Metabolites of C-reactive protein and vimentin are associated with disease activity of axial spondyloarthritis

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Abstract

Objective

Non-radiographic (nr-axSpA) and radiographic (AS) forms of axial spondyloarthritis (axSpA) share clinical features, but have different radiographic patterns. Radiographic progression is not associated with the current disease activity biomarkers. We investigated a matrix metalloproteinase mediated metabolite of C-reactive protein (CRPM) and two biomarkers of citrullinated vimentin (VICM and anti-MCV) as novel biomarkers of disease activity.

Methods

AxSpA patients (n=121 nr-axSpA and n=72 AS) were characterised by activity (AS disease activity score with CRP [ASDAS-CRP], Bath AS disease activity index [BASDAI] and functional index [BASFI]), radiographic scores and quality of life questionnaires. CRPM, VICM and anti-MCV levels were analysed by ELISA in serum. Asymptomatic controls (n=100) were used as reference. Multiple regression investigated association with disease activity and diagnostic potential.

Results

CRPM and VICM levels were increased in AS compared to nr-axSpA (11.9nM vs. 10.2nM, $p<0.001$ and 4.92nM vs. 3.77nM, $p=0.0025$). Anti-MCV was similar in both axSpA subgroups, but lowered in controls. In nr-axSpA, CRPM correlated with CRP ($\varrho=0.33$, $p<0.001$) and VICM ($\varrho=0.29$, $p=0.001$); in AS, VICM correlated with CRP ($\varrho=0.34$, $p=0.0032$) and ESR ($\varrho=0.38$, $p<0.001$). ASDAS-CRP correlated with CRPM and anti-MCV, but when adjusting for CRP the correlation only remained with CRPM. CRPM and VICM separated the subgroups with odds ratios of 1.19 and 1.10 adjusted for age, gender, BMI, and disease duration. VICM lost significance when adjusting for CRP.

Conclusion

CRPM was associated with disease activity in axSpA, and CRPM and VICM separated the axSpA groups. This study indicates that serological biomarkers may be novel biomarkers in axSpA.

Key words

biomarkers, axial spondyloarthritis, ankylosing spondylitis, inflammation, vimentin

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Introduction

Axial spondyloarthritis (axSpA) is a chronic inflammatory disorder with various musculoskeletal manifestations, such as inflammatory back pain, arthritis, and enthesitis (1). Clinical symptoms, HLA B27 status, and response to pharmacological therapy are represented similarly in both axSpA forms (2-4). Patients with the non-radiographic form of axSpA (nr-axSpA) have inflammatory changes of the sacroiliac joints (SIJ) and/or HLA B27 positivity with other clinical axSpA signs (1). The radiographic form (AS) has definite radiographic features. Nr-axSpA compared to AS patients are usually women with lower levels of inflammatory markers, such as C-reactive protein (CRP) (3, 4).

Studies of registries with early axSpA cohorts have demonstrated that after the first two years 12% and, after 15 years 26% of nr-axSpA patients have developed AS (5, 6). These radiographic changes included bone changes on SIJ according to the New York criteria for AS (7). Suggested risk factors for radiographic progression are male gender (5), smoking, HLA B27 status (8), and initial inflammatory activity locally on SIJ or systemically assessed by biomarkers (such as elevated CRP or erythrocyte sedimentation rate (ESR)) (5, 8). The systemic inflammatory biomarkers may reflect the pathogenic processes of axSpA and are an important component for the axSpA diagnosis, evaluation of disease activity and prognosis. However, several pathologies influence the variability of CRP or ESR levels, and only up to half of AS patients with a higher disease activity have elevated CRP (4, 9). A study found that CRP moderately reflected the improvement of spinal and SIJ inflammation after tumour necrosis factor inhibitors (TNFi) therapy (10), indicating CRP as a useful biomarker of treatment efficacy in SpA.

A microenvironment that may promote inflammation is present at sites of axSpA enthesitis and/or synovitis of spinal, SIJ, and peripheral joints. Proteases, such as matrix metalloproteinases (MMPs), support cell proliferation, differentiation and apoptosis and are involved in the turnover of extracellular matrix (ECM). The expression of MMP-3 and -9 and MMPs regulators, tissue specific MMP inhibitors (TIMPs) 1 and 2 in particular, has been found to correlate with the inflammatory cell infiltration, vascularity and cartilage breakdown in the inflamed SpA joints (11). Moreover, the serum levels of MMP-3, -8, and -9 were shown to reflect the increased disease activity and structural progression in AS (12, 13); thus MMPs are associated with axSpA. MMPs degrade the ECM and protein metabolites released into circulation may serve as pathologically relevant biomarkers. The MMPs mediated metabolite of CRP (CRPM) is suggested as a local tissue inflammation biomarker (14) and seems to be more specific for AS than full-length CRP (15). The MMP derived metabolite of citrullinated vimentin (VICM) have been associated with disease activity and radiographic progression in AS (16). Vimentin is a type III intermediate filament protein involved in cell integrity, migration and signalling, but it is secreted to the extracellular space by activated macrophages (2, 17). In a cellular manner, vimentin is prone to citrulline modification, leading to a loss of protein functions and subsequent apoptosis (18).

