BMJ Open

Cross-sectional study of patients with axial spondyloarthritis fulfilling imaging arm of ASAS classification criteria: baseline clinical characteristics and subset differences in a single-centre cohort

Kristyna Bubová,[1] Šárka Forejtová,[1] Kateřina Zegzulková,[1] Monika Gregová,[1] Markéta Hušáková,[1] Mária Filková,[1] Jana Hořínková,[1] Jindřiška Gatterová,[1] Michal Tomčík,[1] Lenka Szczuková,[2] Karel Pavelka,[1] Ladislav Šenolt[1]

To cite: Bubová K, Forejtová Š, Zegzulková K, *et al*. Cross-sectional study of patients with axial spondyloarthritis fulfilling imaging arm of ASAS classification criteria: baseline clinical characteristics and subset differences in a single-centre cohort. *BMJ Open* 2019;**9**:e024713. doi:10.1136/bmjopen-2018-024713

Received 20 June 2018
Revised 1 December 2018
Accepted 22 January 2019

[1]Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine, Charles University, Prague, Czech Republic
[2]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Correspondence to
Ladislav Šenolt;
senolt@revma.cz

ABSTRACT

Objective This study compared demographic, clinical and laboratory characteristics between patients with radiographic and non-radiographic axial spondyloarthritis (axSpA).

Methods In this single-centre cross-sectional study, a total of 246 patients with axSpA fulfilling the imaging arm of Assessment of SpondyloArthritis International Society classification criteria were recruited. A total of 140 patients were diagnosed as non-radiographic axial spondyloarthritis (nr-axSpA), and 106 patients had ankylosing spondylitis (AS). Sociodemographic characteristics, disease manifestations, clinical and laboratory disease activity and their differences between subsets were analysed. P values below 0.05 with CI 95% were considered statistically significant.

Results More nr-axSpA patients were women (61.4%) compared with 24.7% of AS patients. First symptoms developed earlier in AS patients compared with nr-axSpA (23.0 (IQR 17.5–30.0) vs 27.8 (IQR 21.0–33.7) years, p=0.001). Disease manifestations did not differ, but patients with nr-axSpA experienced peripheral arthritis more frequently (35.7% vs 17.0%, p=0.001) with less hip involvement (8.6% vs 18.9%, p=0.022) compared with patients with AS. Patients with AS exhibited worse spinal mobility and physical function compared with nr-axSpA. AS Disease Activity Scores and CRP levels were significantly higher in patients with AS compared with nr-axSpA (2.4 (IQR 1.7–2.8) vs 2.0 (IQR 1.1–2.3), p=0.022 and 7.1 (IQR 2.6–14.9) vs 2.5 (IQR 0.8–8.2) mg/L, p<0.001, respectively).

Conclusions Our data demonstrated some known and also novel differences between the two imaging arm fulfilling axSpA subgroups. Non-radiographic patients were mostly women who had experienced shorter disease duration, milder disease activity and better functional status with less hip involvement but more peripheral arthritis compared with patients with AS.

Strengths and limitations of this study

- A strength of this study is the large sample size.
- This is the first study investigating the differences between axial spondyloarthritis patients in the Czech Republic.
- We included only patients fulfilling imaging arm of Assessment of SpondyloArthritis International Society classification criteria (ankylosing spondylitis and non-radiographic axial spondyloarthritis), patients fulfilling only clinical arm were not included.
- One of the limitations was that the MRI was performed in several imaging centres.

INTRODUCTION

Spondyloarthritis (SpA) is a frequent chronic inflammatory disease that primarily affects the axial skeleton and causes a typical lower back pain. SpA is a heterogeneous group of disorders that share common clinical features, including peripheral arthritis, enthesitis and extra-articular manifestations, such as uveitis, inflammatory bowel disease (IBD) or psoriasis.[1] SpA is divided into predominantly axial or predominantly peripheral disease based on the sites of inflammation.[2] Ankylosing spondylitis (AS) is a prototype of axial spondyloarthritis (axSpA). The prevalence of axSpA is approximately 0.7%–1.4% in the general population.[3] Patients generally develop signs of inflammatory back pain that correspond to sacroiliitis (or spondylitis) as detected by imaging. AS is a slowly progressive disease that is defined using modified New York classification criteria, in which conventional radiographs of the sacroiliac (SI) joints exhibit definite structural changes.[4]

Many patients develop similar axial symptoms but lack the typical changes on radiographs, which potentially causes delayed or missed diagnosis.[5] MRI is used to visualise the radio,graphic changes that typically occur several years after SI joint inflammation. MRI is also included in the new Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axSpA to enable the diagnosis of non-radiographic axSpA (nr-axSpA).[6] Nr-axSpA may be a prestage of AS; however, not all of these patients develop the destructive joint changes that are typical of long-standing disease. Only approximately 10%–20% of patients with nr-axSpA develop structural changes and AS over in the subsequent 2 years, and approximately half of the patients exhibit radiographic sacroiliitis after 5 years of the disease.[7]

Some recent studies investigated differences between these two subgroups.[8–10] These studies varied in male-to-female ratios, the proportion of patients with objective signs of inflammation (such as bone marrow oedema), and the proportion of patients with increased levels of C-reactive protein (CRP), all of which are higher in patients with AS.[8–10] Clinical characteristics such as disease activity, physical impairment and quality of life were comparable between these two subgroups.[11 12] However, some inconsistencies exist. Therefore, our study described the baseline demographic, clinical and laboratory characteristics of axSpA patients and examined differences between AS and nr-axSpA subgroups fulfilling the imaging arm of ASAS classification criteria.

