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ORIGINAL ARTICLE

Is radiographic progression in radiographic axial spondyloarthritis related to matrix metalloproteinase degradation of extracellular matrix?

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ABSTRACT

Background Radiographic axial spondyloarthritis (r-axSpA) is associated with extracellular matrix (ECM) remodelling of affected tissues. We investigated whether there was a relationship between biomarkers of ECM remodelling and 2-year radiographic progression in r-axSpA.

Methods Patients from the Outcome in Ankylosing Spondylitis International Study (OASIS) were included if they had serum, clinical and spinal radiographic assessments available at baseline and 2 years later. Two readers independently scored the radiographs according to the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS). The average score was used. Type I, V and VI collagen metabolites (C1M, C5M and C6M) and citrullinated and matrix metalloproteinase-degraded vimentin (VICM) were assessed in serum by ELISAs. The relationship between serum biomarkers and 2-year radiographic progression was investigated using linear regression analyses adjusted for potential confounders. Interactions were tested.

Results Patients included (n=122) had a mean age of 45 years (SD 12), 70% were male and 82% were human leucocyte antigen-B27 positive. The mean 2-year mSASSS progression was 2.1 (2.9) units. Only C1M was significantly associated with mSASSS progression (β=0.01, 95% CI 0.00 to 0.03). The effect disappeared after adjustment for confounders. C5M, C6M and VICM showed no relationship with mSASSS progression.

Conclusion We did not find evidence that degradation of ECM is related to radiographic progression in patients with r-axSpA.

Key messages

What is already known about this subject?

- Radiographic axial spondyloarthritis (r-axSpA) is associated with extracellular matrix (ECM) remodelling of affected tissues.
- Biomarkers of extracellular matrix turnover have shown potential as biomarkers of radiographic progression in rheumatoid arthritis.

What does this study add?

- We found no evidence that matrix metalloproteinase-driven type I, V and VI collagen or vimentin degradation is related to radiographic progression in patients with r-axSpA.

How might this impact on clinical practice?

- Due to the negative findings of this study, the study does not impact clinical practice.

INTRODUCTION

Radiographic axial spondyloarthritis (r-axSpA) is a chronic inflammatory disease predominately affecting the axial skeleton, characterised by the presence of radiographic sacroiliitis and previously known as ankylosing spondylitis. The disease is characterised by excessive connective tissue remodelling, where bone, cartilage and the synovial membrane undergo changes.[1] R-axSpA may affect the whole axial skeleton with imaging revealing sacroiliitis, syndesmophytes and changes in the zygapophyseal joints, which could eventually lead to a bamboo spine. Currently, the most frequently used technique to monitor disease progression is imaging, namely, spinal radiographs quantified by the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS).[2] Even though imaging is sensitive, the development of changes in r-axSpA is slow, and therefore time is needed to capture change. An alternative and attractive method as a surrogate for radiographic progression and, most importantly to predict this early, would be a serological biomarker of tissue remodelling, which is both fast and objective to assess.[3] If we can identify patients who will progress early, we can specifically target inhibition of radiographic progression in an attempt to modify the course of the disease.

Tissue remodelling results in the release of a range of protein fragments (neoepitopes)

of extracellular matrix (ECM) proteins and intracellular proteins generated by proteases produced locally within the tissue. These fragments can be both degradation fragments and propeptides of proteins. The protein fragments released into the circulation reflect ECM changes at the sites of disease activity. These protein fragments can be used as biomarkers of tissue remodelling. The excessive connective tissue remodelling can be assessed by serological quantification of type I collagen degradation fragments (C1M) generated by matrix metalloproteinase (MMP). C1M has previously shown to be increased in patients with r-axSpA compared with controls[4] and to be a biomarker of radiographic progression in rheumatoid arthritis.[5] Type V collagen is a fibril-forming collagen, and a degradation fragment of this collagen (C5M) has been shown to be elevated in patients with r-axSpA compared with controls.[6] A biomarker of type VI collagen degradation (C6M) has been found to be elevated in patients with r-axSpA.[4] The cartilage degradation marker, CTX-II, was elevated in patients with r-axSpA compared with controls and decreased after treatment with tumour necrosis factor inhibitors (TNFi).[1 7 8] Cartilage degradation has additionally been studied in patients with r-axSpA by C2M (MMP-dependent type II collagen degradation), and the mean level of C2M and C3M (MMP-degraded type III collagen; a synovial biomarker) was elevated in patients with r-axSpA compared with controls.[4] A combination of the biomarkers C2M, C3M and C6M in the studied cohort increased the diagnostic discriminative value and was furthermore highly correlated with radiographic damage as assessed by the mSASSS.[4] Thus, there is evidence of serological assessment of ECM as possible biomarkers of radiographic damage in rheumatic diseases.