As described nr-axSpA in the early stages similar manifested clinically to AS, but the systemic inflammatory level is usually lower. However, local inflammation may be present in early axSpA. In this study, we investigated serum biomarkers (CRPM, VICM and anti-MCV) and their association with disease activity and severity in recent onset axSpA.

Materials and methods

Patients

Recently diagnosed axSpA patients (n=193) were included in the Prague Axial SPondyloArthritis Cohort (PRASPAC). The inclusion criteria were recent or a maximum of a three-year diagnosis of axSpA and relevant information about the data and characteristics of the first symptoms and disease course of axSpA.

All patients were characterised by the following clinical assessment: personal and family history, current and previous

therapy, smoking history, body mass index (BMI), evaluation of peripheral enthesis involvement by Maastrich Enthesitis Score (MASES)(1), disease activity according AS-disease activity score (ASDAS-CRP) (19), Bath AS disease activity index (BASDAI) (20), and function according to Bath AS functional index (BASFI) (21). All patients were examined with x-ray of the SIJ and spine; x-rays were evaluated by two independent radiologists and one rheumatologist trained for evaluation of x-rays in axSpA. Patients with radiographic sacroiliitis according to the mNYc (7) were characterised as having AS (n=72). In the case of negative findings of SIJ on x-ray, magnetic resonance (MRI) of SIJ was performed and independently analysed according the Assessment of SpondyloArthritis International Society (ASAS) (1) by one radiologist and one rheumatologist with training for MRI assessment in axSpA. Patients without radiographic changes on the SIJ, who fulfilled the ASAS criteria (1), were classified as nr-axSpA (n=121). The severity of MRI or x-rays findings on the SIJ or spine was determined by following radiologic scoring systems, Spondyloarthritis Research Consortium of Canada (SPARCC) and Berlin MRI grading system (22, 23) or modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) (24). The patient-reported outcomes, such as quality of life (AS Quality of Life (ASQoL) and European Quality of Life (EQ-5D)) (25, 26) were included. All patients had information about CRP and ESR. The fasting blood samples (serum) for biomarker evaluation were collected from all patients and stored at -70°C until assayed.

One-hundred age- and sex-matched asymptomatic individuals without any autoimmune or other inflammatory disorders or current infection or surgery were used as a control group for biomarker analyses.

All patients signed the informed consent form to be included into this clinical and laboratory database. The inform consent and study design with the database were approved by the local Ethical Committee and Scientific Board of the Institute of Rheumatology in Prague and carried out in accordance with the principles of the Declaration Helsinki.

Biomarker assessments

A panel of protein fingerprint biomarkers was measured in fasting serum by validated competitive ELISAs. The serological levels of the MMP-mediated CRP metabolite (CRPM) (15), and citrullinated and MMP-degraded vimentin (VICM)(27) were assessed in accordance with manufacture instructions.

A commercially available ELISA containing mutated citrulinated vimentin (MCV) as antigen was used for the IgG anti-MCV autoantibodies analysis (OrgenTec Diagnostica GmbH, Mainz, Germany) (3). The test was performed according to the manufacturer's instructions; the cut-off value was determined to be 20 U/ml.

Statistics

All graphical illustrations were performed using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. All data analysis was performed in MedCalc, MedCalc Statistical Software v. 17.5 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017).

Data is presented as mean + 95% CI if not other vice stated. The Mann-Whitney U-test tested for differences in biomarker levels and clinical features between AS, nr-axSpA, and healthy as the biomarker data was not normally distributed. Spearman's ranked correlation investigated correlations between clinical biomarkers and clinical features, as the biomarker data were not normally distributed and some data was non-continuous. Fisher's exact test investigated the difference in the positivity level of anti-MCV between the groups. Multiple regression analysis investigated correlations between biomarkers and clinical features with adjustment for age, gender, BMI, and disease duration. Logistic regression tested if the biomarkers had the capability to separate radiographic axSpA (cases) from non-radiographic axSpA (controls) with adjustment for the age, gender, BMI, disease duration, and CRP.

