Suppression of active, but not total MMP-3, is associated with treatment response in a phase III clinical study of rheumatoid arthritis

A.S. Siebuhr[1], C.F. Kjelgaard-Petersen[1,3], S. Sun[1], I. Byrjalsen[1], C. Christiansen[2], M.A. Karsdal[1], A.C. Bay-Jensen[1]

[1]Biomarkers and Research, Nordic Bioscience, Herlev, Denmark;
[2]Centre of Clinical and Basic Research, Ballerup, Denmark;
[3]Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark.

Abstract

Objective

Biologics for rheumatoid arthritis (RA) patients with moderate to severe disease may preserve joint function. Matrix metalloproteinase 3 (MMP-3), a key tissue degrading protease, is highly elevated in RA. MMP-3, which measures the total pool of circulating MMP-3 species (cMMP3), is a commonly measured biomarker in rheumatology. The aim was to investigate the association of activated MMP-3 (actMMP3) species with treatment response compared to cMMP-3.

Methods

The LITHE biomarker study (n=741) was a 1-year phase III, double-blind, placebo-controlled, parallel group study of TCZ in RA patients on stable methotrexate. cMMP-3 and actMMP-3 were assessed in fasting serum at baseline, week 4, 16, 24 and 52. Patients not achieving ACR20 remission at week 16 or 28 received rescue treatment (escapers). Spearman's correlation was analysed between biomarker baseline level or biomarker delta and clinical measures. Changes in biomarker levels were studied as a function of time and treatment.

Results

ActMMP-3 16-week change in treatment groups was predictive of 1-year radiographic progression; a small change in actMMP3 was equal to worsening radiographics. Baseline cMMP-3 was associated with 52-weeks' radiographic status and cMMP3 16-weeks' change was predictive of 1-year change in disease activity. ActMMP-3 was dose-dependently decreased by TCZ, and escapers decreased in actMMP-3 upon treatment.

Conclusion

ActMMP-3 and cMMP-3 were found to be efficacy biomarkers of TCZ and actMMP-3 were able to differentiated doses. Moreover, the suppression of actMMP3, but not cMMP3 was associated with treatment response. This study illustrates that two biomarkers of the same protein may have different predictive capacities.

Key words

active protease, MMP-3, protein fingerprint biomarker, treatment efficacy, tocilizumab

Anne S. Siebuhr, PhD
Cecilie F. Kjelgaard-Petersen, MSc
Shu Sun, PhD
Inger Byrjalsen, MD
Claus Christiansen, MD, DMSc
Morten A. Karsdal, PhD
Anne-Christine Bay-Jensen, PhD

Please address correspondence and reprint requests to:
Dr Anne Sofie Siebuhr,
Nordic Bioscience A/S,
Herlev Hovedgade 205-207,
DK-2730 Herlev, Denmark.
E-mail: aso@nordicbio.com

Received on December 19, 2016; accepted in revised form on May 24, 2017.

Trial registration: NCT00106535

Funding: The LITHE study was supported by Hoffmann-La Roche and the investigation of acMMP-3 of the LITHE biomarker study was supported by the Danish Research Foundation.

Competing interests: A.S. Siebuhr, S. Sun, A.C. Bay-Jensen, I. Byrjalsen and M. Karsdal are full-time employees at Nordic Bioscience;
C. Christiansen and M. Karsdal hold stocks in the company;
C.F. Kjelgaard-Petersen worked at Nordic Bioscience during the completion of this manuscript. Nordic Bioscience is a privately-owned; small-medium size enterprise partly focused the development of biomarkers for rheumatic and fibrotic diseases. None of the authors received fees, bonuses or other benefits for the work described in the manuscript.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by inflammation and massive tissue destruction in multiple synovial joints. Tissue destruction is a consequence of an unbalanced tissue remodelling, in which the increase in protease activity results in increased tissue degradation that is not compensated with tissue formation. Several studies have shown that matrix metalloproteinase 3 (MMP-3) is elevated in RA and other arthritic diseases (1-4). Thus, MMP-3 could be a biomarker of disease activity, and progression and a treatment efficacy in arthritic diseases. Biologic treatments for RA patients with moderate to severe disease have shown to preserve the joint (5). However, since biologics are also associated with side effects there is a medical need for early identification of those patients that are progressing toward joint destruction (structural progressors) and who will benefit the most from treatment with biologics. Clinical measures such as radiography and disease activity scores have shown to be ineffective in early identification of patients who benefit from a given treatment. Thus, developing and identifying biomarkers for early identification of progressors and those who respond to treatment are urgently needed. This will ensure clinical benefit for the right patients and will improve the benefit/risk ratio of therapies and enhance the quality of life for the patients.