Patients and methods

This study is a descriptive, single-centre, cross-sectional, ongoing study of the Prague Spondyloarthritis Cohort (PRASPAC), which included 246 patients who fulfilled the ASAS classification criteria for axSpA.[13] Patients with a suspicion of SpA were referred to our specialised early-SpA centre in the outpatient department of the Institute of Rheumatology mostly by general practitioners/ophthalmologists/rheumatologists (minority of patients by other specialists) from the central region of the Czech Republic. Patients were further classified as AS or nr-axSpA based on radiographic findings, and irrespective of the presence of psoriasis or IBD. Patients were classified as nr-axSpA if radiographic changes in the SI joints of at least grade II bilaterally or grade III or IV unilaterally were lacking, and positive MRI (ie, characteristic bone marrow oedema) was present with at least one SpA feature. Patients were classified as AS according to New York classification criteria.[4] Patients who fulfilled only clinical arm of ASAS classification criteria were included in the PRASPAC and underwent the same examination protocol. However, these patients were not included in our analyses. No restrictions for disease duration or treatment protocol were used at inclusion.

All patients were recruited from October 2012 to March 2016 at the outpatient rheumatology department of the Institute of Rheumatology in Prague and were followed every 6 months for the first 2 years. Trained rheumatologists obtained data related to the disease status according to recommended standardised methodologies: metrology (modified Schober, occiput to wall, chin–chest distance and chest expansion), Maastricht Ankylosing Spondylitis Enthesitis Score,[14] swollen joint count and tender joint count (SJC and TJC), physician global assessment (MDGAS), Ankylosing Spondylitis Disease Activity Score (ASDAS-CRP),[15] Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[16] and Bath Ankylosing Spondylitis Functional Index (BASFI).[17] Laboratory parameters (CRP and erythrocyte sedimentation rate [ESR]) were analysed from blood samples at each visit. Additional data related to the diagnosis were obtained at the recruitment visit, including age at the onset of first symptoms, type of first symptom (eg, back pain, peripheral arthritis and extra-articular manifestations), age at diagnosis, family history (AS, IBD and psoriasis), inflammatory back pain, occurrence of peripheral or hip arthritis and extra-articular manifestations, previous and current medications (non-steroidal anti-inflammatory drugs [NSAIDs], conventional synthetic disease-modifying antirheumatic drugs [csDMARDs], glucocorticoids, biological treatment [bDMARDs]), HLA-B27 positivity and sociodemographic data (age, gender, Body Mass Index [BMI]), current/ex/non-smoker). Both axSpA subsets were treated according to the EULAR recommendations for the management of SpA. Patients with mild disease were treated with NSAIDs on demand. Most of the patients with previously developed peripheral arthritis were treated with csDMARDs. Patients with severe disease were treated with bDMARDs. The study was initiated before anti-TNF treatment was approved for nr-axSpA by local authorities. Data presented in this study were collected from the recruitment visit.

Patient and public involvement

The patients and/or public were not involved in the design, recruitment or conduct of the study.

Imaging

Radiographs of the SI joints and lumbar and cervical spine from all patients were obtained prior to recruitment, and a trained rheumatologist and/or central radiologist scored the radiographs for the initial disease classification. Radiographic sacroiliitis was scored from grade 0 (normal) to grade 4 (ankylosis) according to the Bennett scoring system.[18] Cervical and lumbar spines were scored according to the modified Stoke AS Spine Score.[19] A trained rheumatologist scored MRI images from nr-axSpA patients obtained at recruitment.

Laboratory analysis

Fasting blood samples were collected from all patients on the same day as the clinical examination. CRP levels were measured using turbidimetry (Beckman Coulter, Pasadena, CA, USA), and ESR was measured according to the Fahraeus Westergren method in a routine clinical laboratory. HLA-B27 was detected using flow cytometry

kits (IOTest HLA-B27-FITC/HLA-B7-PE, Beckman Coulter—Immunotech SAS, Marseille, France) and BDTM HLA-B27 Kit (BD Bioscience, San Jose, CA, USA) according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.1. A Kolmogorov-Smirnov test of normality was performed for all variables. Categorical variables were compared between groups using Fisher's exact test. Data for continuous variables are presented as the median with IQR, and variables were compared using Mann-Whitney tests if not stated otherwise. P values below 0.05 with CI 95% were considered statistically significant for all statistical evaluations.