Results

Demographic description

AS patients (n=72) had a mean age of 34.5 years; 32% were female, they had a BMI of 24.4 and 83% were HLA-B27 positive. In nr-axSpA patients (n=121) the mean age was 37.5 years, 60% were female, they had a BMI of 25.1 and 93% were HLA-B27 positive (Table I). The mean duration since the first symptom was 10.0 years in AS and significantly shorter in nr-axSpA (7.9 years, $p<0.001$). The disease activity was high in AS with a mean ASDAS-CRP of 2.21, but moderate in nr-axSpA with a mean ASDAS-CRP of 1.98. AS patients had an increased CRP of 7.7 mg/L compared to healthy reference level (<5), while the nr-axSpA patients did not have increased CRP (2.6 mg/L). BASDAI was 2.6 in AS and 3.0 in nr-axSpA. BASFI was 1.3 in AS and 1.0 in nr-axSpA. The ASQoL and EQ-5D were 5.7 and 0.66 in AS, and 5.3 and 0.66 in nr-axSpA. The swollen joint count was 0.17 in AS and 0.43 in nr-axSpA. AS patients had significantly increased MRI scores (mSASSS, SPARCC MRI and Berlin MRI) compared to patients with nr-AxSpA, but this was due to the sub-grouping criteria.

Serological biomarker assessment

Compared to asymptomatic controls, the level of CRPM was significantly higher in nr-axSpA and AS ($p\leq0.001$; Fig. 1). The level of CRPM was significantly increased in AS compared to nr-axSpA patients ($p<0.001$).

The level of VICM was significantly lower in nr-axSpA (3.77 [3.34–4.28]) compared to asymptomatic controls (5.31 [4.48–6.07]; $p<0.01$). VICM was not different in AS patients compared to asymptomatic controls. The VICM level was significantly lower in nr-axSpA compared to AS ($p<0.0025$).

The mean levels of anti-MCV autoantibodies were significantly higher in nr-axSpA (9.2 [8.2–10.4]; $p<0.01$) and AS patients (9.1 [8.0–10.8]; $p<0.001$) compared to asymptomatic controls (7.2 [6.1–8.3]). There were no differences between AS and nr-axSpA in anti-MCV. Positivity of anti-MCV autoantibodies was detected in 14% of patients with AS, 10% of nr-axSpA, and

Table I. The variation in the demographics in AS, nr-axSpA and controls.

	AS		nr-axSpA		Controls		AS *vs.* nr-axSpA
	Mean	95% CI	Mean	95% CI	Mean	95% CI	*p*-value
n	72		121		100		
Age at diagnosis (years)	34.5	32.3- 36.8	37.5	35.5-39.6	37.0	35.4-39.6	ns
Gender (% female)	32*		60		51		0.0003
Duration (years) since first symptom	10.0	7.9- 12.1	7.9	6.3-9.5	-	-	0.0009
BMI	24.4	23.5-25.4	25.1	24.2-26.0	-	-	ns
ASDAS-CRP	2.21	1.98- 2.44	1.98	1.79-2.16	-	-	ns
ASQoL	5.7	4.5- 6.8	5.3	4.4-6.2	-	-	ns
BASDAI	2.6	2.1- 3.0	3.0	2.6-3.4	-	-	ns
BASFI†	1.30	0.99- 1.92	1.00	0.72-1.40	-	-	ns
EQ-5D	0.66	0.60-0.72	0.66	0.61-0.71	-	-	ns
HLA-B27 (% positive)	83		93		-	-	ns
MASES†	0.0	0.0-0.0	0.0	0.0-1.0	-	-	ns
SJC	0.17	-0.014- 0.35	0.43	0.24-0.62	-	-	0.0093
Berlin MRI†	4.5	2.4-7.0	2.0	1.0-2.0	-	-	0.0028
mSASSS	4.83	1.47-8.19	0.74	0.17-1.31	-	-	0.018
SPARCC MRI	14.71	10.17-19.26	6.82	5.17-8.47	-	-	0.0030

Statistical analysis: Mann-Whitney U-test evaluated the differences between the disease groups as well as between the disease groups and asymptomatic controls. *Statistical significance between the patient group and asymptomatic controls. †Median + 95% CI, as the parameter was not normally distributed. AS: ankylosing Spondylitis, nr-axSpA: non-radiographic axial spondyloarthritis, BMI: body mass index. ASDAS-CRP: ankylosing spondylitis disease activity score with C-reactive protein. ASQoL: Ankylosing spondylitis quality of life. BASDAI: Bath ankylosing spondylitis disease activity index. BASFI: Bath ankylosing spondylitis functional index, EQ-5D: Euro quality of life questionnaire, SJC: Swollen joint count. MRI: magnetic resonance imagining, mSASSS: Modified stoke ankylosing spondylitis spinal score. SPARCC MRI: Spondyloarthritis Research Consortium of Canada MRI scoring system, ns: not significant.

