.70 at a significance level of 0.05. ROC analysis was performed to compare the accuracy (AUC) of change in serum MMP-3 levels to predict ASAS20 response after etanercept treatment with that of change in BASDAI, ESR, CRP, or ASDAS scores. P values < 0.05 were considered statistically significant.

RESULTS

Mean age of the 92 patients with AS was 41.2 years (SD ± 9.9), median disease duration was 16 years (range 2–41), and 74% were male. At baseline, male patients showed significantly higher serum MMP-3 levels ($p < 0.001$) and lower BASDAI and patient GDA scores ($p < 0.05$) compared to female patients. Both groups were comparable for age, disease duration, HLA-B27 status, concomitant presence of extraarticular manifestations or peripheral arthritis, comedication, and baseline ESR, CRP, ASDAS, physician GDA, and BASFI scores (Table 1). Since baseline serum MMP-3 levels were significantly different between male and female AS patients, further data analysis was split for gender.

As shown in Table 2, all clinical assessments of disease activity and physical function significantly improved after 3 and 12 months of etanercept treatment in both male and female patients ($p < 0.01$). Further, etanercept treatment resulted in a significant reduction in serum MMP-3 levels in male patients after 3 months ($p < 0.05$). The percentage of patients that achieved ASAS20 response after 3 and 12 months of etanercept treatment was 79% and 66% for male patients, respectively, and 50% and 46% for female patients. The percentage of patients that reached ASAS40 response after 3 and 12 months of etanercept treatment was 50% and 47% for male patients, and 42% and 42% for female patients. The percentage of patients that discontinued etanercept treatment after 3 and 12 months was 10% and 19% for male patients, and 25% and 33% for female patients.

Concomitant peripheral arthritis. At baseline, peripheral arthritis (defined as at least one swollen joint) was observed in 15 (22%) male and 6 (25%) female patients. After 3 and 12

Table 1. Baseline characteristics of the ankylosing spondylitis (AS) study population. Values are mean ± SD or median (range) unless otherwise indicated.

Characteristic	Total	Male	Female	p*
No.	92	68	24	—
Age, yrs	41.2 ± 9.9	42.5 ± 10.4	37.9 ± 7.5	0.053
Duration of symptoms, yrs	16 (2–41)	16 (2–41)	14 (3–36)	0.690
Time since diagnosis, yrs	9 (0–37)	9 (0–37)	9 (0–26)	0.685
HLA-B27+ (%)	76 (83)	55 (81)	21 (86)	0.501
History of IBD (%)	4 (4)	3 (4)	1 (4)	1.000
History of uveitis (%)	29 (32)	22 (32)	7 (29)	0.773
History of psoriasis (%)	4 (4)	4 (6)	0 (0)	0.570
Peripheral arthritis (%)	21 (23)	15 (22)	6 (25)	0.782
Concomitant NSAID use (%)	81 (88)	59 (87)	22 (92)	0.722
Concomitant DMARD use (%)	20 (22)	14 (21)	6 (25)	0.652
BASDAI (range 0–10)	6.2 ± 1.8	6.0 ± 1.7	6.8 ± 1.8	0.048
ESR, mm/h	21 (2–101)	19 (2–101)	27 (5–67)	0.294
CRP, mg/l	15 (2–99)	15 (2–99)	12 (2–55)	0.800
ASDAS	3.8 ± 0.8	3.8 ± 0.9	4.1 ± 0.5	0.174
Physician GDA (range 0–10)	6 (1–9)	6 (1–9)	7 (1–8)	0.516
Patient GDA (range 0–10)	7 (1–10)	7 (1–10)	8 (2–10)	0.034
BASFI (range 0–10)	5.9 ± 2.0	6.0 ± 2.0	5.7 ± 2.2	0.567
MMP-3, ng/ml	10.8 (1.8–47.5)	13.6 (4.3–47.5)	5.9 (1.8–26.2)	0.000

* Male compared to female patients. IBD: inflammatory bowel disease; NSAID: nonsteroidal antiinflammatory drug; DMARD: disease-modifying antirheumatic drug; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ASDAS: Ankylosing Spondylitis Disease Activity Score; GDA: global disease activity; BASFI: Bath Ankylosing Spondylitis Functional Index; MMP-3: matrix metalloproteinase-3.

Table 2. Clinical and laboratory assessments at baseline and after 3 and 12 months of etanercept treatment. Values are mean ± SD or median (range).

