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Original Article

Matrix metalloproteinase-3 and -9: possible biomarkers of infliximab efficacy in ankylosing spondylitis (AS)?

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Abstract: Objective: To investigate the concentration of serum Matrix Metalloproteinase (MMP)-3 and -9 in active ankylosing spondylitis (AS) during infliximab treatment and to evaluate whether MMP-3 and MMP-9 were associated with clinical measures of disease activity and function in AS. Method: Baseline and sequential serum MMP-3 and MMP-9 were examined at week 12 of infliximab treatment in 44 AS patients. The clinical sores were evaluated by ASDAS, BASFI, BASMI, ESR and CRP. The paired T-test and Spearman correlation analysis were used for statistic analysis. Results: ① Serum MMP-3 and MMP-9 and clinical scores including ASDAS, BASFI, BASMI, ESR and CRP of 44 AS patients significantly decreased when they were treated with infliximab for 12 weeks ($P<0.05$). ② AS with peripheral arthritis (PA) patients had higher MMP-3 level than AS without PA patients ($P<0.01$). Contrastly, AS without PA patients had higher MMP-9 level ($P<0.05$). ③ The association was found between MMP-3 and ESR, CRP, ASDAS and BASFI ($P<0.01$), and MMP-9 was associated with CRP, ASDAS, BASFI and BASMI ($P<0.05$). ④ After 12 weeks, the decrease of MMP-3 was associated with the improvement of ASDAS, BASFI, ESR, CRP, but not with BASMI. The decline of MMP-9 was correlated with the improvement of ASDAS, BASMI and CRP. ⑤ There were thirty-seven responders and seven non-responders according to the improvement of ASDAS criteria at week 12 after infliximab infusion. Responders have higher baseline serum MMP-3 than that of non-responders ($P<0.01$), but not MMP-9. Conclusions: Serum MMP-3 and MMP-9 decreased significantly in active AS patients during infliximab therapy and their decline was associated with the improvement of clinical syndromes. Infliximab responders have higher baseline serum MMP-3 not MMP-9 level than that in infliximab non-responders.

Keywords: Ankylosing spondylitis, matrix metalloproteinase, infliximab, biomarker

Introduction

Ankylosing spondylitis (AS) is characterized by inflammatory spinal pain and occasionally by peripheral joint and entheses swelling [1]. The pain and its resultant impaired function often affect their daily life and work. It always comes back and forth, sometimes resulting in disability. Although genetic factors are important in the disease development [2], there is still no any biomarker to predict what kind of patients will suffer severe disease [3]. The blood tests currently used in clinical practice are the two acute phase reactants, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), which correlate weakly with disease activity in AS and can't fully reflect the disease process [4], so their value in clinical trials is limited. In addition, these clinical evaluations, such as ASDAS, BASDAI and BASFI, rely mostly on subjective self-evaluation by the patients. Therefore, the analysis of serum biomarkers reflecting inflammation, bone destruction, and new bone formation could be helpful to predict disease activity more objectively.

MMPs participate in extracellular matrix (ECM) degradation by cleavage of ECM constituents such as collagens and proteoglycans. All the MMP-derived collagen biomarkers significantly elevated in AS patients compared with age-matched controls [5]. To date, more than 20 MMPs have been recognized in inflammatory arthritis [6]. Some researches stained for MMP-3, MMP-9 in the synovial lining and sublining layers and showed a cellular and interstitial pattern that was similar both RA and SpA [7, 8], but a more pronounced staining for MMP-3 in the sublining layer than in the lining layer, and a more pronounced peri- and intravascular stain-

ing for MMP-9. Involvement of MMPs in SpA synovitis was suggested by the correlation with cellular infiltration, vascularization, and cartilage degradation. Using microarray as a screening strategy in SpA synovitis, they also found the synovial down-regulation of MMP-3 and MMP-9 in almost all infiltrating cell types after infliximab treatment [7] or etanercept [8]. Recent publications suggest that MMP-3 is closely related to inflammation and high disease activity in AS [9-11]. In addition, serum concentration of MMP-3 in AS is a possible prognostic indicator of radiological progression [12]. MMP-9 is also the mediator of joint destruction, but additionally has a particular role in angiogenesis [13]. Therefore, the change of serum MMP-3 and MMP-9 may be biomarkers for disease activity of AS patients.