MMP-3, also known as stromelysin-1, is a zinc-dependent protease that is mainly secreted by fibroblasts. The main function of the protease is degradation of extracellular matrix (ECM) and activation of other proteases (6–8). MMP-3 is secreted as an inactive protease, which is activated upon removal of the propeptide by proteases (6). The inactive form of MMP-3 is found in circulation, but the activated form of MMP-3 (containing the catalytic domain) is in circulation mainly found as a complex with the plasma proteinase inhibitor alpha-2-macroglobulin (9). This complex with alpha-2-macroglobulin is not detected in the conventional MMP-3 assessment. Thus, assessment of MMP-3 in circulation only measures the inactive circulating form MMP-3 (cMMP3). Hence, the conventional assessment does not give information about the amount of active MMP-3, which mediates tissue destruction. Assessment of the activity of MMP-3 can be done by using a substrate, while the amount of activated MMP-3 can be assessed by the neo-epitope, which is exposed when the pro-peptide is cleaved off. This neo-epitope is only accessible in circulation, when the catalytic domain is disrupted, such when further degradation of the active form of MMP-3 occurs. This fragment of activated MMP-3 (actMMP3) is released into circulation and may be quantified. An ELISA has been developed quantifying the exposed neo-epitope of MMP-3 upon removal of the pro-peptide in serum. This ELISA is specific to the activated form of MMP-3 in circulation (10).

The hypothesis of the current study was that assessment of the activated MMP-3 is associated with tissue degradation and thus associated with treatment response in RA. The aim of the study was to investigate whether biomarkers of MMP3 (actMMP-3 and cMMP3) were affected by tocilizumab (TCZ) treatment and if they were associated with disease activity and treatment response.

Materials and methods

The LITHE study

The LITHE biomarker study was a part of the LITHE study (Trial registration: NCT00106535), which was a 2-year, phase III, double-blind, placebo-controlled, parallel group study of TCZ (4 mg/kg or 8 mg/kg every 4 weeks) + methotrexate (MTX; 10 to 25 mg/week) in patients with moderate to severe active RA with an inadequate response to MTX. Patients were diagnosed with RA according to the American College of Rheumatology criteria (11) and had a disease duration of at least 6 months prior to enrolment into the study. Moreover, the patients had to have radiographically confirmed joint erosion.

The LITHE biomarker study included 741 patients followed for the first 52 weeks from the first dose, but only 734 patients were available for acMMP3 or cMMP3 assessment. The 741 biomarker sub-study patients were equally distribut-

ed among the treatment groups: placebo (n=256), 4 mg/kg TCZ (TCZ4; n=241) and 8 mg/kg TCZ (TCZ8; n=244). Stable non-steroidal anti-inflammatory drug and corticosteroid (≤10 mg/day prednisolone or equivalent) doses were continued throughout the study. Every 4 weeks, patients received an infusion of TCZ 4 mg/kg, 8 mg/kg or placebo for a total of up to 13 infusions in 52 weeks. Sera for biomarker assessment were collected following overnight fasting at baseline and at weeks 4, 16, 24 and 52. All samples were stored below -70°C until they were assayed.

Patients who failed to respond to treatment during the study, that is experienced ≤20% improvement from baseline in both swollen joint counts (SJCs) and tender joint counts (TJCs) at week 16, could receive blinded rescue therapy from week 16 and subsequently 12 weeks later (at week 28) if there was still ≤20% improvement. First-step rescue patients receiving placebo + MTX were switched at week 16 to TCZ4 + MTX. Patients receiving TCZ4 + MTX were switched to TCZ8 + MTX, and treatment of patients with TCZ8 + MTX was discontinued. Second-step rescue at week 28 consisted of TCZ8 + MTX, which was offered through week 52 if inadequate response persisted after three doses of first-step rescue therapy. Treatment was discontinued in patients who did not respond after three doses of second-step rescue. The patients who received rescue therapy were designated escapers. Half of the patients receiving placebo + MTX at baseline were receiving first-step rescue therapy at week 52. Only 6 patients were on second-step rescue therapy at week 52. Around 25% of patients in the TCZ4 group received rescue treatment. Only 15% of the patients in the TCZ group failed and were excluded from the remainder of the study.

Visual analogue scale (VAS) score for pain and Disease Activity Score in 28 joints erythrocyte sedimentation rate (DAS28-ESR) were assessed at baseline and weeks 24 and 52. The patients' self-reported general health was assessed by having them complete the Health Assessment Questionnaire (HAQ) at baseline and at weeks 24 and 52.

The LITHE study was approved by ethics committees at each participating institution (5). All patients in the LITHE study provided their written informed consent to participate. The study was conducted according to the principles of Good Clinical Practice and according to the Declaration of Helsinki.

Biomarkers

ActMMP-3 was assessed by an in-house ELISA previously described (10). In short, the assay is performed as follows: A 96-well streptavidin coated plate was coated with biotinylated screening peptide and incubated for 30 min at 20°C. Thirty µL of calibrator, control and sample were added to appropriate wells followed by horseradish peroxidase (HRP) conjugated antibody and was incubated at 4°C for 20 hours. Finally, 100 µL tetramethylbenzinidine (TMB) was added and incubated at 20°C for 15 min in darkness. The enzyme reaction was terminated with 100µL 0.1% H_2SO_4 and the absorbance was read with a SpectraMax. All incubation steps were at shaking 300 rpm and in between the incubation steps the plate was washed in TBST.

The cMMP-3 was measured by a 2-site ELISA using two polyclonal antibodies raised against human MMP-3 (Quantikine, Human Total MMP-3 immunoassay, R&D Systems, Inc, Minneapolis MN).