Vimentin is a type III intermediate filament protein that is expressed by various cells and is an important part of the cytoskeleton.[9] It has been shown to be secreted by activated macrophages, which accounts for its presence in the ECM and potentially in the circulation.[10] As anticitrullinated protein antibodies have been found in patients with r-axSpA,[11] a specific MMP mediated and citrullinated fragment of vimentin (VICM) could be an antigen for these anticitrullinated protein antibodies. In addition, it is known that smoking causes inflammation,[12] tissue damage[12] and post-translational modifications, especially citrullinations[13 14]; thus, it is anticipated that VICM is higher in inflammatory diseases and especially in smokers with these diseases. Nevertheless, the role of these biomarkers (C1M, C5M, C6M and VICM) in predicting spinal radiographic progression in r-axSpA has never been investigated.

In this study, we aimed to investigate the relationship between selected ECM biomarkers (C1M, C5M, C6M and VICM) previously assessed in r-axSpA and 2-year spinal radiographic progression in patients with r-axSpA and to determine whether they can be used to identify patients with spinal radiographic progression.

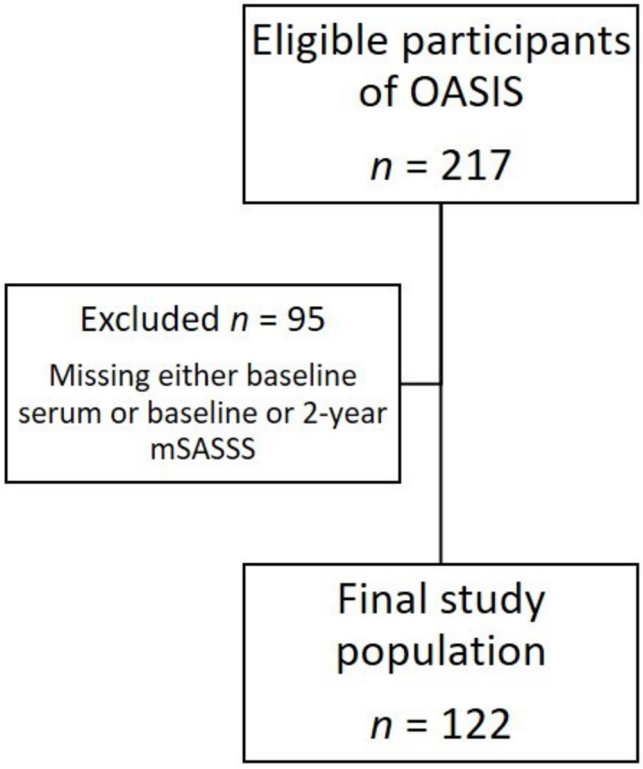

Figure 1 Standards for Reporting of Diagnostic Accuracy Studies (STARD) flow diagram. OASIS, Outcome in Ankylosing Spondylitis International Study.

METHODS

Patients

Patient data from the Outcome in Ankylosing Spondylitis International Study (OASIS) were used for this analysis. OASIS started in 1996 and is a prevalence cohort including 217 consecutive patients with r-axSpA from the Netherlands, Belgium and France.[15] Clinical and radiographic data have been collected in 2-year intervals. The ethics committees from all participating hospitals have approved the study and all patients have given their informed consent to participate in the study. In the present analysis, patients (n=122) have been included if they had serum, as well as clinical and spinal radiographic assessments available at the same visit and the latter also available 2 years later (Figure 1). The baseline visit for this study corresponds to the visit in which the biomarker was assessed from the available serum and does not necessarily correspond to the baseline from the OASIS study. Each patient was only included once and the first visit fulfilling the inclusion criteria was selected. All patients provided written informed consent and the study was performed in accordance with the Declaration of Helsinki.