Several placebo-controlled studies have shown that the clinical symptoms, but not in all AS patients, respond very significantly to treatment with TNFα blockers such as infliximab [14]. However, these TNFα blockers are very costly and their uses are not supported by health providers in China. If there are more objective predictors available, physicians could rapidly and reliably distinguish well from poor responders. So MMP-3 and MMP-9 are important inflammatory factors in the pathological mechanism of SpA patients. However, whether MMP-3 and MMP-9 could predict the disease activity of AS or response to the treatment remains unclear.

Materials and methods

Patients

Patients with AS (n=44) were recruited from the rheumatology departments of lihuli hospital in Ningbo. Patients invited to take part in the study were over 18 years of age, had AS according to the modified New York criteria [15]. The majority of patients had been on a stable dose of nonsteroidal anti-inflammatory drugs for at least the previous 4 weeks. All Patients were in the active stage (ASDAS>1.3) and none of them were on anti-TNF therapy, systemic steroid use in the previous 3 months or bisphosphonates in the previous 6 months. Infliximab i.v. was administrated by intravenous infusion at a dose 3 mg/kg at the baseline, then at 2 weeks, 6 weeks and 12 weeks.

The patients were informed about the research study, and signed an informed consent. The study was approved by the Ethical Committee of the Lihuili hospital of Ningbo.

Clinical outcome and laboratory methods

All patients underwent general physical examination and routine blood test at baseline and 12 weeks after the initial treatment. The following clinical scores were obtained at baseline and 12 weeks follow-up visit: 1) The Ankylosing Spondylitis Disease Activity Score (ASDAS) [16], which includes five components: patient global assessment, back pain, peripheral pain and swelling, duration of morning stiff and ESR, which is a measure of axial spondyloarthritis (SpA) disease activity and better than the Bath ankylosing spondylitis disease activity index (BASDAI) [17]. 2) The Bath Ankylosing Spondylitis Functional Index (BASFI) [18] is used to assess the functional capacity. This self-assessment instrument was designed by a team of medical professionals and patients, and it consists of eight specific questions regarding physical function in AS and two questions reflecting the patient's ability to cope with everyday life. 3) The Bath Ankylosing Spondylitis Metrology Index (BASMI) [19] is a quantitative, physician assessed measure of the spinal mobility limitations experienced by a patient with AS. BASMI is a validated index consisting of five clinical measurements including cervical rotation, tragus-to-wall distance, lateral spine flexion, lumbar flexion and intermalleolar distance, which reflects axial segmental involvement. Response to treatment at week 12 was defined by the improvement of ASDAS [16]. Selected cut-offs for improvement were: change ≥1.1 units for clinically important improvement and change ≥2.0 units for major improvement. So response criteria are defined as follows: Responders: Cut-off ≥1.1 units. Non-responders: Cut-off <1.1 units. With respect to joint distribution, patients were categorized into AS with peripheral arthritis (PA) and AS without peripheral arthritisaxial (i.e., disease confined to the spine and hips).