Statistics

Summary statistics were used to generate general demographics. Univariate analysis by Spearman's ranked correlation was conducted on the baseline level of the biomarkers (actMMP-3 and cMMP-3) and baseline clinical parameters. In addition, associations between the change from baseline to week 16 in biomarkers and 1-year change in clinical parameters were investigated by Spearman's ranked correlation. Kruskal-Wallis with Dunn's multiple comparisons test was used to test the changes from baseline of the biomarkers as a function of time or treatment, as biomarkers were not normally distributed. The change from baseline of actMMP-3 for escape and non-escape patients in the 4 mg/kg group was assessed by Kruskal-Wallis with Dunn's multiple comparisons test. Statistical analysis and graphical illustration of results were performed in MedCalc Statistical Software v. 14.12.0 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014). There were no forward imputation for missing data.

Results

Patient demographics at baseline

A total of 734 patients were included in the MMP-3 study. Eight patients were excluded, due to missing serum samples for assessment of actMMP. The baseline characteristics are listed in Table I. There were no differences in the demographics at baseline between the treatment groups, except for patients in the 8 mg/kg group being statistical significantly older than both the placebo (PBO) and 4 mg/kg (p=0.034 and p=0.031, respectively).

Univariate analysis at baseline

There were no correlation between actMMP-3 and age or BMI, whereas cMMP-3 was weakly and negatively correlated with BMI (rho -0.09; Table II). Neither actMMP-3 nor cMMP-3 were correlated with disease duration. cMMP-3, but not actMMP-3, was weakly correlated with HAQ and VAS-pain (rho: 0.16 and 0.15, respectively). Both actMMP-3 and cMMP-3 were weakly correlated to DAS (rho: 0.09 and 0.20) and to CRP (rho: 0.26 and 0.48). Lastly, there was a weak correlation between actMMP-3 and cMMP-3 (rho: 0.16).

Univariate analysis between baseline levels of biomarkers and change in disease parameters were investigated in the placebo group to investigate if the baseline level of the biomarkers were associated disease progression (Table III). Baseline level of actMMP-3 was weakly correlated to 24-weeks change in VAS-pain (rho: 0.19). There were no correlations between baseline actMMP-3 levels and the 52-week change in disease parameters.

Univariate analysis of change to week 16 in biomarkers and 24 and 52 weeks change in disease parameters

The 16-week changes in biomarkers

Table I. Baseline demographics in the population and in the randomised groups.

	All			PBO			4 mg/kg			8 mg/mL		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Age (years)	734	52.5	12.3	255	51.7	12.4	241	51.9	13.0	238	54.0	11.3
Sex (% female)	738	83		253	83		241	86		244	80	
BMI (kg/m^2)	725	27.9	6.5	253	28.2	7.4	237	27.6	6.2	235	27.7	5.7
Disease duration (years)	734	9.6	8.2	255	9.2	8.1	241	10.1	8.0	238	9.6	8.6
DAS-ESR	719	6.5	0.93	248	6.5	0.93	238	6.5	0.93	233	6.5	0.92
VAS-pain	725	54.5	22.0	251	54.8	21.3	240	52.7	22.3	234	56.0	22.4
HAQ	668	1.5	0.62	232	1.5	0.61	220	1.5	0.65	216	1.5	0.61

BMI: bone mass index; DAS: Disease Activity score; HAQ: Health Assessment Questionnaire, ERN: erosion; JSN: joint space narrowing; PBO: placebo.

Table II. Correlation between the baseline demographics and the biomarkers. Spearmann's rho, the corresponding *p*-value and the number of subjects in the analysis are presented.

	actMMP-3			cMMP-3		
	rho	*p*-value	n	rho	*p*-value	n
Age	-0.07	-	644	-0.04	-	672
BMI	-0.00	-	637	-0.09	0.02	665
Disease duration	-0.01	-	644	0.04	-	672
HAQ	0.06	-	583	0.16	0.0001	608
DAS	0.09	0.03	630	0.20	<0.0001	658
VAS-pain	0.02	-	636	0.15	0.0001	664
CRP	0.26	<0.0001	643	0.48	<0.0001	672
cMMP-3	0.16	0.0001	625	-		

were investigated for association with change in disease parameters at 24 and 52 weeks (Table IV). There was no correlation between actMMP-3 and any of the disease parameter at either time point in the placebo group. cMMP-3 was associated with DAS at both 24 and 52 weeks change (rho: 0.20 and 0.33, respectively) and HAQ at 24 weeks (rho: 0.25).

In the 4 mg/kg group, the 16-week change in actMMP-3 was associated with the 52-week change in HAQ and VAS-pain (rho 0.29 and 0.23, respectively). The 16-week change in cMMP-3 was associated with disease activity, HAQ and VAS-pain at both 24 weeks (rho 0.24, 0.24 and 0.22) and 52 weeks (rho 0.25, 0.21 and 0.22). In the 8 mg/kg group there was no association with the change in actMMP-3 cMMP-3 to week 16 and clinical parameters.