RESULTS

Demographic data

A total of 246 patients who fulfilled ASAS classification criteria for axSpA were included in this study. Table 1 shows patients' demographic data. The entire group consisted of 106 patients with AS (43.1%) and 140 patients with nr-axSpA (56.9%). There was no gender predominance in the entire group (male-to-female ratio: 53.3% vs 46.7%). However, most of the nr-axSpA patients were women compared with the AS patients (p<0.001). There were no significant differences in age, BMI or smoking history between AS and nr-axSpA patients.

Mean age at the diagnosis was 33.2 years, and the disease duration from first symptoms was 7.8 years for the entire axSpA group. The first clinical symptoms developed earlier in patients with AS compared with patients with nr-axSpA (p=0.001). AS patients were younger at the time of diagnosis than nr-axSpA patients (p=0.023).

Clinical parameters

Disease activity as determined by ASDAS-CRP was 2.2 in the entire axSpA group, and it was significantly higher in AS patients compared with nr-axSpA patients (p=0.022). The mean BASDAI was 2.6 in the entire axSpA group, but it did not significantly differ between AS and nr-axSpA subgroups. AS patients exhibited significantly worse spinal mobility compared with nr-axSpA patients. AS patients exhibited worse BASFI compared with nr-axSpA patients (p=0.030).

Peripheral arthritis and hip arthritis were present in 27.6% and 13.0% of all axSpA patients, respectively. Patients with nr-axSpA exhibited peripheral arthritis more frequently and hip arthritis less frequently compared with AS patients (p=0.001 and p=0.022, respectively). SJC and TJC were significantly higher in nr-axSpA patients compared with AS patients (p=0.021 and p=0.015, respectively). There were no significant differences in the first symptoms of the disease, extra-articular manifestations, or current and previous medications. Division of axSpA according to gender to compare joint variables (peripheral arthritis, hip arthritis, SJC and TJC) revealed a significant difference only in hip arthritis that was more frequent in male patients compared with female patients (p<0.001). Tables 1 and 2 present all clinical parameters.

Laboratory parameters

CRP serum levels (p<0.001) and ESR (p=0.007) were significantly higher in AS patients than nr-axSpA patients. HLA-B27 was found in most of the patients in this study (87.4%), and the prevalence of HLA-B27 was not significantly higher in AS patients than nr-axSpA patients. Tables 1 and 2 show all of the laboratory parameters.

DISCUSSION

This study investigated similarities and differences between AS and nr-axSpA subgroups fulfilling the imaging arm of ASAS classification criteria in a single-centre axSpA cohort in Prague.

Demographic characteristics were comparable in both axSpA subgroups, except the male-to-female ratio, which was higher in AS patients than nr-axSpA patients, which is consistent with previous studies.[8–11] The nr-axSpA subgroup consisted of more female patients than the AS subgroup. Our data also demonstrated that nr-axSpA patients presented first symptoms of the disease later than AS patients, which is also consistent with some previous studies.[20 21] Male gender and early onset of the disease in AS were proposed prognostic factors for severe radiographic damage,[22 23] and female gender was associated with milder disease and later onset.[24] Female predominance and later disease onset in nr-axSpA may underlie the lower percentage of nr-axSpA female patients progressing to AS.[11]

Positive family history is a common finding in SpA. For example, siblings of HLA-B27-positive AS patients exhibit a 50-fold increased risk of developing AS compared with the general population.[25] Many patients, especially HLA-B27-positive patients, have a positive family history of SpA or related diseases. More than one-third of all cases had first-degree relatives with AS, psoriasis or IBD in our study. Furthermore, recent findings even suggest that a substantial proportion of healthy first-degree relatives of HLA-B27-positive AS patients exhibit clinical and/or imaging abnormalities suggestive of SpA, and almost 33% may be classified as SpA especially as nr-axSpA.[26] Comparison of first-degree relatives across gender did not reveal any differences, but a significantly greater frequency of positive family history was previously described in women.[27 28] This result contrasts one study of the occurrence of SpA in first-degree relatives of patients in which no gender differences were demonstrated.[29]

The disease activity of axSpA patients, using ASDAS score and CRP levels, differed between subgroups but remained similar when BASDAI was used in the present study. AS patients exhibited significantly higher disease activity as determined by ASDAS and acute phase reactants compared with nr-axSpA patients, which is consistent with previous studies.[11 30] Elevated CRP may predict the

Table 1 Baseline characteristics: demographic and clinical features of spondyloarthritis