At baseline and week 12, the blood sample was drawn immediately before the infusion of infliximab was initiated and then centrifuged; the supernatants were harvested, and frozen at -70°C until assays were performed. Serum

Table 1. MMP-3, MMP-9 concentrations and clinical scores at baseline and week 12 in patients with AS treated by infliximab, All values are expressed as mean ± SD

	Baseline	At week 12	*P* value
MMP-3 (pg/ml)	688.23 ± 344.96	269.71 ± 200.27	*<0.01*
MMP-9 (pg/ml)	535.81 ± 402.38	204.69 ± 149.64	*<0.01*
ASDAS	2.56 ± 0.69	0.94 ± 0.55	*<0.01*
BASFI	3.98 ± 2.01	1.51 ± 1.04	*<0.01*
BASMI	4.059 ± 2.30	1.94 ± 1.57	*<0.05*
ESR (mm/h)	45.68 ± 25.54	13.35 ± 6.71	*<0.01*
CRP (mg/dl)	29.22 ± 19.8	12.16 ± 7.81	*<0.01*

MMP-3 and MMP-9 were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits: Quantikine® Human Total MMP-3 Immunoassay (R&D Systems, Minneapolis, MN) and Quantikine® Human MMP-9 Immunoassay (R&D Systems, Minneapolis, MN). Analysis was performed in accordance with the instructions of the manufacturers. The ESR and the CRP level were measured by the Westergren method and by nephelometry, respectively.

Statistical analysis

Statistical analysis was performed using SPSS software for Windows version 12.0. All values are given as median and mean ± SD. Differences in biomarker values between baseline and each time point after treatment were analyzed using paired *t*-tests. Correlations between clinical variables, laboratory values, and MMP3, MMP-9 were conducted by Spearman correlation test. *P* values less than 0.05 were considered significant.

Results

Serum MMP-3, MMP-9 concentrations and clinical scores at baseline and week 12 after infliximab treatment

In our study, the serum MMP-3 and MMP-9 at baseline were 688.23 ± 344.96 pg/ml and 535.81 ± 402.38 pg/ml, respectively, and then they declined to 269.71 ± 200.27 pg/ml and 204.69 ± 149.64 pg/ml at 12 weeks after infliximab treatment. The decline was statistically significant ($P<0.01$). Meanwhile, the levels of ASDAS ($P<0.05$), BASFI ($P<0.01$), BASMI ($P<0.01$), ESR ($P<0.01$), and CRP ($P<0.01$) at week 12 were significantly lower in the patients treated with infliximab as compared to the results before the treatment (**Table 1**).

Baseline serum MMP-3 and MMP-9 difference between AS with peripheral arthritis (PA) and AS without PA patients

There were 25 AS patients with PA and 19 AS patients without PA. There was a difference in MMP-3, MMP-9 between the two groups at baseline. AS with PA patients had higher MMP-3 level than AS without PA patients ($P<0.01$). Contrastly, AS without PA patients had higher MMP-9 level ($P<0.05$). Need to put out that there were discriminating clinical measures of CRP or ESR between AS with PA and AS without PA at baseline ($P<0.05$) (**Figure 1**).

Association between concentrations of MMP-3, MMP-9 and clinical scores at baseline

There was a strong correlation between baseline MMP-3 and ASDAS (**Figure 2A**), BASFI (**Figure 2B**), ESR (**Figure 2D**), CRP (**Figure 2E**) respectively ($P<0.01$) in active AS patient. In contrast, baseline MMP-3 didn't correlate with BASMI (**Figure 2C**) ($P=0.07$). The concentration of MMP-9 correlated with ASDAS (**Figure 2F**), BASFI (**Figure 2G**), BASMI (**Figure 2H**) and CRP (**Figure 2I**), but not with ESR in the baseline.

Correlation between decrease of MMP-3, MMP-9 and the improvement of clinical scores at 12 week during infliximab treatment

The decrease of MMP-3 was correlated with the improvement of ASDAS (**Figure 3A**), BASFI (**Figure 3B**), CRP (**Figure 3D**), ESR (**Figure 3E**), but not with the improvement of BASMI (**Figure 3C**). The decrease of MMP-9 was correlated with the improvement of ASDAS (**Figure 3F**), BASMI (**Figure 3H**), CRP (**Figure 3I**), but not with the improvement of BASFI (**Figure 3G**) and ESR (**Figure 3**).