Biomarker level with time and treatment

Patients receiving 8 mg/kg TCZ had a rapid and significant decrease (within the first 4 weeks) in actMMP-3 level (p=0.006) compared to baseline (Fig. 1A). This lower level of actMMP-3 was kept throughout the rest of the study, with the exception of week 24, where the level was not different from baseline. Patients receiving 4 mg/kg did not differ from baseline in actMMP-3 throughout the study. In the placebo group, the level of actMMP-3 compared to baseline was increased albeit non-significantly throughout the study. The 8 mg/kg group had significantly lowered actMMP3 compared to placebo at week 4 (p<0.0001), 16 (p=0.0009), 24 (p<0.0001) and 52 (p<0.0001). In addition, the 8 mg/kg group had significantly lower actMMP3 levels than the 4 mg/kg at week 4 (p<0.0001) and 16 (p=0.03).

cMMP-3 was significantly decreased throughout the study in both TCZ groups (p<0.0001) compared with both baseline level and placebo (p=<0.001; Fig. 1B). There was no significant difference between the 4 mg/kg and 8mg/kg groups in cMMP3.

The level of actMMP-3 did not differ between escapers and non-escapers in the placebo group at baseline or throughout the study (Fig. 2A). However, in the 4 mg/kg group escape patients had significantly lower actMMP3 level at week 52 compared to non-escapers, but the baseline level of actMMP-3 was the same in escapers and non-escapers (Fig. 2B). As the escapers in the 8 mg/kg group discontinued the study, only

Table III. Association between baseline biomarker levels and 1-year change in disease parameters in the placebo group. Spearmann's rho, the *p*-value and the number of subjects in the analysis are presented.

	Baseline actMMP-3						Baseline cMMP-3					
	24 weeks			52 weeks			24 weeks			52 weeks		
	rho	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n
ΔDAS	0.11	-	125	-0.06	-	87	-0.14	-	127	-0.13	-	89
ΔHAQ	0.05	-	113	0.02	-	84	-0.19	-	114	-0.10	-	86
ΔVAS-pain	0.19	0.03	129	0.12	-	95	0.04	-	131	-0.07	-	97

Table IV. Association between 16 weeks change (ng/mL) in biomarker level and 1-year change in disease parameters. Spearmann's rho, the *p*-value and the number of subjects in the analysis are presented.

Placebo

	ΔactMMP-3 week 16						ΔcMMP-3 week 16					
	24 weeks			52 weeks			24 weeks			52 weeks		
	rho	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n
ΔDAS	-0.08	-	109	0.05	-	77	0.20	**0.02**	123	0.33	**0.002**	97
ΔHAQ	-0.14	-	97	-0.08	-	74	0.25	**0.008**	110	0.18	-	84
ΔVAS-pain	-0.09	-	112	-0.06	-	85	0.10	-	127	0.16	-	95

4 mg/kg

	ΔactMMP-3 week 16						ΔcMMP-3 week 16					
	24 weeks			52 weeks			24 weeks			52 weeks		
	rho	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n
ΔDAS	0.02	-	128	0.08	-	91	0.24	**0.002**	162	0.25	**0.007**	120
ΔHAQ	0.13	-	120	0.28	**0.008**	91	0.24	**0.004**	151	0.21	**0.02**	118
ΔVAS-pain	0.00	-	132	0.23	**0.02**	101	0.22	**0.004**	166	0.22	**0.01**	131

8 mg/kg

	ΔactMMP-3 week 16						ΔcMMP-3 week 16					
	24 weeks			52 weeks			24 weeks			52 weeks		
	rho	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n	*rho*	*p*- value	n
ΔDAS	-0.05	-	130	0.06	-	106	-0.05	-	125	0.06	-	106
ΔHAQ	-0.02	-	119	-0.09	-	101	-0.01	-	114	-0.09	-	101
ΔVAS-pain	0.05	-	131	0.01	-	114	0.05	-	126	0.01	-	114

the baseline level was assessed in this group and no significant difference was observed (data not shown).

There was no significant difference in the level of cMMP3 between escapers and non-escapers in both the placebo group and the 4 mg/kg group throughout the study (Fig. 2C-D) or at baseline (data not shown). However, in the placebo group from week 24 cMMP3 levels tend to decrease in escapers, but this was not observed in the 4 mg/kg group.

Discussion

The assessment of MMP-3 has been highly used in clinical trials within rheumatology. We investigated serological biomarkers of MMP-3 (the active form (actMMP-3) and the circulating form (cMMP3)) in a phase III clinical trial. We found that actMMP-3 was dose-dependently decreased with treatment and that cMMP-3 was associated with pain and disease activity. In addition, we found that an early change in cMMP3 was predictive of 1-year change in disease activity. Thus, the MMP-3 biomarkers had different capacities and this study illustrates that assessment of a protein by different biomarkers provide different information, also exemplified by the weak correlation between the two biomarkers of MMP-3.