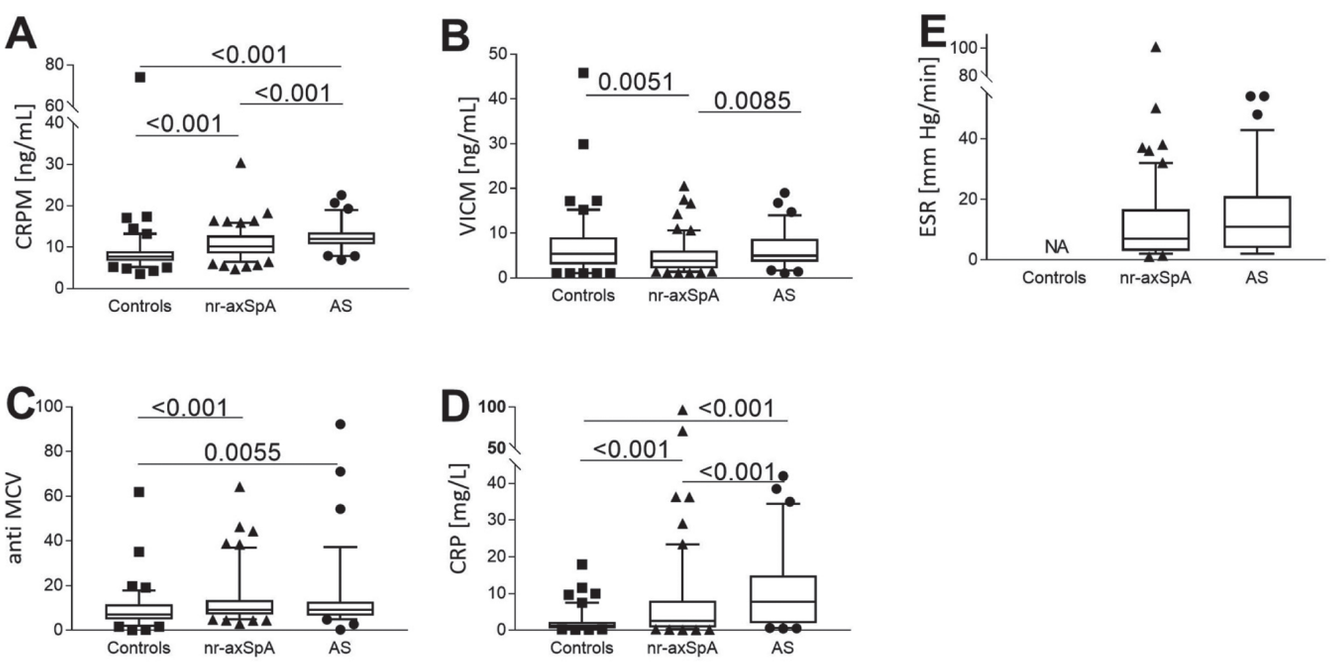

Fig. 1. Biomarker levels in the AS, nr-axSpA group and asymptomatic controls.
A: Tissue inflammation - MMP-degraded C-reactive protein, **B**: Macrophage activation - Citrullinated and MMP-degraded vimentin; **C**: Anti-mutated citrullinated vimentin; **D**: Systemic inflammation - C-reactive protein; **E**: Systemic inflammation - Erythrocyte sedimentation rate.
Statistical analysis: the Kruskal-Wallis test was used to test for differences between the three groups with Dunn's multi comparisons test. Data are box and whiskers plots. AS: ankylosing spondylitis; nr-axSpa: non-radiographic axial spondyloarthritis; MMP- metalloproteinase, CRP: C-reactive protein; CRPM: metalloproteinase degraded C-reactive protein; VICM: metalloproteinase degraded vimentin; EST: erythrocyte sedimentaion rate; anti-MCV: autoantibodies against modified citrullinated vimentin; NA: not available.

2% of asymptomatic controls. There were significantly more patients with positive anti-MCV in AS and nr-axSpA compared to asymptomatic controls (p=0.0045 and p=0.023, respectively), but there was no difference between AS and nr-axSpA (p=0.49). Only ESR was significantly different between AS patients with positive anti-MCV compared to AS patients with negative anti-MCV (p=0.012; data not shown). There was no difference in the ESR level between subgroups and asymptomatic controls, but CRP was significantly higher in AS compared to nr-axSpA

Table II. Spearman's correlation between serological biomarkers and clinical assessments.