Baseline serum MMP-3 and MMP-9 differences between responders and non-responders to infliximab therapy

The mean ASDAS score was 2.56 at baseline including 9 patients in moderate disease activity, 21 patients in high disease activity and 4

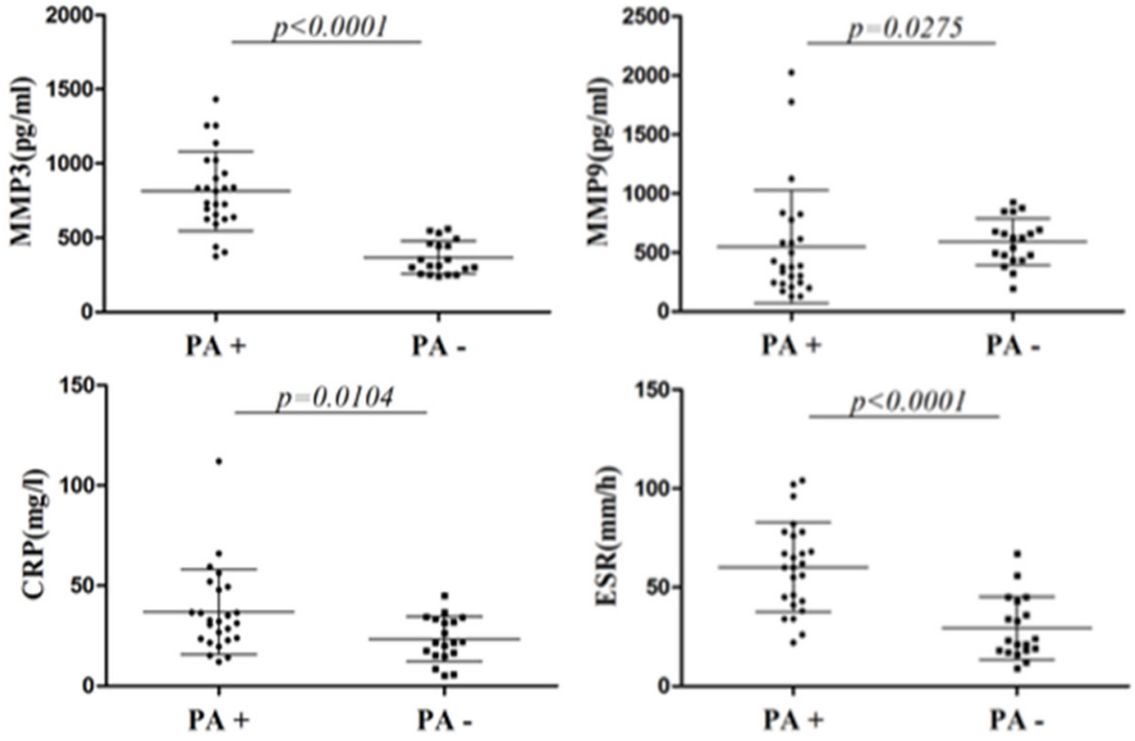

Figure 1. Baseline serum MMP-3 and MMP-9 difference between AS with PA and AS without PA patients.