MMP-3 is considered an important player in joint damage and is a major player in tissue destruction, not only degrading ECM, but also functioning as an activator of other proteases (12, 13). IL-6 is a pleiotropic pro-inflammatory, multi-functional cytokine produced by a variety of cell types including lymphocytes and fibroblast. Hashizume *et al*. (14) showed that IL-6 induces phosphorylation of STAT3 and ERK1/2 in RA fibroblast, the pathway involved in secretion of MMP-3. In alignment, IL-6 was found to induce the expression of MMP-3 in chondrocytes (15) and fibroblasts (16). The IL-6 receptor has not been found on fibroblasts, but fibroblasts are thought to respond to IL-6 through trans signalling. sIL-6Rs exist in serum and bind to IL-6 forming a complex. This complex bind to the gp130 receptor, which is expressed on fibroblasts (17). Thus, by inhibiting IL-6 binding to the receptor, TCZ could lower the MMP-3 level. Furthermore, the final step in MMP-3 activation is dependent on active MMP-3 itself, thus lowering the MMP-3 level triggers a feedback loop resulting in lowering of activated MMP-3.

Lowering levels of activated MMP-3 presumably reflects the general inhibition of tissue destruction. As an example this was seen in the collagen-induced arthritis model in mice, where MMP-3 was reduced by MTX and infliximab, which resulted in decreased bone and cartilage destruction (18). Theoretically, MMP-3 is a good indicator of the destructive status of the disease-affected tissue, as a decrease in the MMP-3 level should lower the amount of ECM degradation. It has been shown that ECM degradation fragments (matrikines) are local irritants, which induces inflammation and further tissue destruction (19, 20). A matrikine of aggrecan was shown to induce MMP-3 expression

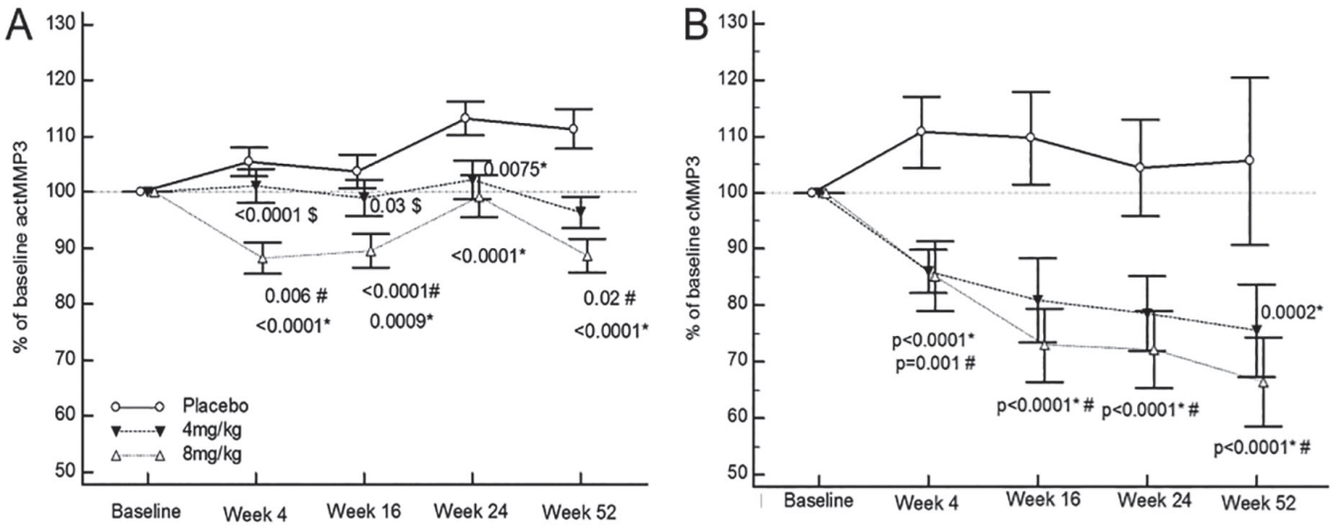

Fig. 1. Percentage change from baseline in A: actMMP-3 and B: cMMP-3 during the study. Data is shown as mean with 95% CI. Statistics was done by Kruskal-Wallis with Dunn's multiple comparisons test for investigating biomarker level compared to baseline. Independent *t*-test was used to investigate the difference in the biomarker levels between 2 groups at each time point. #: compared to baseline. *: Compared to placebo. $: compared to 8 mg/kg.