		CRPM		VICM		Anti-MCV		CRP		ESR	
		AS	nr-axSpA	AS	nr-axSpA	AS	nr-axSpA	AS	nr-axSpA	AS	nr-axSpA
Anti-MCV	ϱ P	-	-	0.27 0.023	-	-	-	0.27 0.023	-	**0.37** **0.0014**	**0.32** **<0.001**
CRP	ϱ P	-	**0.33** **<0.001**	-	-	0.27 0.023	-	-	-	**0.64** **<0.001**	**0.68** **<0.001**
CRPM	ϱ P	-	-	-	-	-	-	-	-	**0.39** **<0.001**	**0.27** **0.0033**
VICM	ϱ P	-	-	-	-	-	0.19 0.045	-	-	**0.38** **<0.001**	-
Age at diagnosis (years)	ϱ P	-	-	-	-	0.26 0.027	-	-	-	0.29 0.014	0.19 0.038
ASDAS-CRP	ϱ P	0.32 0.0075	-	**0.70** **<0.001**	**0.64** **<0.001**	-	-	**0.70** **<0.001**	**0.64** **<0.001**	**0.54** **<0.001**	**0.48** **<0.001**
ASQoL	ϱ P	-	-	0.26 0.030	-	-	-	0.26 0.030	-	**0.35** **0.0026**	-
BASDAI	ϱ P	0.25 0.034	-	-	-	-	-	-	-	-	0.22 0.017
BASFI	ϱ P	**0.37** **0.0018**	-	**0.35** **0.0023**	-	-	-	**0.35** **0.0023**	-	**0.37** **0.0014**	0.20 0.033
Berlin MRI	ϱ P	-	-	0.40 0.036	-	-	-	0.40 0.036	-	-	-
Dis.dur	ϱ P	-	-	-	-	-	-	-	-	-	-
EQ-5D	ϱ P	**-0.36** **0.0024**	-	-0.26 0.025	-	-	-	-0.26 0.025	-	-0.27 0.023	-
mSASSS	ϱ P	-	-	0.28 0.029	-	-	-	0.28 0.029	-	-	-
MASES	ϱ P	-	-	-	-	-	0.23 0.011	-	-	0.31 0.0081	-
SJC	ϱ P	-	-	-	0.25 0.0063	-	-	-	0.25 0.0063	-	0.22 0.014
SPARCC MRI	ϱ P	-	-	0.41 0.031	-	-	-			-	-

Statistical analysis: Spearman's ranked correlation. Bold indicates correlations that were significant after Bonferroni correction with an alpha value of 0.005 (0.05/10). Non-bold indicates correlations that were significant ($p<0.05$) and that did not remain significant after Bonferroni correction.
BMI: body mass index; CRPM: metalloproteinase degraded CRP; VICM: metalloproteinase degraded citrullinated vimentin; anti-MCV: autoantibodies against modified citrullinated vimentin; CRP: C- reactive protein; ESR: erythrocyte sedimentation rate; ASDAS-CRP: ankylosing spondylitis disease activity score with C-reactive protein; ASQoL: ankylosing spondylitis quality of life; BASDAI: Bath ankylosing spondylitis disease activity index; BASFI: Bath ankylosing spondylitis functional index; Dis.dur: disease duration; MRI: magnetic resonance imagining; EQ-5D: Euro Quality of life; SJC: Swollen joint count; mSASSS: Modified Stoke Ankylosing Spondylitis spinal score; SPARCC MRI: Spondyloarthritis Research Consortium of Canada MRI scoring system.

(7.72 [4.37–11.99] *vs*. 2.56 [1.65–3-97]; $p<0.001$).

Association between biomarkers and clinical assessments

In nr-axSpA, CRPM correlated with CRP and VICM (Table II). The level of VICM correlated to CRP and ESR in AS. ESR correlated to CRP, CRPM, and anti-MCV in both AS and nr-axSpA. There was a trend for a positive relationship between anti-MCV and VICM in nr-axSpA and anti-MCV and CRP in AS, but it lost significance after adjustments.