Assessment	Baseline	3 Months	p*	12 Months	p**
Males (n = 68)					
BASDAI (range 0–10)	6.0 ± 1.7	3.0 ± 2.0	0.000	3.1 ± 2.3	0.000
ESR, mm/h	19 (2–101)	4 (2–51)	0.000	5 (2–50)	0.000
CRP, mg/l	15 (2–99)	3 (2–56)	0.000	3 (2–56)	0.000
ASDAS	3.8 ± 0.9	1.9 ± 0.8	0.000	2.1 ± 1.1	0.000
Physician GDA (range 0–10)	6 (1–9)	2 (0–5)	0.000	1 (0–8)	0.000
Patient GDA (range 0–10)	7 (1–10)	3 (0–9)	0.000	2 (0–10)	0.000
BASFI (range 0–10)	6.0 ± 2.0	3.8 ± 2.4	0.000	3.6 ± 2.6	0.000
MMP-3, ng/ml	13.6 (4.3–47.5)	13.1 (5.0–35.7)	0.016	12.7 (3.3–37.6)	0.053
Females (n = 24)					
BASDAI (range 0–10)	6.8 ± 1.8	3.8 ± 1.9	0.000	3.6 ± 2.0	0.000
ESR, mm/h	27 (5–67)	15 (2–40)	0.000	17 (3–46)	0.012
CRP, mg/l	12 (2–55)	4 (2–24)	0.001	6 (2–196)	0.019
ASDAS	4.1 ± 0.5	2.5 ± 1.0	0.000	2.6 ± 1.1	0.000
Physician GDA (range 0–10)	7 (1–8)	2 (0–7)	0.000	1 (0–9)	0.001
Patient GDA (range 0–10)	8 (2–10)	4 (0–10)	0.002	4 (1–9)	0.004
BASFI (range 0–10)	5.7 ± 2.2	3.2 ± 2.1	0.000	3.1 ± 2.3	0.001
MMP-3, ng/ml	5.9 (1.8–26.2)	5.6 (2.4–11.0)	0.983	7.2 (3.3–14.6)	0.191

See Table 1 for definitions. * Values at baseline compared to 3 months. ** Values at baseline compared to 12 months.

months of etanercept treatment, serum MMP-3 levels decreased significantly in male patients with concomitant peripheral arthritis at baseline ($p < 0.05$), but not in male patients with only axial disease (Table 3).

Correlations between serum MMP-3 levels and clinical assessments. At baseline, no statistically significant correlations were found between serum MMP-3 levels and clinical assessments of disease activity or physical function in male patients. In female patients, baseline serum MMP-3 levels correlated positively with baseline CRP and ASDAS scores

Table 3. Clinical and laboratory assessments at baseline and after 3 and 12 months of etanercept treatment in male AS patients with concomitant peripheral arthritis and with axial disease only. Values are mean ± SD or median (range).

Assessment	Baseline	3 Months	p*	12 Months	p**
Males with concomitant peripheral arthritis (n = 15)					
BASDAI (range 0–10)	6.7 ± 1.5†	3.0 ± 2.0	0.000	2.5 ± 1.8	0.000
ESR, mm/h	26 (2–101)	4 (2–26)	0.001	5 (2–50)	0.001
CRP, mg/l	24 (4–99)†	3 (2–14)	0.001	3 (2–15)	0.001
ASDAS	4.3 ± 0.8	1.8 ± 0.7	0.000	1.7 ± 0.7	0.000
MMP-3, ng/ml	17.0 (4.3–47.5)	14.7 (7.2–35.7)	0.016	10.9 (3.3–21.3)	0.004
Males with axial disease only (n = 53)					
BASDAI (range 0–10)	5.8 ± 1.8	3.0 ± 2.0	0.000	3.3 ± 2.4	0.000
ESR, mm/h	15 (2–80)	5 (2–51)	0.000	6 (2–43)	0.000
CRP, mg/l	12 (2–69)	2 (2–56)	0.000	3 (2–56)	0.000
ASDAS	3.6 ± 0.9	2.0 ± 0.8	0.000	2.2 ± 1.1	0.000
MMP-3, ng/ml	13.2 (4.9–37.2)	12.3 (5.0–29.6)	0.218	12.7 (4.6–37.6)	0.818

See Table 1 for definitions. † Statistically significant difference ($p < 0.05$) compared to values of males with axial disease only. * Values at baseline compared to 3 months. ** Values at baseline compared to 12 months.

($p < 0.05$). After 3 and 12 months of etanercept treatment, changes in serum MMP-3 levels correlated positively with changes in BASDAI, ESR, CRP, ASDAS, physician GDA, and patient GDA scores in male patients ($p < 0.05$). In female patients, there were significant positive correlations between changes in serum MMP-3 levels and changes in physician GDA scores after 3 months as well as changes in BASDAI, CRP, ASDAS, and BASFI scores after 12 months ($p < 0.05$; Table 4).