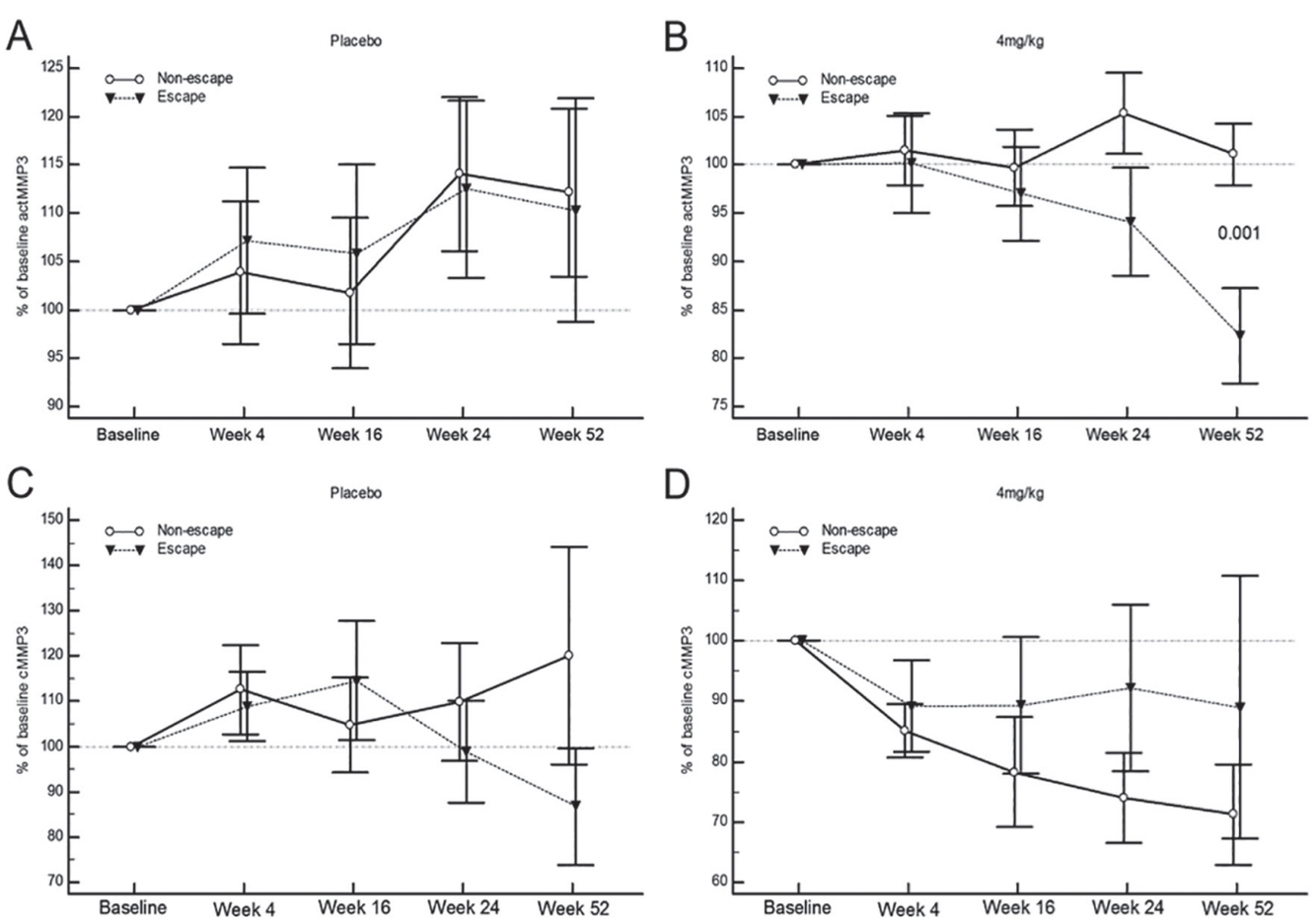

Fig. 2. Percent of baseline of actMMP-3 and cMMP3 levels in the PBO and 4 mg/kg groups' escapers and non-escapers during the study. Escape patients are defined as placebo patients who at week 16 did not achieve ACR20 received rescue treatment (first 4 mg/kg) and if they still did not achieve ACR20 at week 24, they received 8 mg/kg during the rest of the study. Placebo patients that first at week 24 did not achieve ACR20 received 4mg/kg during the rest of the study. Patients of the 4 mg/kg group that did not achieve ACR20 at either week 16 or 24 received rescue treatment (TCZ 8 mg/kg) throughout the rest of the study. **A**: actMMP3 in percent of baseline in the placebo patients grouped as escape and non-escape patients. **B**: actMMP3 in percent of baseline in the 4 mg/kg patients grouped as escape and non-escape patients. **C**: cMMP3 in percent of baseline in the placebo patients grouped as escape and non-escape patients. **D**: cMMP3 in percent of baseline in the 4 mg/kg patients grouped as escape and non-escape patients. Data are shown as mean + 95% CI. Statistics was done by Individual samples *t*-test.

and pro-inflammatory cytokines in primary chondrocytes (19) and a 29kDa fibronectin fragment was shown to increase MMP-3 level in chondrocytes in a dose-dependent manner (20). These studies indicate a negative feedback loop of elevated levels of MMP-3, as MMP-3 directly or indirectly aid in the production of the matrikines investigated. Thus, lowering MMP-3 due to treatment should limit tissue destruction and inflammation, which for a patient would be translated to a lower disease activity, a halt in disease progression and inflammation, which would give a better health assessment and quality of life. This is reflected in the current study, where a lowering of MMP-3 was associated with a lowering of DAS, and HAQ and furthermore VAS pain. Several studies have investigated the potential of MMP-3 as a biomarker of disease activity and as a prognostic biomarker (1, 21-30). cMMP-3 has been assessed before, but the conclusion of the usage of MMP-3 as a biomarker of disease activity and prognostic capacity is debatable. Several studies have found that cMMP-3 is a biomarker of disease activity and radiographic progression, where other studies have shown limited or no usage of cMMP-3 as a biomarker of disease activity, radiographic progression and a biomarker with prognostic capacities (1, 21, 24-27, 29). The data of the current study support cMMP-3's association with disease activity and prognostic capacity of disease activity (1, 21, 26, 29). The baseline level of cMMP-3 was associated with disease activity and the change in cMMP-3 after 16 weeks was significantly associated with the change in disease activity after 24 and 52 weeks. Contrary, the baseline level of actMMP-3 was not associated with disease activity neither after 24 nor 52 weeks. When assessing the change in the MMP-3 biomarkers in response to treatment, actMMP-3 was dose-dependently decreased from week 4, whereas cMMP-3 was decreased by treatment, albeit not dose-dependently. An earlier study of the treatment efficacy of cMMP-3 with TCZ treatment found that cMMP-3 was significantly and dose-dependently decreased at week 16 (25). The difference between these results is unknown. The current study suggests that assessment of MMP-3 by different biomarkers, in the current case two distinct epitopes of MMP-3, yields different information.