Characteristic		SpA	nr-axSpA	AS	M-W/FET p value
Age (years), median (IQR)		34.7 (29.3–43.5)	36.9 (29.2–46.9)	36.0 (29.3–44.1)	**0.551**
Gender	Male number (%)	131 (53.3)	54 (38.6)	77 (72.6)	**<0.001**
BMI (kg/m^2), median (IQR)		24.3 (21.7–27.4)	24.8 (21.6–28.2)	24.2 (22.6–26.6)	0.946
History of smoking					
	Ever-smoker, number (%)	107 (43.7)	52 (37.1)	55 (52.4)	0.138
HLA-B27 positive, number (%)		215 (87.4)	117 (83.6)	98 (92.5)	0.051
Disease duration, years (IQR)		7.8 (3.1–14.5)	5.6 (2.6–12.2)	10.2 (5.1–15.5)	**0.001**
First symptom					0.086
	Back pain, number (%)	195 (79.3)	104 (74.3)	91 (85.8)	
	Peripheral arthritis, number (%)	27 (11.0)	19 (13.6)	8 (7.5)	
	Extra-articular manifestations, number (%)	24 (9.8)	17 (12.1)	7 (6.6)	
Family history					
	First-degree relatives, number (%)	86 (35.0)	38 (27.1)	24 (22.6)	0.460
	Second-degree relatives, number (%)	28 (11.4)	18 (12.9)	10 (9.4)	0.426
History of					
	Peripheral arthritis, number (%)	68 (27.6)	50 (35.7)	18 (17)	**0.001**
	Hip arthritis, number (%)	32 (13.0)	12 (8.6)	20 (18.9)	**0.022**
	Uveitis, number (%)	63 (25.6)	39 (27.9)	24 (22.6)	0.379
	IBD, number (%)	13 (5.3)	9 (6.4)	4 (3.8)	
	Psoriasis, number (%)	1 (0.4)	0 (0)	1 (0.9)	
	Other, number (%)	3 (1.2)	1 (0.7)	2 (1.9)	
Current symptoms					
	MASES, median (IQR)	0.0 (0.0–2.0)	0.0 (0.0–2.0)	0.0 (0.0–1.3)	0.289
	SJC, mean (±SD)	0.4 (1.4)	0.5 (1.5)	0.3 (1.4)	**0.021**
	TJC, mean (±SD)	0.4 (1.4)	0.5 (1.4)	0.3 (1.7)	**0.015**
Current medication					
	NSAIDs, number (%)	126 (51.2)	71 (50.7)	55 (51.9)	**0.898**
	CsDMARDs, number (%)	39 (15.9)	26 (18.6)	13 (12.3)	**0.218**
	Corticosteroids, number (%)	5 (2)	2 (1.4)	3 (2.8)	
	BoDMARDs/bsDMARDs, number (%)*	6 (2.4)	2 (1.4)	4 (3.8)	

*Adalimumab two patients; certolizumab one patient; golimumab two patients; infliximab one patient.
AS, ankylosing spondylitis; BMI, Body Mass Index; BoDMARDs, biological original disease modifying antirheumatic drugs; bsDMARDs, biosimilar disease modifying antirheumatic drugs; csDMARDs, conventional synthetic disease modifying antirheumatic drugs; IBD, inflammatory bowel disease; MASES, Maastricht Ankylosing Spondylitis Enthesitis Score; M-W/FETp, Mann-Whitney/Fisher exact test p value; nr-axSpA, non-radiographic axial spondyloarthritis; NSAIDs, non-steroidal anti-inflammatory drugs; SJC, swollen joint count; SpA, spondyloarthritis; TJC, tender joint count.

development of radiographic changes.[8] However, recent findings demonstrated similar disease activity as determined by the BASDAI index between AS and nr-axSpA subgroups.[8] The BASDAI may not be a reliable index for evaluating disease activity in axSpA because it reflects subjective perceptions of the disease. Spinal mobility measures and BASFI reflecting movement functions were significantly worse in the AS patients, which is consistent with the results of the German Spondyloarthritis Inception cohort.[11] These results are most likely due to advanced structural changes in the spine of AS patients.

A recent meta-analysis found that arthritis and extra-articular manifestations were equally prevalent in AS and nr-axSpA subgroups, except uveitis, which is slightly more

Table 2 Baseline characteristics: activity and metrology

Characteristic	SpA	nr-axSpA	AS	M-W/FET p value
ASDAS-CRP, median (IQR)	2.2 (1.4–2.8)	2.0 (1.1–2.3)	2.4 (1.7–2.8)	**0.022**
BASDAI, median (IQR)	2.6 (1.2–4.7)	2.7 (1.0–4.9)	2.4 (1.2–3.7)	0.362
CRP (mg/L)	4.3 (1.2–12.0)	2.5 (0.8–8.2)	7.1 (2.6–14.9)	**<0.001**
ESR (mm/h)	8.0 (4.0–17.8)	7.0 (3.0–16.0)	12.0 (4.4–22.0)	**0.007**
BASFI, median (IQR)	1.4 (0.5–2.1)	1.1 (0.3–2.9)	1.8 (0.7–3.3)	**0.030**
Metrology				
Modified Schober (cm), median (IQR)	4.0 (3.0–5.0)	4.5 (3.5–5.5)	4.0 (3.0–5.0)	**<0.001**
Occiput to wall (cm), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	**<0.001**
Chin–chest distance (cm), median (IQR)	1.5 (0.0–3.0)	1.0 (0.0–2.0)	2.0 (0.0–3.0)	**0.008**
Chest expansion (cm), median (IQR)	4.0 (3.0–6.0)	4.0 (3.0–6.0)	3.8 (2.0–6.0)	0.136

AS, ankylosing spondylitis; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score based on CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; M-W/FETp, Mann-Whitney/Fisher exact test p value; nr-axSpA, non-radiographic axial spondyloarthritis; SpA, spondyloarthritis.