Radiographic progression

Spinal radiographs, cervical and lumbar, have been collected every 2 years and have been scored using the mSASSS (0–72)[2] by two independent readers, who were blinded to demographics and clinical data, but were aware

of the chronological order, as this is the most sensitive method.[16] The average score of the two readers was used. Details of the radiograph readings, imputation of missing vertebral corners and reliability have been reported elsewhere.[17] The radiographs from the visit of biomarker assessment (here designated as 'baseline', see below) and 2 years later were used. Radiographic progression was calculated as the difference in the mSASSS between the two radiographs (baseline subtracted from year 2). Patients were classified into progressors if they showed any radiographic progression, that is, a 2-year progression above 0. Secondarily, analyses were run with 2-year mSASSS >2 and 2-year mSASSS >5. In another sensitivity analysis, all available follow-up radiographs were included and every patient contributed with as many progression intervals as (s)he had available up to 12 years.

Serological biomarkers

MMP-mediated type I collagen degradation was quantified using the C1M ELISA.[18] This assay is a competitive ELISA based on a monoclonal antibody recognising the C-terminal of the fragment GSPGKDGVRG at position 755–764 in the mature type I collagen. C5M assessing MMP-mediated type V collagen degradation was quantified using the C5M ELISA.[6] The assay is a competitive ELISA assay based on a monoclonal antibody recognising the C-terminal fragment HMGREGREGE at position 1317–1326 in type V collagen. MMP-degradation of type VI collagen was assessed by C6M ELISA.[19] The competitive assay assesses the fragment YRGPEGPQGP at position 573–582 in type VI collagen. A vimentin fragment, RLRSSVPGVR, at position 69–78, has been found to be cleaved with MMP's at both termini. The arginine residues at both termini are prone to be converted to citrulline. A competitive ELISA with a monoclonal antibody was developed to quantify the fragment, where the C-terminal arginine was converted to citrulline.[20] Thus, the ELISA assessed citrullinated and MMP-degraded vimentin. All biomarkers were quantified in serum as nmol/L.

Other variables of interest

When investigating the relationship between the biomarkers and 2-year radiographic progression, we took other variables into account as potential confounders or effect modifiers, such as age, gender, symptom duration, human leucocyte antigen (HLA)-B27 status, baseline smoking status (yes vs no), baseline mSASSS, C-reactive protein (CRP, in mg/L) and disease activity as measured with the Ankylosing Spondylitis Disease Activity Score (ASDAS) (added in a separate model than the ones with CRP). Information on the dosage of non-steroidal anti-inflammatory drug (NSAID) intake or whether the intake was continuous was not available in this cohort.

Statistical analysis

The levels of the biomarkers were compared between progressors and non-progressors with t-test or Mann-Whitney test, as appropriate. We analysed the correlation between each of the different biomarkers at baseline and radiographic damage at baseline as well as 2-year mSASSS progression. For this, Spearman's ranked correlation was used. We also investigated the correlation between CRP and radiographic damage at baseline as well as 2-year mSASSS progression to understand whether the relationships possibly captured by the ECM biomarkers reflected a relationship with inflammation. To further investigate the relationship between each of the biomarkers and 2-year radiographic progression, we conducted a linear regression analysis. Sensitivity analyses with dichotomous outcomes (eg, mSASSS progression >0) were conducted with logistic regression and the analyses including all follow-up radiographs available beyond baseline were conducted with generalised estimating equations, which take within subject correlations into account. First, interactions with gender, HLA-B27 status, symptom duration, baseline ASDAS and baseline mSASSS were tested. Analyses were adjusted for potential confounders, in case there were no effect modifiers. P values <0.05 were considered significant. Analysis was performed using Stata V.12.