Although the clinical variables of disease activity (ASDAS-CRP and BASDAI), function (BASFI), and quality of life (ASQoL and EQ-5D) were similar in AS and nr-axSpA (Table I), there were variations in the correlation of disease activity between the axSpA subgroups and all assessed biomarkers. In AS, there was a positive correlation between the BASFI and CRP, CRPM, and ESR (ϱ=0.35, ϱ=0.37, and ϱ=0.37, respectively) and negative relationship between EQ-5D and CRPM (ϱ= -0.36)- This was not the case in nr-axSpA. Not surprisingly, both CRP and ESR correlated positively with ASDAS-CRP in AS and nr-axSpA (Spearman's ϱ≥0.48). In addition, there were no correlations with the age, disease duration, BASDAI, Berlin MRI, mSASSS, SJC, and SPARCC MRI with any of the

Table III. Biomarker association with disease activity by ASDAS-CRP.

	AS						nr-axSpA					
	CRPM		VICM		Anti-MCV		CRPM		VICM		Anti-MCV	
	Beta (SD)	r-partial	Beta (SD)	r-partial	Beta (SD)	r-partial	Beta (SD)	r-partial	Beta (SD)	r-partial	Beta (SD)	r-partial
Unadjusted	0.08 (0.04)	0.27*	0.05 (0.03)	0.22	0.02 (0.01)	0.26*	0.06 (0.03)	0.19*	0.03 (0.03)	0.10	0.02 (0.01)	0.18*
Adjusted for age, gender, BMI, dis.dur	0.09 (0.04)	0.27*	0.06 (0.03)	0.22	0.02 (0.01)	0.25*	0.06 (0.03)	0.19*	0.04 (0.03)	0.12	0.02 (0.01)	0.18

Statistical analysis: multiple regression analysis. Beta (SD) and r-partial are provided. Statistical significance was considered when alpha <0.05 and is assigned *p<0.05.
AS: ankylosing spondylitis; nr-axSpA: non-radiographic axial spondyloarthritis; BMI: body mass index; Dis.dur: disease duration; CRPM: metalloproteinase degraded CRP; VICM: metalloproteinase degraded citrullinated vimentin; anti-MCV: autoantibodies against modified citrullinated vimentin; ASDAS-CRP: ankylosing spondylitis disease activity score.

tested biomarkers in both axSpA subgroups.

Relationship between the biomarkers and disease activity (ASDAS-CRP)

In the unadjusted models, VICM was not associated with ASDAS-CRP, while CRPM and anti-MCV were (Table III). This differed from the univariate analysis (Spearman's ranked correlation), where CRPM and anti-MCV were not correlated with ASDAS-CRP. When biomarkers were adjusted for the age, gender, BMI, and disease duration, the association with ASDAS-CRP remained at the same level as without the adjustment in both groups of patients. However, the statistical significance was lost for anti-MCV in nr-axSpA. However, for these analyses, the effect-size (beta) was minimal (<0.1); therefore, the results may not be clinically relevant.

The biomarkers, VICM and CRPM, may differentiate between nr-axSpA and AS

We then investigated if the serological biomarkers could separate patients into the two disease subgroups (AS vs. nr-axSpA) by logistic regression of biomarkers with adjustments for the age, gender, BMI, disease duration, and CRP (Table IV). CRPM had a statistically significant, but minimal clinically significant, odds ratio for separating AS and nr-axSpA patients. Even after adjustment, the odds ratio remained significant (≥1.19). VICM also had a significant odds ratio (>1.10) for separating patients into axSpA groups, even after adjusting for the age, gender, BMI, and disease duration. However, the association was lost when adjusting for CRP. The ability of CRP and anti-MCV to separate patients into disease groups was also tested, but no significant odds ratio was found with or without adjustment for the age, gender, BMI, disease duration, and CRP.

Table IV. Biomarker capability of segregating axSpA disease groups .

		Unadjusted	Adjusted for age, gender, BMI, dis.dur	Adjusted for age, gender, BMI, dis.dur, CRP
CRPM	Beta (SD)	**0.18 (0.05)**	**0.18 (0.06)**	**0.17 (0.06)**
	p-value	**0.0006**	**0.0013**	**0.0033**
	Odds (95% CI)	**1.19 (1.09-1.32)**	**1.20 (1.08-1.34)**	**1.19 (1.06-1.33)**
VICM	Beta (SD)	**0.11 (0.04)**	**0.09 (0.05)**	0.07 (0.05)
	p-value	**0.0063**	**0.047**	0.13
	Odds (95% CI)	**1.12 (1.03-1.22)**	**1.10 (1.00-1.20)**	1.08 (0.98-1.18)
Anti-MCV	Beta (SD)	0.01 (0.01)	0.02 (0.01)	0.02 (0.01)
	p-value	0.62	0.17	0.26
	Odds (95% CI)	1.01 (0.98-1.03)	1.02 (0.99-1.05)	1.01 (0.99-1.05)
CRP	Beta (SD)	0.02 (0.01)	0.02 (0.01)	NA
	p-value	0.087	0.094	NA
	Odds (95% CI)	1.02 (1.00-1.05)	1.03 (1.00-1.06)	NA