prevalent in AS patients.[12] However, our study demonstrated a significant difference between the occurrence of peripheral arthritis, which was more frequent in the nr-axSpA than AS subgroup. A large SpA cohort recently demonstrated more peripheral involvement in women,[27] which may explain the higher prevalence of peripheral arthritis in nr-axSpA with the larger female predominance, at least in the present study. Hip involvement was more frequent in AS patients than the nr-axSpA patients in our cohort. Hip involvement is more prevalent in patients with a younger disease onset, which may be associated with more severe axial disease, and it represents a prognostic factor for severe outcome.[23 31]

Our study has some limitations. First, four assessors examined the patients, which may cause possible inter-rater variability. Second, MRI was performed in several centres, and two MRI sequences were not available for reassessment at the time of data analysis. Therefore, we followed the written report from the MRI examination to divide the patients into AS or nr-axSpA subgroups. We have tried to reduce possible bias by excluding patients fulfilling only the clinical arm of ASAS classification criteria and included only patients with sacroiliitis confirmed by MR (nr-axSpA) or conventional X-ray (AS). Patients fulfilling only the clinical arm had lower participation in the study and fulfilling only clinical arm of ASAS classification criteria provide relatively low sensitivity and specificity and sometimes causing questionable or borderline diagnosis.

CONCLUSIONS

In summary, although disease activity, as determined by ASDAS and acute phase reactants, and functional limitations are worse in AS compared with nr-axSpA patients fulfilling the imaging arm of ASAS classification criteria, we confirmed that patients with nr-axSpA and patients with AS share some similar disease manifestations. However, they differ in gender ratio where women are more prevalent in nr-axSpA than in AS subset and surprisingly, peripheral arthritis, unlike hip joint involvement, was more prevalent in nr-axSpA compared with AS subset. To conclude, patients with nr-axSpA and AS exhibited many similarities despite the issue of classification, which suggests a common therapeutic approach.

Acknowledgements The authors would like to thank nurse Jana Gistrová and nurse Jiřina Kvítková for assistance during the patients examinations. Furthermore, the authors would like to thank the Department of molecular biology and immunogenetics for technical assistance during the laboratory analyses.

Contributors MH, KB, LS and KP designed the study. MH, KB, SF, KZ, MG, JH, MF, MT, KP and LS prepared the clinical database or took clinical care of axSpA patients in the PRASPAC cohort. KB, MT and LS did the data analysis. KB, MT and LS drafted the manuscript. KB and JG determined the radiographic and MRI scores. All authors contributed to and approved the final manuscript.

Funding This study has been supported by the Project for Conceptual Development for the institution of Ministry of Health Czech Republic—Institute of Rheumatology (number 023728), by SVV for LF1 UK (number 260373) and grant MHCR (number 17-33127A).

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval The local ethics committee (Mgr. Ivana Půtova) of the Institute of Rheumatology in Prague approved this study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available. All data from the PRASPAC cohort are available to all qualified researchers/research groups on request.

REFERENCES

1. Sieper J, Poddubnyy D. Axial spondyloarthritis. *Lancet* 2017;390:73–84.
2. Taurog JD, Chhabra A, Colbert RA. Ankylosing spondylitis and axial spondyloarthritis. *N Engl J Med* 2016;375:2563–74.