RESULTS

Baseline patient description

The studied population consisted of 122 patients with r-axSpA, of which 70% were males and the mean age was 45 years; 9% was on tumour necrosis factor inhibiting (TNFi) therapy and 78% on NSAIDs at baseline. Patients had a mean symptom duration of 23 (SD 12) years and disease duration was 14 (9) years. The mean mSASSS was 13.9 (17.6) units. The mean 2-year mSASSS progression was 2.1 (2.9) units. The biomarker levels were C1M 59.2 (38.2) nmol/L, C5M 5.6 (3.9) nmol/L, C6M 10.6 (19.7) nmol/L and VICM 11.7 (16.6) nmol/L. The only difference between the included and excluded OASIS patients of this study was the number of patients on TNFi therapy, as all these patients (n=11) were included in the present analysis (see online Supplementary table 1).

Patients were separated into progressors (2-year mSASSS >0) and non-progressors (mSASSS=0). The groups were balanced on most baseline clinical descriptives (table 1). Exemptions were age, ASDAS; progressors were on average 5 years older (p=0.04) and had a 0.4 higher ASDAS (p=0.02). Progressors had significantly higher mean CRP and C1M (p<0.05), whereas there were no differences in the mean level of the other soluble markers (table 1). Baseline mSASSS was significantly higher in the progressor group (11.6 vs 15.1, p=0.002). In addition, there were significantly more patients with baseline mSASSS >0 in the progressors group (74 vs 94%, p=0.003). The mean average 2-year change in mSASSS was 3.2 (3.0).

Correlation between biomarkers, structural damage and inflammation

C1M, C6M and VICM were significantly correlated with CRP (Spearman's ρ 0.75, 0.73 and 0.37, respectively), whereas C5M was not. There were no associations between

Table 1 Demographics of study population

	All	Non-progressors, 2-year mSASSS change=0	Progressors, 2-year mSASSS change>0	P values
N	122	43	79	
	Mean (SD) or n (%)			
Male	86 (70%)	30 (70%)	56 (71%)	0.90
Age (years)	45 (12)	42 (12)	47 (12)	0.04
HLA-B27 positive	97 (82%)	36 (84%)	61 (80%)	0.64
Symptom duration (years)	23 (12)	21 (14)	25 (11)	0.23
Disease duration (years)	14 (9)	13 (11)	15 (8)	0.24
NSAIDs	95 (78%)	35 (81%)	60 (76%)	0.49
TNF-a treated	11 (9%)	3 (7%)	8 (10%)	0.56
ASDAS-CRP	2.6 (1.0)	2.3 (1.0)	2.7 (1.0)	0.02
BASDAI (0–10)	3.4 (2.0)	3.0 (2.0)	3.6 (2.0)	0.11
Patient's global assessment of disease activity (0–10)	3.7 (2.6)	3.4 (2.7)	4.0 (2.6)	0.19
Spinal pain (0–10)	3.5 (2.3)	3.1 (2.5)	3.7 (2.2)	0.17
ESR (mm/h)	14.4 (14.6)	12.0 (12.0)	15.7 (15.8)	0.07
CRP (mg/L)	13.7 (20.2)	8.1 (9.2)	16.7 (23.6)	0.03
C1M (nmol/L)	59.2 (38.2)	48.0 (21.9)	65.4 (43.5)	0.04
C5M (nmol/L)	5.6 (3.9)	5.3 (1.8)	5.7 (4.7)	0.76
C6M (nmol/L)	10.6 (19.7)	11.5 (29.2)	10.1 (7.5)	0.54
VICM (nmol/L)	11.7 (16.6)	9.6 (7.8)	12.8 (19.8)	0.86
mSASSS (0–72)	13.9 (17.6)	11.6 (20.1)	15.1 (16.2)	0.002
mSASSS>0	106 (87%)	32 (74%)	74 (94%)	0.003

Baseline refers to the baseline measurement of this study, when the biomarkers were assessed, which does not necessarily correspond to the baseline of the Outcome in Ankylosing Spondylitis International Study cohort.
ASDAS-CRP, ankylosing spondylitis disease activity score-C-reactive protein; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; C1M, C5M and C6M, MMP-mediated degradation of type I, V and VI collagen, respectively; ESR, erythrocyte sedimentation rate; HLA, human leucocyte antigen; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; MMP, matrix metalloproteinase; NSAIDs, non-steroidal anti-inflammatory drugs; TNF-a antitumour necrosis factor; VICM, citrullinated and MMP-degraded vimentin.

baseline mSASSS and any of the soluble markers, indicating that none of the markers was associated with the level of structural damage.