Accuracy of baseline serum MMP-3 levels in predicting response. Since the number of female patients was relatively small (n = 24), ROC analysis was performed only in male patients. The accuracy of baseline serum MMP-3 levels to predict response after 3 and 12 months of etanercept treatment was poor, with an AUC of 0.685 (95% CI 0.551–0.819) and 0.655 (95% CI 0.515–0.796), respectively, for ASAS20 response; and 0.568 (95% CI 0.425–0.710) and 0.607 (95% CI 0.468–0.747), respectively, for ASAS40 response.

Accuracy of change in serum MMP-3 levels in predicting response. The accuracy of change in serum MMP-3 levels from baseline to 3 months to predict the ASAS20 response after 3 and 12 months of etanercept treatment was moderate, with an AUC of 0.752 (95% CI 0.618–0.886) and 0.744 (95% CI 0.607–0.882), respectively. The best cutoff value of change in serum MMP-3 levels from baseline to 3 months to discriminate between ASAS20 responders and nonresponders had a sensitivity of 72% and specificity of 75%, respectively, after 3 months, and 73% and 72% after 12 months. When AUC val-

Table 4. Spearman correlations between baseline or change in serum MMP-3 levels and clinical assessments after 3 and 12 months of etanercept treatment.

		Baseline Δ0–3/12 BASDAI	Baseline Δ0–3/12 ESR	Baseline Δ0–3/12 CRP	Baseline Δ0–3/12 ASDAS	Baseline Δ0–3/12 PhyGDA	Baseline Δ0–3/12 PatGDA	Baseline Δ0–3/12 BASFI
Males (n = 68)								
Baseline MMP-3	ρ	–0.164	0.086	0.163	–0.016	0.173	–0.122	–0.050
	p	0.190	0.495	0.194	0.898	0.172	0.334	0.695
Δ0–3 MMP-3	ρ	0.410	0.346	0.359	0.540	0.264	0.319	0.250
	p	0.001	0.008	0.006	0.000	0.047	0.015	0.058
Δ0–12 MMP-3	ρ	0.319	0.343	0.394	0.330	0.328	0.277	0.251
	p	0.018	0.013	0.003	0.015	0.015	0.041	0.065
Females (n = 24)								
Baseline MMP-3	ρ	–0.027	0.370	0.639	0.492	–0.065	–0.217	0.076
	p	0.905	0.090	0.001	0.023	0.774	0.346	0.738
Δ0–3 MMP-3	ρ	0.298	0.236	0.261	0.397	0.492	0.340	0.276
	p	0.229	0.347	0.296	0.115	0.038	0.182	0.268
Δ0–12 MMP-3	ρ	0.561	0.098	0.602	0.700	0.444	0.402	0.549
	p	0.037	0.727	0.018	0.004	0.112	0.138	0.034

Δ0–3: baseline to 3 months change; Δ0–12: baseline to 12 months change; PhyGDA: physician global disease activity; PatGDA: patient global disease activity. See Table 1 for other definitions.

ues were compared, the AUC value of baseline to 3 months change in serum MMP-3 levels to predict ASAS20 response after 3 months was significantly lower than that of baseline to 3 months change in BASDAI ($p < 0.001$). Further, no significant differences were found between AUC values of baseline to 3 months change in serum MMP-3 levels to predict ASAS20 response after 3 or 12 months and those of baseline to 3 months change in ESR or CRP levels.

The accuracy of change in serum MMP-3 levels from baseline to 3 months to predict ASAS40 response after 3 and 12 months of etanercept treatment was poor, with an AUC of 0.610 (95% CI 0.458–0.762) and 0.670 (95% CI 0.528–0.813), respectively (Table 5).

DISCUSSION

This longitudinal observational study in daily clinical practice did not confirm the potential usefulness of serum MMP-3 levels as a biomarker for monitoring response to TNF-α-blocking therapy in AS, as suggested[9]. At baseline, serum MMP-3 levels correlated positively with CRP and ASDAS scores in female patients with AS. However, no statistically significant correlations were found between baseline serum MMP-3 levels and clinical assessments of disease activity or physical function in male AS patients. Changes in serum MMP-3 levels after 3 or 12 months of etanercept treatment correlated positively with changes in clinical assessments of disease activity and physical function in both male and female patients. However, ROC analysis in male patients showed that baseline to 3 months change in serum MMP-3 levels had poor accuracy to discriminate between ASAS40 responders and nonresponders after 3 months of etanercept treatment. The accuracy of baseline to 3 months change in serum MMP-3 levels to predict ASAS20 response after 3 months was moderate, but not superior to the accuracy of change in the objective biomarkers ESR and CRP that are currently used.