Limitations

Firstly, the non-responders (15%) to TCZ in the 8 mg/kg group was discontinued and there was no activity measures of these at week 52. Thus, the group with the worst outcome was not included in this study, which weakened the study. Furthermore, the study population investigated had a long disease duration and already profound joint damage. The profound joint damage could limit the progression of disease, which does not make this the ideal study for investigation of prognostic biomarkers of disease progression. Another limitation is the effect of the concomitant intake of corticosteroid and non-steroidal anti-inflammatory drugs. This could influence the MMP-3 level and it is known that especially prednisolone intake has an influence on joint degradation (31, 32). However, patients were on stable doses of co-medications at study inclusion, thus the effect of the concomitant drugs might be obliterated in the baseline level of MMP-3.

Conclusion

In conclusion, we found that actMMP3 was an efficacy biomarker of TCZ treatment in RA and cMMP was an early predictive biomarker of disease activity after 24 and 52 weeks. This is in line with the result that there was only a week association between the two biomarkers of MMP-3. Thus, the two MMP-3 biomarkers had different capacities and this study illustrates that assessment of a protein by different biomarkers provides different information.

Key message

- actMMP3 change after 16 weeks was predictive of 1-year radiographic progression.
- cMMP3 change after 16 weeks was predictive of HAQ and DAS improvement after 1-year.
- Assessment of the different forms of MMP3 provides different clinical information.

Acknowledgements

We would like to thank all the LITHE investigators for the sublime work on recruiting patients and collecting samples for the study.

References

1. HOUSEMAN M, POTTER C, MARSHALL N *et al*.: Baseline serum MMP-3 levels in patients with rheumatoid arthritis are still independently predictive of radiographic progression in a longitudinal observational cohort at 8 years follow up. *Arthritis ResTher* 2012; 14: R30.
2. KOBAYASHI A, NAITO S, ENOMOTO H *et al*.: Serum levels of matrix metalloproteinase 3 (stromelysin 1) for monitoring synovitis in rheumatoid arthritis. *ArchPatholLab Med* 2007; 131: 563-70.
3. MAKSYMOWYCH WP, LANDEWÉ R, CONNER-SPADY B *et al*.: Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum* 2007; 56: 1846-53.
4. POSTHUMUS MD, LIMBURG PC, WESTRA J *et al*.: Serum levels of matrix metalloproteinase-3 in relation to the development of radiological damage in patients with early rheumatoid arthritis. *Rheumatology* (Oxford) 1999; 38: 1081-7.
5. KREMER JM, BLANCO R, BRZOSKO M *et al*.: Tocilizumab inhibits structural joint damage in rheumatoid arthritis patients with inadequate responses to methotrexate: results from the double-blind treatment phase of a randomized placebo-controlled trial of tocilizumab safety and prevention of structu. *Arthritis Rheum* 2011; 63: 609-21.
6. NAGASE H, VISSE R, MURPHY G: Structure and function of matrix metalloproteinases and TIMPs. *CardiovascRes* 2006; 69: 562-73.
7. SHAPIRO SD, FLISZAR CJ, BROEKELMANN TJ *et al*.: Activation of the 92-kDa gelatinase by stromelysin and 4-aminophenylmercuric acetate. Differential processing and stabilization of the carboxyl-terminal domain by tissue inhibitor of metalloproteinases (TIMP). *J Biol Chem* 1995; 270: 6351-6.
8. SUZUKI K, ENGHILD JJ, MORODOMI T *et al*.: Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry* 1990; 29: 10261-70.
9. NAGASE H: Activation mechanisms of matrix metalloproteinases. *Biol Chem* 1997; 378: 151-60.
10. SUN S, BAY-JENSEN A-C, KARSDAL MA *et al*.: The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. *BMC Musculoskelet Disord* 2014; 15: 93.
11. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
12. WU JJ, LARK MW, CHUN LE *et al*.: Sites of stromelysin cleavage in collagen types II, IX, X, and XI of cartilage. *J Biol Chem* 1991; 266: 5625-8.