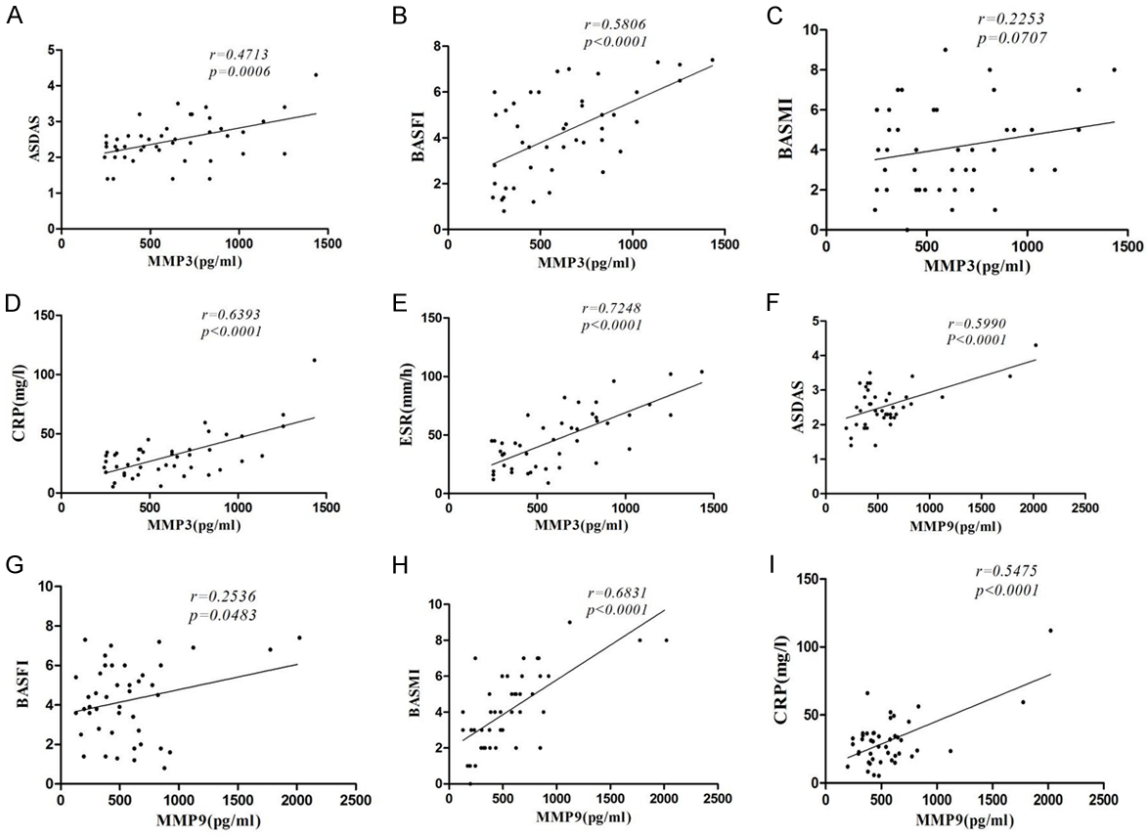

Figure 2. Correlations between MMP-3, MMP-9 and clinical scores in the 44 AS patients. Data were log-transformed, and the correlations were calculated using Spearman's correlation test.