3. Paramarta JE, Baeten D. Spondyloarthritis: from unifying concepts to improved treatment. *Rheumatology* 2014;53:1547–59.
4. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984;27:361–8.
5. Dincer U, Cakar E, Kiralp MZ, *et al*. Diagnosis delay in patients with ankylosing spondylitis: possible reasons and proposals for new diagnostic criteria. *Clin Rheumatol* 2008;27:457–62.
6. Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis international society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
7. Poddubnyy D, Sieper J. Radiographic progression in ankylosing spondylitis/axial spondyloarthritis: how fast and how clinically meaningful? *Curr Opin Rheumatol* 2012;24:363–9.
8. Poddubnyy D, Sieper J. Similarities and differences between nonradiographic and radiographic axial spondyloarthritis: a clinical, epidemiological and therapeutic assessment. *Curr Opin Rheumatol* 2014;26:377–83.
9. Kiltz U, Baraliakos X, Karakostas P, *et al*. Do patients with non-radiographic axial spondylarthritis differ from patients with ankylosing spondylitis? *Arthritis Care Res* 2012;64:1415–22.
10. Rudwaleit M, Haibel H, Baraliakos X, *et al*. The early disease stage in axial spondylarthritis: results from the German Spondyloarthritis Inception Cohort. *Arthritis Rheum* 2009;60:717–27.
11. Baraliakos X, Braun J. Non-radiographic axial spondyloarthritis and ankylosing spondylitis: what are the similarities and differences? *RMD Open* 2015;1:e000053.
12. de Winter JJ, van Mens LJ, van der Heijde D, *et al*. Prevalence of peripheral and extra-articular disease in ankylosing spondylitis versus non-radiographic axial spondyloarthritis: a meta-analysis. *Arthritis Res Ther* 2016;18:196.
13. Rudwaleit M, Braun J, Sieper J. [ASAS classification criteria for axial spondyloarthritis]. *Z Rheumatol* 2009;68:591–3.
14. Heuft-Dorenbosch L, Spoorenberg A, van Tubergen A, *et al*. Assessment of enthesitis in ankylosing spondylitis. *Ann Rheum Dis* 2003;62:127–32.
15. Lukas C, Landewé R, Sieper J, *et al*. Development of an ASAS-endorsed disease activity score (ASDAS) in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009;68:18–24.
16. Garrett S, Jenkinson T, Kennedy LG, *et al*. A new approach to defining disease status in ankylosing spondylitis: the bath ankylosing spondylitis disease activity index. *J Rheumatol* 1994;21:2286–91.
17. Jones SD, Calin A, Steiner A. An update on the Bath Ankylosing Spondylitis Disease Activity and Functional Indices (BASDAI, BASFI): excellent Cronbach's alpha scores. *J Rheumatol* 1996;23:407.
18. Bennett PH BT. *Population studies of the rheumatic diseases*. Amsterdam: Excerpta Medica Foundation, 1968.
19. Wanders AJ, Landewé RB, Spoorenberg A, *et al*. What is the most appropriate radiologic scoring method for ankylosing spondylitis? A comparison of the available methods based on the Outcome Measures in Rheumatology Clinical Trials filter. *Arthritis Rheum* 2004;50:2622–32.
20. Jeong H, Yoon JY, Park EJ, *et al*. Clinical characteristics of nonradiographic axial spondyloarthritis in Korea: a comparison with ankylosing spondylitis. *Int J Rheum Dis* 2015;18:661–8.
21. Wallis D, Haroon N, Ayearst R, *et al*. Ankylosing spondylitis and nonradiographic axial spondyloarthritis: part of a common spectrum or distinct diseases? *J Rheumatol* 2013;40:2038–41.
22. Lee W, Reveille JD, Davis JC, *et al*. Are there gender differences in severity of ankylosing spondylitis? Results from the PSOAS cohort. *Ann Rheum Dis* 2007;66:633–8.
23. Amor B, Santos RS, Nahal R, *et al*. Predictive factors for the longterm outcome of spondyloarthropathies. *J Rheumatol* 1994;21:1883–7.
24. Pradeep DJ, Keat A, Gaffney K. Predicting outcome in ankylosing spondylitis. *Rheumatology* 2008;47:942–5.
25. Carter N, Williamson L, Kennedy LG, *et al*. Susceptibility to ankylosing spondylitis. *Rheumatology* 2000;39:445.
26. Turina MC, de Winter JJ, Paramarta JE, *et al*. Clinical and Imaging Signs of Spondyloarthritis in First-Degree Relatives of HLA-B27-Positive Ankylosing Spondylitis Patients: The Pre-Spondyloarthritis (Pre-SpA) Cohort Study. *Arthritis Rheumatol* 2016;68:2444–55.
27. de Carvalho HM, Bortoluzzo AB, Gonçalves CR, *et al*. Gender characterization in a large series of Brazilian patients with spondyloarthritis. *Clin Rheumatol* 2012;31:687–95.
28. Will R, Edmunds L, Elswood J, *et al*. Is there sexual inequality in ankylosing spondylitis? A study of 498 women and 1202 men. *J Rheumatol* 1990;17:1649–52.
29. Dernis E, Said-Nahal R, D'Agostino MA, *et al*. Recurrence of spondylarthropathy among first-degree relatives of patients: a systematic cross-sectional study. *Ann Rheum Dis* 2009;68:502–7.
30. Wallman JK, Kapetanovic MC, Petersson IF, *et al*. Comparison of non-radiographic axial spondyloarthritis and ankylosing spondylitis patients-baseline characteristics, treatment adherence, and development of clinical variables during three years of anti-TNF therapy in clinical practice. *Arthritis Res Ther* 2015;17:378.
31. Vander Cruyssen B, Vastesaeger N, Collantes-Estévez E. Hip disease in ankylosing spondylitis. *Curr Opin Rheumatol* 2013;25:448–54.

Rheumatology International
https://doi.org/10.1007/s00296-019-04301-z

Rheumatology
INTERNATIONAL

OBSERVATIONAL RESEARCH

Serum visfatin levels in patients with axial spondyloarthritis and their relationship to disease activity and spinal radiographic damage: a cross-sectional study

Hana Hulejová[1] · Tereza Kropáčková[1,2] · Kristýna Bubová[1,2] · Olga Kryštůfková[1,2] · Mária Filková[1,2] · Heřman Mann[1,2] · Šárka Forejtová[1,2] · Michal Tomčík[1,2] · Jiří Vencovský[1,2] · Karel Pavelka[1,2] · Ladislav Šenolt[1,2]