The second aim of our study was to investigate whether serum MMP-3 levels can predict response to etanercept treatment in AS. Our ROC analysis in male patients showed that baseline serum MMP-3 level had poor accuracy to discriminate between ASAS20 or ASAS40 responders and nonresponders after 3 or 12 months of etanercept treatment in daily clinical practice. This finding is in accord with results from Romero-Sanchez, *et al*, who also reported that serum MMP-3 levels did not predict ASAS20 or ASAS40 response at the same timepoint or at later timepoints[19]. Further, in our study, the accuracy of change in serum MMP-3 levels from baseline to 3 months to predict ASAS20 or ASAS40 response after 12 months was moderate and poor, respectively, and not superior to the accuracy of change in ESR or CRP levels from baseline to 3 months.

A possible explanation for our results may be that serum MMP-3 levels primarily reflect peripheral joint inflammation, which occurs in only a relatively small proportion of AS patients. In our study, baseline serum MMP-3 levels seemed

Table 5. Receiver operating characteristic analysis of baseline to 3 months change in clinical and laboratory assessments predicting response after 3 and 12 months of etanercept treatment in male patients with AS (n = 68).

	AUC (95% CI)	p*	Optimal Cutoff	Sensitivity, %	Specificity, %
ASAS20 response after 3 months					
Δ0–3 MMP-3	0.752 (0.618–0.886)	—	0.0	72	75
Δ0–3 ESR	0.708 (0.544–0.872)	0.556	6.5	77	64
Δ0–3 CRP	0.609 (0.436–0.781)	0.120	6.5	69	57
Δ0–3 BASDAI	0.953 (0.898–1.000)	0.000	1.9	90	86
Δ0–3 ASDAS	0.864 (0.744–0.984)	0.107	1.3	89	79
ASAS40 response after 3 months					
Δ0–3 MMP-3	0.610 (0.458–0.762)	—	0.1	76	55
Δ0–3 ESR	0.679 (0.546–0.811)	0.387	6.5	88	53
Δ0–3 CRP	0.584 (0.442–0.726)	0.761	10.5	64	67
Δ0–3 BASDAI	0.831 (0.731–0.932)	0.001	2.5	91	67
Δ0–3 ASDAS	0.777 (0.663–0.891)	0.007	1.3	97	52
ASAS20 response after 12 months					
Δ0–3 MMP-3	0.744 (0.607–0.882)	—	0.2	73	72
Δ0–3 ESR	0.779 (0.659–0.899)	0.662	6.5	88	70
Δ0–3 CRP	0.616 (0.472–0.761)	0.161	9.5	63	74
Δ0–3 BASDAI	0.816 (0.699–0.934)	0.395	2.6	81	83
Δ0–3 ASDAS	0.810 (0.694–0.926)	0.356	1.3	91	61
ASAS40 response after 12 months					
Δ0–3 MMP-3	0.670 (0.528–0.813)	—	0.5	74	61
Δ0–3 ESR	0.683 (0.552–0.814)	0.882	6.5	90	51
Δ0–3 CRP	0.578 (0.437–0.719)	0.268	9.5	63	61
Δ0–3 BASDAI	0.790 (0.680–0.900)	0.137	2.7	83	69
Δ0–3 ASDAS	0.739 (0.618–0.860)	0.325	1.9	70	72

* Compared to AUC of Δ0–3 MMP-3. AUC: area under the curve. See Table 1 for other definitions.

somewhat higher in male patients with concomitant peripheral arthritis compared to male patients with only axial disease. In addition, etanercept treatment significantly decreased serum MMP-3 levels only in male patients with concomitant peripheral arthritis. Previous studies also reported differences in serum MMP-3 levels between patients with and those without concomitant peripheral arthritis[13,15,29]. Moreover, serum MMP-3 levels were found to be markedly elevated in patients with RA, a disease characterized by chronic inflammation of the joints[30,31,32]. Unfortunately, the number of AS patients with concomitant peripheral arthritis in our study was too small to investigate the usefulness of serum MMP-3 levels as a biomarker for monitoring and predicting response to etanercept treatment in this particular group of AS patients.

Since baseline serum MMP-3 levels were significantly higher in male compared to female AS patients, we decided to split further data analysis for gender. Our finding that male sex is associated with higher serum MMP-3 levels is in accord with other findings in AS[33] and in healthy controls[30,31,32]. In addition, Natoli, *et al* showed that the male sex steroid testosterone increased both gene and protein expression of MMP-3[34]. A drawback of splitting our analysis for gender was that analyses in female patients were limited by their relatively small number.

Although significant changes in serum MMP-3 levels after etanercept treatment were found, especially in male AS patients with concomitant peripheral arthritis, further data analysis indicated that measuring serum MMP-3 levels was not very useful for monitoring and predicting response to etanercept treatment in AS patients in daily clinical practice.

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