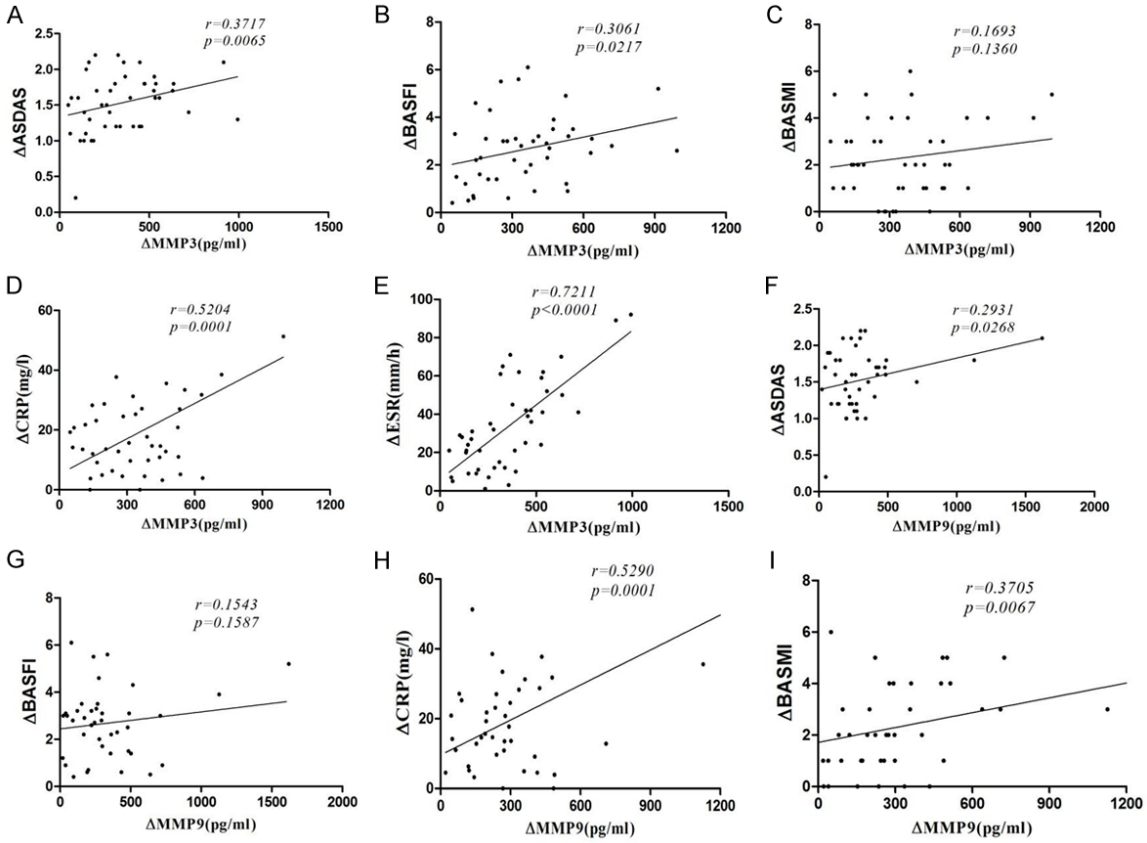

Figure 3. Correlations between decrease of MMP-3, MMP-9 and the improvement of clinical scores at 12 week after infliximab treatment in 44 AS patients. Data were log-transformed, and the correlations were calculated using Spearman's correlation test.

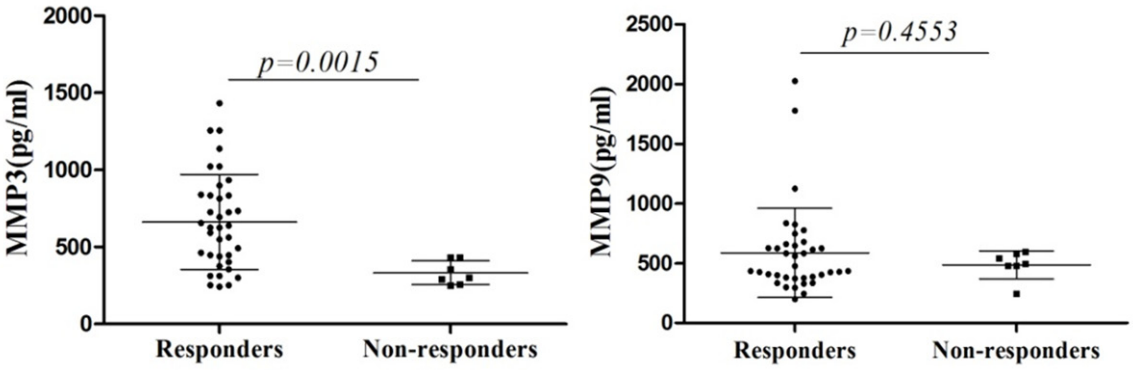

Figure 4. Baseline serum MMP-3 and MMP-9 differences between responders and non-responders to infliximab therapy.

patients in very high disease activity. At week 12 after infliximab infusion, the mean ASDAS score declined to 0.92, including 26 inactive patients, 16 moderate patients and 2 high disease activity patients. There were thirty-seven responders and seven non-responders according to the improvement of ASDAS criteria. Both responders and non-responders were of similar age (26.2 ± 7.1 and 28.3 ± 5.5, respectively). Responders had a numerical shorter mean disease duration (40 ± 9.2 months) than non-responders (56 ± 11.4 months), which approached statistical significance (*P= 0.04*). There was no difference in the use of either NSAIDs or disease modifying antirheumatic drugs (DMARDs) between the two groups.