Received: 4 February 2019 / Accepted: 9 April 2019

Abstract

The purpose of this cross-sectional study was to assess the visfatin levels in patients with axial spondyloarthritis (axSpA) and to investigate the association between visfatin, disease activity and radiographic spinal damage. Serum visfatin levels were determined by enzyme-linked immunosorbent assay in 64 patients with axSpA (46 with radiographic axSpA (r-axSpA) and 18 with non-radiographic axSpA (nr-axSpA)) and 61 age-/sex-matched healthy individuals. Patients with r-axSpA were further divided into two subsets based on radiographic spinal damage using modified Stoke Ankylosing Spondylitis Spine Score (mSASSS = 0 and mSASSS ≥ 1). The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was used to assess disease activity. C-reactive protein (CRP) levels and human leukocyte antigen (HLA)-B27 were determined. Visfatin levels were significantly higher in patients with axSpA and in the subgroup of patients with r-axSpA than in healthy individuals ($p = 0.010$ and $p = 0.005$, respectively), with no difference between patients with r-axSpA and with nr-axSpA. In general, disease activity was high (mean BASDAI 5.01) and was moderately correlated with visfatin levels ($r = 0.585$; $p = 0.011$) in patients with nr-axSpA. Visfatin levels correlated with mSASSS ($r = 0.281$; $p = 0.026$) and were significantly higher in axSpA patients with mSASSS ≥ 1 than in those with mSASSS = 0 ($p = 0.025$). Our study showed that circulating visfatin levels are elevated in axSpA patients, may be associated with disease activity in early phase of the disease and with the degree of radiographic spinal involvement.

Keywords Axial spondyloarthritis · Visfatin · Disease activity · Radiographic damage

Abbreviations

ASDAS	Ankylosing Spondylitis Disease Activity Score
axSpA	Axial spondyloarthritis
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BMI	Body mass index
csDMARDs	Conventional synthetic disease-modifying anti-rheumatic drugs
bDMARDs	Biological disease-modifying anti-rheumatic drugs
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
HLA	Human leukocyte antigen
IQR	Interquartile range
MRI	Magnetic resonance imaging
mSASSS	Modified Stoke Ankylosing Spondylitis Spine Score
NAMPT	Nicotinamide phosphoribosyltransferase
nr-axSpA	Non-radiographic axial spondyloarthritis
NSAIDs	Non-steroidal anti-inflammatory drugs
PBEF	Pre-B cell colony-enhancing factor
r-axSpA	Radiographic axial spondyloarthritis
SIJ	Sacroiliac joints

Hana Hulejová and Tereza Kropáčková contributed equally to this manuscript.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00296-019-04301-z) contains supplementary material, which is available to authorized users.

✉ Hana Hulejová
hulejova@revma.cz

Extended author information available on the last page of the article

Published online: 25 April 2019

Introduction

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease predominantly affecting the axial skeleton (sacroiliac joints (SIJ) and spine). Peripheral and extra-articular manifestations, such as dactylitis, enthesitis, uveitis, inflammatory bowel disease and psoriasis, can also be associated with axSpA. Depending on the presence of radiographic sacroiliitis, patients with axSpA are further classified as having non-radiographic axSpA (nr-axSpA) or radiographic axSpA (r-axSpA) [1].

However, the diagnosis of the early phases of axSpA remains challenging. In recent years, great effort has been made to find a reliable biomarker of the disease [2–4]. A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [5]. In patients with axSpA, such a biomarker could be used not only for the early detection of the disease but also for the evaluation of disease activity, disease progression and treatment response [3]. Currently, human leukocyte antigen (HLA)-B27 is considered to be the marker with the highest diagnostic value, while C-reactive protein (CRP) is useful for assessing disease activity and predicting disease progression or treatment response [4].

Adipokines are biologically active substances that are produced and released predominantly by adipose tissue. They have many regulatory functions in energy metabolism, as well as in inflammation and joint tissue metabolism [6, 7]. Several studies have already reported associations between some adipokines and r-axSpA [8, 9]. However, their exact role in the pathogenesis of the disease and their potential to serve as a disease biomarker is still unclear.

Visfatin, which is also known as pre-B-cell colony-enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (NAMPT), is a 52-kDa adipokine that is expressed in a wide range of tissues, including the bone marrow, liver and muscle [10]. Visfatin has proinflammatory properties [11], and its expression is enhanced in a variety of metabolic and inflammatory diseases [12]. In previous studies, visfatin was shown to play a role in bone homeostasis by stimulating osteoblast proliferation [13] and inhibiting osteoclastogenesis [14, 15].

Recently, serum visfatin levels were found to be increased in patients with r-axSpA compared with healthy controls, and higher baseline visfatin levels were associated with subsequent progression of radiographic damage in r-axSpA [8]. Although several other studies have analysed visfatin in r-axSpA [16, 17], the data regarding patients with non-radiographic axSpA are lacking. Therefore, the aim of our study was to assess the serum visfatin levels in patients with r-axSpA and nr-axSpA and to investigate the association between visfatin, disease activity and radiographic damage.