Statistic analysis: Logistic regression. Beta (SD), *p*-value and odds ratio are provided and bold indicates a significant association (p<0.05) of the biomarker to separate disease subgroups (AS *vs*. nr-axSpA).
BMI: body mass index; CRP: C-reactive protein; Dis.dur: disease duration; NA: not applicable; CRPM: metalloproteinase degraded CRP; VICM: metalloproteinase degraded citrullinated vimentin; anti-MCV: autoantibodies against modified citrullinated vimentin; ASDAS-CRP: ankylosing spondylitis disease activity score.

Discussion

Our study demonstrates, for the first time, a variation in the serological levels of VICM, CRPM, and anti-MCV autoantibodies in nr-axSpA and AS. Furthermore, CRPM was associated with disease activity and could discriminate AS from nr-axSpA. This suggests that CRPM, VICM, and anti-MCV are associated with both axSpA forms, while only CRPM are associated with ASDAS-CRP and may discriminate between AS and nr-axSpA.

CRPM is a biomarker detecting a CRP metabolite produced by MMP activity in the microenvironment of tissues. Therefore CRPM may reflect local inflammatory activity (14, 15). MMP-1 and -8 was found to be responsible for cleaving CRP to CRPM during the validation of CRPM ELISA (28). Four MMPs (-1, -3, -8 and -9) are associated with axSpA (11-13), while both MMP-8 and -9 strongly reflect the disease activity (12). CRP, on the other hand, is a biomarker of systemic inflammation, as several simple pathologies such as a common cold induce an increased

CRP level. The CRP levels do not follow axSpA disease pathogenesis as a recent study reported that neither CRP nor ESR was higher in early axSpA patients compared to patients with lower pack pain but no axSpA diagnosis (29). However, in AS patients with a longer disease duration, the CRP level was increased compared to healthy controls, albeit to a lower extent than in patients with rheumatoid arthritis (RA) (16, 30, 31). In agreement, we found increased CRP levels in AS patients compared to nr-axSpA patients and the level in nr-axSpA patients was within the normal range. In contrast, we found elevated CRPM levels in axSpA (both AS and nr-axSpA) compared to asymptomatic controls, but the levels were higher in AS patients than in nr-axSpA patients. Therefore, our results indicate that CRPM may serve as a biomarker, reflecting the axSpA related inflammation better than full-length CRP (14, 15, 30). Previously elevated CRPM levels was found to some extend estimate radiographic progression in osteoarthritis (32). Furthermore, the potential for CRPM to be a biomarker of inflammatory joint disorders has been observed in RA (33), where its non-decreasing value predicted poor response to tocilizumab treatment (34). This RA study found the mean CRPM level in RA patients to be 14.6 ng/mL, which is higher than the mean in the current study of axSpA patients, indicating the level of CRPM to be slightly lower in axSpA compared to RA (35). Together the studies indicate that CRPM may be a tissue inflammation biomarker in several rheumatic diseases.

The complex disease activity scoring systems in axSpA, such as ASDAS-CRP, are intended as useful tools for disease activity assessments in both axSpA forms (36). Although several biomarkers, such as calprotectin, have been suggested for response to therapy in AS, this type of biomarkers reflecting the disease activity are still missing (37). Our results indicate the potential of CRPM as a new sensitive, serological biomarker for monitoring disease activity in axSpA patients. This finding is important in light of the recent study by Baraliakos *et al*. (38) in which nr-axSpA patients with clinically active disease, but without objective inflammatory signs, such as elevated CRP or inflammation on MRI SIJ, developed AS.

The definition of nr-axSpA and AS is the difference in radiographic status, but previous clinical data suggest a similar disease activity level and clinical manifestations, such as fatigue and extra-articular manifestations, in nr-axSpA and AS (2, 4). In the current study, there were no available data from MRI of the whole spine in AS and nr-axSpA, but there were data of mSASSS and SPARCC. Althoff *et al*. (39) showed that the active inflammatory lesions within the SIJ, spine, and non-axial locations are more common in fully advanced AS than in nr-axSpA when evaluating whole-body MRI (39). In the present study, we found no association between mSASSS or MRI indexes of SIJ involvement and CRPM levels. However, the variation in the mSASSS and MRI indexes was small in the current study; therefore, a future study with a larger spread in the mSASSS and MRI indexes should further investigate the CRPM association with MRI evaluation of joint inflammation.