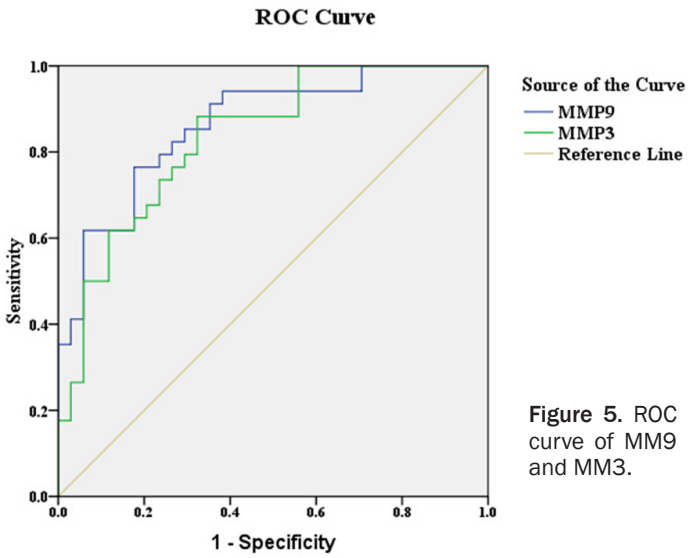

Figure 5. ROC curve of MM9 and MM3.

Furthermore, there were no discriminating clinical measures of disease activity or function between responders and non-responders at baseline. There was a difference in MMP-3 between the two groups at baseline, with responders having higher MMP-3 levels than non-responders (*P<0.01*) (**Figure 4**).

ROC curve analysis showed that both MMP-3 andMMP-9 were of good specificity and sensitivity. There were no significant difference between these two indicators (**Figure 5**, *P*=0.58).

Discussion

In this study, we investigate serum MMP-3 and MMP-9 as biomarkers for disease activity in Chinese AS patients during Infliximab treatment. We also evaluate the association between MMPs and the clinical scores. Our results showed the concentration of MMP-3, MMP-9 in serum statistically significant decrease during infliximab treatment. Since some researchers found that the serum MMP-3, MMP-9 was higher in AS than normal, the increased MMPs in synovial membrane, fluid and blood may mirror the inflammatory process [7, 8], we didn't enroll the normal control. Meantime, our results showed that the basic serum MMP-3, MMP-9 and the decrease of two biomarks were correlated with ASDAS. The decrease of MMPs with effective treatment was strong associated with ASDAS, which reflected the disease activity. Other studies also have suggested that serum MMP-3 is closely related to inflammation and high disease activity in AS and is a useful marker of disease activity in AS, [12, 20, 21]. This relationship is due to the fact that MMP-3 directly participates in the degradation of the extracellular matrix, and its elevation can activate multiple MMP factor precursors to further accelerate the degradation of joint cartilage matrix, destroy cartilage, and advance the progression of AS. It was further supported by the finding that synovial fluid and serum MMP-3 levels had stronger correlation with synovial inflammatory infiltration than with systemic inflammatory parameters such as the CRP level and ESR [20].

We have a consistent result that serum MMP-3, MMP-9 increased for the active AS, but if they are the biomarkers to predict the severity and effectiveness in response to TNF antagonists. Our results showed that the decrease of MMPs was strong associated with ASDAS, CRP, ESR and good responders had higher MMP-3, A number of studies have shown that MMP-3 level reduced in response to TNF antagonists [12, 20, 21, 22]. Infliximab infusions led to rapid and significant suppression of serum MMP-3 levels, if serum MMP-3 levels did not distinguish responders from non-responders? Related researchers were few. A recent study also noted that the change of serum MMP-3 level from baseline to 3 months in predicting response was poor for ASAS40 or moderate for ASAS20, and was not superior to the predicting value of change in the currently used objective biomarkers [21]. MMP-3 values as a marker of response is debatable.