A statistically significant but weak correlation between baseline C1M and 2-year mSASSS change (Spearman's ρ 0.22) was found (table 2). CRP was weakly correlated

Table 2 Association between biomarkers, radiographic damage and change and CRP

		C1M	C5M	C6M	VICM	CRP
CRP	ρ	0.75	−0.01	0.73	0.37	
	p	<0.0001	ns	<0.0001	<0.0001	
	N	120	114	118	120	
Baseline mSASSS	ρ	0.10	−0.07	−0.09	0.02	−0.02
	p	ns	ns	ns	ns	ns
	N	122	116	120	122	120
2 year mSASSS change	ρ	0.22	−0.07	0.08	−0.04	0.22
	p	0.013	ns	ns	ns	0.017
	N	122	116	120	122	120

Values are Spearman' s correlation coefficients.
CRP, C-reactive protein; C1M, C5M and C6M, MMP-mediated degradation of type I, V and VI collagen, respectively; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; MMP, matrix metalloproteinase; VICM, citrullinated and MMP-degraded vimentin, NS, non-significant

Table 3 Relationship between each of the biomarkers (C1M, C5M, C6M and VICM) and 2-year mSASSS change (unadjusted and adjusted models)

	C1M	C5M	C6M	VICM
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Unadjusted	0.01 (0.00 to 0.03)	−0.00 (-0.12 to 0.11)	−0.00 (-0.03 to 0.02)	−0.00 (-0.03 to 0.02)
Adjusted for age, gender and symptom duration	0.02 (-0.00 to 0.04)	−0.02 (-0.15 to 0.10)	−0.00 (-0.03 to 0.03)	−0.00 (-0.03 to 0.03)
Adjusted for age, gender, symptom duration and baseline mSASSS	0.02 (-0.00 to 0.03)	−0.01 (-0.14 to 0.11)	−0.00 (-0.03 to 0.03)	0.00 (-0.03 to 0.03)
Adjusted for age, gender, symptom duration, mSASSS and CRP	0.00 (-0.03 to 0.03)	−0.00 (-0.12 to 0.12)	−0.01 (-0.04 to 0.02)	−0.03 (-0.06 to 0.00)
Adjusted for age, gender, symptom duration, mSASSS and ASDAS	0.02 (-0.01 to 0.04)	−0.01 (-0.14 to 0.11)	−0.00 (-0.03 to 0.02)	−0.00 (-0.03 to 0.03)

ASDAS, Ankylosing Spondylitis Disease Activity Index; C1M, C5M and C6M, MMP-mediated degradation of type I, V and VI collagen; respectively; CRP, C-reactive protein; mSASSS, modified Stoke Ankylosing Spondylitis Spine; MMP, matrix metalloproteinase; VICM, citrullinated and MMP-degraded vimentin.

with 2-year radiographic progression (Spearman's ρ 0.22). There were no associations between C5M, C6M and VICM with 2-year mSASSS progression (table 2).

Relationship between biomarkers and radiographic progression

In univariable analysis, baseline C1M was significantly associated with 2-year mSASSS progression, but with a low effect size (β=0.01, 95% CI 0.00 to 0.03; table 3). This effect just lost its significance when adjusted for confounders, namely, age, gender, symptom duration and baseline mSASSS. When further adjusting for CRP or ASDAS, no effect was found. Similar results were obtained, when investigating 2-year mSASSS >2, 2-year mSASSS >5, as well as mSASSS progression over time, taking all follow-up radiographs into account (data not shown).