In contrast to a previous study by Bay-Jensen *et al*. (16), we did not find the increased serum levels of VICM in AS patients compared to asymptomatic controls. Furthermore, the mean VICM levels in the current AS group were lower (6.3 nmol/l) than in the previous study (16.4 nmol/l). The levels are furthermore lower than what was previously found in an RA study (34). The differences in biomarker level may be due to different clinical characteristics of AS patients, as the disease duration of the current cohort was shorter and BASDAI, CRP, ESR, and mSASSS were lower. Therefore, the overall activity of the diseased population in the current study is lower, which indicates a lower inflammatory state, which would be reflected in both VICM and CRPM. Furthermore, nr-axSpA patients had lower levels of VICM as well as mSASSS, CRP, and ESR compared to AS. As the nr-axSpA patients had similar disease activity as AS patients, our observation suggests VICM as a biomarker for axSpA patients with radiographic disease. The serological VICM level was previously found to correlate with the mSASSS, disease duration, and both CRP and ESR in AS patients (16). Furthermore, the VICM levels were similar in early RA patients and patients with undifferentiated arthritis (33). It has also been shown that VICM was not associated with the erosive course of the rheumatic disease after two years (33). We did not find a correlation with either mSASSS or disease duration in the current study, which could be attributed to the difference in the assessed level of these compared to the previous report.

The role of B cells in the immunepathological background of axSpA has not been clearly elucidated. However, an elevated proportion of plasma cells and several autoantibodies have been found to be present in AS and to be associated with disease onset or activity (40). One of these antibodies could be reacting against the antigen detected by the VICM biomarker, but this remains to be investigated. Bodnar *et al*. (41) found that anti-MCV autoantibodies could be identified in AS and that they correlated with ESR, but not disease activity. In addition, there were no differences in the presence of these autoantibodies in peripheral, axial, or extra-articular manifestations. In our study, the anti-MCV autoantibodies were higher in both nr-axSpA and AS than in asymptomatic controls. As in a previous study, we found a correlation between ESR and anti-MCV in AS and nr-axSpA (41). However, we did not find a correlation between VICM and anti-MCV with disease activity or radiographic findings. When we investigated the relationships between the VICM levels and anti-MCV autoantibodies, there were only trends for correlation in the nr-axSpA group, but there were no correlations in AS and asymptomatic controls. This raises the question about the ability of the immune system of axSpA react to citrullinated peptides. Beltrami *et al*. (42) demonstrated the capacity of two HLA B27 subtypes, HLA-B*2705 and *2709, to bind citrullinated peptides and present the peptide with distinct conformations, which may lead to aberrant immune response. The

true connection between citrullinated peptides and autoantibodies production in axSpA is still unknown. It is well known that autoantibodies against citrullinated peptides (ACPAs) and anti-MCV are associated with RA pathogenesis and diagnostic for RA, but they are poor prognosis biomarkers for RA (43). Lifestyle factors, and smoking in particular, are known to produce ACPAs. In axSpA, smoking is one of the factors associated with rapid radiographic progression (5). In our study, however, we did not find a relationship between smoking and anti-MCV production (data not shown).

Some limitations should be considered, when evaluating the results of this study. First, the AS patients had a longer disease duration since the first symptoms than their non-radiographic counterparts, although the criteria for including patients in our cohort included a diagnosis established fewer than 3 years prior to the study. Although this supports the efficacy of ASAS criteria for earlier axSpA diagnosis (1) and nr-axSpA may be accepted as the early phase of AS, our results should be evaluated better as differences in patients with the radiographic and non-radiographic forms. Another limit of our study is the lack of whole body MRI in AS and nr-axSpA to evaluate correlations between CRPM and local inflammation. Lastly, in the control group, only individuals without clinical signs of inflammatory disease were included, but they did not undergo clinical investigations, such as with x-ray.

Conclusion

In conclusion, our study demonstrates the potential of CRPM and VICM as useful biomarkers for both the radiographic and non-radiographic forms of axSpA. Although the CRPM and VICM levels may discriminate between the AS and nr-axSpA forms, only CRPM seems to be a prospective laboratory tool for the disease activity assessment for axSpA. Our results indicate that serological assessment of metabolites of pathological important proteins (CRP and vimentin), are novel biomarkers of disease activity and radiographic status in axSpA patients.

Key messages

- Our study demonstrates the potential of CRPM and VICM as useful biomarkers for both the radiographic and non-radiographic forms of axSpA.
- CRPM seems to be a prospective laboratory tool for the disease activity assessment for axSpA.
- Serological assessment of metabolites of pathological important proteins (CRP and vimentin), are novel biomarkers of disease activity and radiographic status in axSpA patients.

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