The debate on MMP-3 predicting value may reflect differences in the patient groups since it has also been suggested that MMP-3 level was higher in patients with peripheral arthritis than those with axial disease only [21-23], although this has not always been observed [12]. Our data showed that AS patients with PA having higher MMP-3 level than AS patients without PA, but we did not analyze the correlation between MMP-3 level and the presence of peripheral arthritis in this study because there were discriminating differences of clinical scores between those with axial involvement only (i.e., disease confined to the spine and

hips) and those with both axial and peripheral involvement at baseline. (Data is not shown). This may reflect differences in the patient groups since it has also been suggested that MMP-3 levels are higher in patients with peripheral arthritis than those with axial disease only [16, 18, 19], although this has not always been observed [23].

Besides MMP-3 and MMP-9 were related with the disease activity score and function, their decreases were significantly correlated with the changing of most clinical scores. Moreover, we found that patients who responded to infliximab as defined by the improvement of ASDAS criteria have higher levels of MMP-3 level before treatment than non-responders. The level of MMP-9 at baseline did not differ significantly between responders and non-responders. Accordingly, MMP-3 may be a better biomarker to monitor AS activity and may be an efficacy predictor of infliximab.

We also had noted that the concentration of MMP-9 was associated with ASDAS and declined with the improvement of ASDAS, which was similar with MMP-3. This indicated that the level of MMP-9 is also associated with the disease activity in AS. According to our results, it seemed that there were some differences between MMP-3 and MMP-9. The association with a component consisting of MMP-3 and MMP-9 were found with BASFI and only MMP-9 was associated with BASMI. At week 12, the decrease of MMP-3 had related with the improvement of BASFI, but not BASMI. MMP-9 was closely related to BASMI in early response to infliximab treatment. BASMI was adopted to assess the flexibility of spine. We also found that AS without peripheral arthritis have higher MMP-9 levels. AS without peripheral arthritis means axial AS in this study. So we suggested that MMP-9 may show the inflammation and function of spine.

MMP-9 in particular has been shown to degrade extracellular matrix, initiate and promote new vessel formation [24]. Angiogenesis plays a central role in diseases simultaneously occurred with bone formation. During formation of bone from cartilage or enchondral ossification, invasion of the cartilage by new blood vessels precedes osteoblastic transformation and ossification. Angiogenesis is also involved in the pathogenesis of SpA. Chondrocytes and osteoblasts can produce angiogenic factors such as VEGF [25], which has been isolated from the joints of AS patients [26]. Serum VEGF levels were correlated strongly with clinical and laboratory indices of disease activity of SpA. Serum VEGF was a potential value for monitoring disease activity and treatment response in SpA patients [25, 27]. VEGF treatment also accelerates smooth muscle cell migration through synthetic barriers, and this response is blocked by MMP inhibition, suggesting that this action is mediated via up-regulation of MMPs [28].

There was a similar conclusion [11] that a profile consisting of high levels of MMP-8 and MMP-9 is associated with increased disease activity in AS, rather than MMP-3 levels. Therefore, we conclude that MMP-9 was associated with axial AS.

Conclusion

Infliximab has a fast onset as an advantage. This research has shown that a profile consisting of high levels of MMP-3 and 9 was associated with increased clinical scores in active AS patients and those they decreased with the improvement of clinical syndromes during infliximab therapy. MMP-3 and MMP-9 could reflect the change of disease in the early response, so they can be used as the biomarkers to evaluate disease activity and response. Further studies with larger numbers of patients are necessary to perform a multiple logistic regression analysis in order to determine if these variables are true predictors of response to infliximab.

Disclosure of conflict of interest

None.

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