DISCUSSION

R-axSpA is an inflammatory disease associated with extensive remodelling of ECM proteins. At the group level, it is a disease characterised by slow spinal radiographic progressing, but some patients show a rapid progression, and it is particularly important to identify them early. In this study, we quantified by serology MMP-derived fragments of type I, V and VI collagen (C1M, C5M and C6M, respectively) and MMP-degraded and citrullinated vimentin (VICM) in order to investigate their relationship with 2-year spinal radiographic progression in patients with r-axSpA. We found signals of a weak, but statistically significant relationship between C1M and 2-year radiographic progression. However, the effect size of this relationship was minimal and disappeared after appropriate adjustments. The remaining biomarkers were not associated with radiographic progression. This means that in the investigated cohort, the presently measured biomarkers do not serve as predictors of radiographic progression.

What does a weak statistically significant relationship between C1M and the 2-year radiographic progression mean? The results show that per one unit increase in C1M, there is an increase of 0.01 in mSASSS. This means that a difference in C1M at baseline of 50 nM nmol/L is equal to a difference in 2-year mSASSS progression of 0.5 mSASSS units. At a group level, we expect a progression of 2 mSASSS units per 2 years in patients with r-axSpA.[17] Although differences of 50 nmol/L in C1M levels between patients are possible and have been seen in previous studies,[4 21] according to the present results, where the mean value of C1M at baseline was 59.2 nmol/L and with an SD of 38.3 and the level of C1M in progressors was significantly higher compared with non-progressors with a mean difference of 17 nmol/L, indicating that a change of 50 nmol/L is not reasonable in the current study.[4] Moreover, a change in mSASSS of 0.5 in 2 years (at the group level) is minimal and not considered a clinically significant change for such a large change in the biomarker level.

The levels of the other biomarkers assessed (C5M, C6M and VICM) were not significantly different in progressors and non-progressors. VICM was expected to be associated with radiographic progression, as this biomarker is a measure of macrophage activity, and citrullinations are known to be increased in r-axSpA.[22] However, we found no association between this biomarker and disease progression. This could be due to the quite long disease duration of the patients in the investigated cohort. It could be that macrophages are involved in the early disease progression or only associated with fast progression, whereas in the later stages of r-axSpA or with low disease progression, other processes are responsible for disease progression. In a previous study, we found that VICM was a potential biomarker of radiographic disease progression in r-axSpA, but this was not replicated in the current study.[23] The reason for the difference in

association between VICM and radiographic disease progression in the two studies is unknown, but may be attributed to lower VICM levels in the current study compared with the previous study (11.7 vs 16.4 mmol/L). Furthermore, different baseline disease activity levels could have had an additional influence. Moreover, in the current study, we found a high variance in the VICM and C5M levels in the progression group. This could indicate that subpopulations exist, which has been shown to be the case in both rheumatoid arthritis and osteoarthritis,[5 21 24 25] but this has still to be investigated for r-axSpA.

The limited sensitivity to change is a known limitation of the existing spinal radiographic scoring methods in r-axSpA, like the mSASSS. Low-dose CT is appearing as a potentially successful and more efficient alternative and raises the possibility of capturing more progression that can eventually, in future studies, be related to degradation of ECM.[26]

A limitation to this study is that the serum samples investigated have been stored for up to 15 years at −80°C. It is a limitation since the biomarkers investigated in this study assess protein fragments, and these protein fragments could be destroyed by long-time storage. It is not known whether the sample storage of >2 years affects the biomarkers levels, due to the novelty of the biomarkers. However, samples stored for 2 years at −80°C do not affect the biomarker levels (provided by manufacturer). The length of the storage could be the reason why the C1M level is lower than assessed previously in a population of patients with r-axSpA.[4]

CONCLUSION

In conclusion, this study did not find any evidence that degradation of ECM (C1M, C5M, C6M and VICM) is related to radiographic progression in patients with AS.

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Contributors All authors have planned and designed the biomarker study. ASS did the biomarker assessment. All authors were involved in the data analysis, scientific discussion and interpretation of the data. Additionally, all authors were involved with drafting the manuscript, approving the manuscript prior to submission and have agreed to be accountable for all published data.

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Competing interests MAK and A-CB-J are full-time employees and shareholder in Nordic Bioscience. ASS is full-time employee of Nordic Bioscience.

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