13. FLANNERY CR, LARK MW, SANDY JD: Identification of a stromelysin cleavage site within the interglobular domain of human aggrecan. Evidence for proteolysis at this site in vivo in human articular cartilage. *J Biol Chem* 1992; 267: 1008-14.
14. HASHIZUME M, HAYAKAWA N, MIHARA M: IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17. *Rheumatology* (Oxford) 2008; 47: 1635-40.
15. LEGENDRE F, BOGDANOWICZ P, BOUMEDIENE K *et al*.: Role of interleukin 6 (IL-6)/IL-6R-induced signal tranducers and activators of transcription and mitogen-activated protein kinase/extracellular. *J Rheumatol* 2005; 32: 1307-16.
16. LINDNER D, ZIETSCH C, MORITZ BECHER P *et al*.: Differential expression of matrix metalloproteases in human fibroblasts with different origins. *Biochem Res Int* 2012; 875742.
17. BARNES TC, ANDERSON ME, MOOTS RJ: The many faces of interleukin-6: the role of IL-6 in inflammation, vasculopathy, and fibrosis in systemic sclerosis. *Int J Rheumatol* 2011; 2011: 721608.
18. LEE A, PARK K, CHOI SJ *et al*.: Prediction of antiarthritic drug efficacies by monitoring active matrix metalloproteinase-3 (MMP-3) levels in collagen-induced arthritic mice using the MMP-3 probe. *MolPharm* 2014; 11: 1450-8.
19. LEES S, GOLUB SB, LAST K *et al*.: Bioactivity in an aggrecan 32-mer fragment is mediated via toll-like receptor 2. *Arthritis Rheumatol* 2015; 67: 1240-9.
20. HWANG HS, PARK SJ, CHEON EJ *et al*.: Fibronectin fragment-induced expression of matrix metalloproteinases is mediated by MyD88-dependent TLR-2 signaling pathway in human chondrocytes. *Arthritis Res Ther* 2015; 17: 320.
21. ALLY MM, HODKINSON B, MEYER PW, MUSENGE E, TIKLY M, ANDERSON R: Serum matrix metalloproteinase-3 in comparison with acute phase proteins as a marker of disease activity and radiographic damage in early rheumatoid arthritis. *Mediators Inflamm* 2013; 2013: 183653.
22. BAY-JENSEN AC, WICHUK S, BYRJALSEN I *et al*.: Circulating protein fragments of cartilage and connective tissue degradation are diagnostic and prognostic markers of rheumatoid arthritis and ankylosing spondylitis. *PLoS One* 2013; 8: 1-7.
23. BAY-JENSEN AC, BYRJALSEN I, SIEBUHR AS *et al*.: Serological biomarkers of joint tissue turnover predict tocilizumab response at baseline. *J Clin Rheumatol* 2014; 20: 332-5.
24. GALIL SM, EL-SHAFEY AM, HAGRASS HA *et al*.: Baseline serum level of matrix metalloproteinase-3 as a biomarker of progressive joint damage in rheumatoid arthritis patients. *Int J Rheum Dis* 2014.
25. KARSDAL MA, SCHETT G, EMERY P *et al*.: IL-6 receptor inhibition positively modulates bone balance in rheumatoid arthritis patients with an inadequate response to anti-tumor necrosis factor therapy: biochemical marker analysis of bone metabolism in the Tocilizumab RADIATE Study (NCT00106522). *Semin Arthritis Rheum* 2012; 42: 131-9.
26. MAMEHARA A, SUGIMOTO T, SUGIYAMA D *et al*.: Serum matrix metalloproteinase-3 as predictor of joint destruction in rheumatoid arthritis, treated with non-biological disease modifying anti-rheumatic drugs. *Kobe J Med Sci* 2010; 56: E98-107.
27. SHIOZAWA K, YAMANE T, MURATA M *et al*.: MMP-3 as a predictor for structural remission in RA patients treated with MTX monotherapy. *Arthritis ResTher* 2016; 18: 55.
28. SIEBUHR AS, BAY-JENSEN AC, LEEMING DJ *et al*.: Serological identification of fast progressors of structural damage with rheumatoid arthritis. *Arthritis Res Ther* 2013; 15: R86.
29. YOUNG-MIN S, CAWSTON T, MARSHALL N *et al*.: Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. *Arthritis Rheum* 2007; 56: 3236-47.
30. GARNERO P, THOMPSON E, WOODWORTH T, SMOLEN JS: Rapid and sustained improvement in bone and cartilage turnover markers with the anti-interleukin-6 receptor inhibitor tocilizumab plus methotrexate in rheumatoid arthritis patients with an inadequate response to methotrexate: Results from a substudy of the multicenter double-blind, placebo-controlled trial of tocilizumab in inadequate responders to methotrexate alone. *Arthritis Rheum* 2010; 62: 33-43.
31. VAN TUYL LH, BOERS M, LEMS WF *et al*.: Survival, comorbidities and joint damage 11 years after the COBRA combination therapy trial in early rheumatoid arthritis. *Ann Rheum Dis* 2010; 69: 807-12.
32. HAFSTROM I, ALBERTSSON K, BOONEN A *et al*.: Remission achieved after 2 years treatment with low-dose prednisolone in addition to disease-modifying anti-rheumatic drugs in early rheumatoid arthritis is associated with reduced joint destruction still present after 4 years: an open 2-year continuation. *Ann Rheum Dis* 2009; 68: 508-13.