Materials and methods

Patients

Sixty-four patients who fulfilled the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axSpA were included in this study [18]. Based on imaging, patients were further classified as r-axSpA ($n=46$) and nr-axSpA ($n=18$). Patients with r-axSpA were divided into two subsets based on radiographic findings using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS: mSASSS = 0 (22 patients with SIJ involvement only) and mSASSS ≥ 1 (24 patients with involvement of both the SIJ and the spine).

The following information was collected: clinical and laboratory disease activity measures; demographic status; disease-related factors, such as the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and CRP levels; current medications (non-steroidal anti-inflammatory drugs (NSAIDs), conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), glucocorticoids, biological treatments (bDMARDs)); age, gender; body mass index (BMI); initial symptoms (e.g., back pain, peripheral arthritis, extra-articular manifestations); age at diagnosis; occurrence of peripheral arthritis and extra-articular manifestations; and the presence of HLA-B27 antigen. Sixty-one age- and sex-matched healthy subjects were enrolled in the study. The local ethics committee of the Institute of Rheumatology in Prague approved this study. Written informed consent was obtained from all individuals prior to initiation of the study.

Imaging

Radiographs of the SIJ, lumbar and cervical spine were obtained from majority of patients (except for one patient) prior to blood collection. A trained rheumatologist and a central radiologist scored the radiographs for the initial disease classification, and both were blinded to all the clinical data. Cervical and lumbar spine radiographs were scored according to the mSASSS [18].

Patients were classified as r-axSpA according to the New York classification criteria [19] and as nr-axSpA if the radiographic findings of the SIJ were negative (grade I bilaterally or grade II unilaterally) and the magnetic resonance imaging (MRI) was positive (i.e., characteristic bone marrow oedema) with at least one feature of SpA, according to the ASAS classification criteria for axial spondyloarthritis [18].

MRI images were obtained prior to recruitment and scored by a trained rheumatologist who was blinded to all the clinical data.

Laboratory analysis

Fasting blood samples were collected from all the individuals and immediately centrifuged. The serum samples were stored at − 80 °C until analysis. Serum visfatin levels were analysed by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biovision, Milpitas, CA, USA) according to the manufacturer's protocol. The absorbance was measured using a Sunrise ELISA reader (Tecan, Salzburg, Austria) with 450 nm as the primary wavelength. The assay sensitivity was 30 pg/mL, and the detection limit (cut-off) was 0.125 ng/mL. The intra- and inter-assay coefficient of variation was 3.5% and 5.3%, respectively.

Serum CRP levels were determined by an immuno-turbidimetric technique using an Olympus AU 680 biochemical analyser (Olympus Optical, Tokyo, Japan), reference range of CRP was 0–5 mg/L, the intra-assay variability was 5.73–0.8%, and the inter-assay variability was 6.5–2.3%.

HLA-B27 was detected by flow cytometry using an IOTest HLA-B27-FITC/HLA-B7-PE (Beckman Coulter-Immunotech SAS, Marseille, France) and a BDTM HLA-B27 Kit (BD Bioscience, San Jose, CA, USA).

Statistical analysis

Statistical analyses were performed with GraphPad Prism 5.1 (GraphPad Software, San Diego, CA, USA). The Kolmogorov–Smirnov test of normality was used for all the variables. Based on distribution, the data are presented as the median [interquartile range (IQR)] or mean ± SD. Because of non-normality of data, the non-parametric tests were used for group comparison and correlational analyses. Spearman's correlation coefficients were calculated to determine the association between visfatin levels and the other variables. For comparison between the groups, the Mann–Whitney *U* test was used. Fisher's exact test was performed for the analysis of categorical variables. *P* values less than 0.05 were considered statistically significant.

Results

Characteristics of patients

The patients' demographic and clinical characteristics are summarized in Table 1. Sixty-four patients with axSpA were enrolled in this study [47 males and 17 females, median (IQR) age 35.1 (29.3–40.1) years, 92% HLA-B27 positive]. The proportion of male patients with r-axSpA was higher

Table 1 Characteristics of patients with axial spondyloarthritis and healthy controls

Characteristic	nr-axSpA	r-axSpA	axSpA	HC
Patients (*n*)	18	46	64	61
Gender (male/female)	8/10	39/7	47/17	44/17
Age (years)	36.3 (29.1–42.8)	34.8 (29.2–39.4)	35.1 (29.3–40.1)	36.0 (31.0–48.0)
BMI (kg/m^2)	24.67 ± 4.06	26.61 ± 3.83	24.08 ± 3.95	27.05 ± 3.98
CRP (mg/L)	4.01 (1.26–12.74)	11.08 (5.04–19.60)	9.67 (3.48–16.43)	0.94 (0.13–7.00)
Visfatin (ng/mL)	2.25 (0.89–3.13)	2.77 (1.25–4.43)	2.69 (1.09–4.27)	1.62 (0.93–2.66)
Disease duration (years)	0.2 (0.0–4.0)	5.0 (2.0–9.0)	4.0 (0.5–8.0)	–
HLA-B27 positivity (*n*, %)	17 (94%)	42 (91%)	59 (92%)	–
BASDAI	4.78 (1.53–6.25)	5.38 (3.15–7.57)	5.01 (2.68–7.29)	–
csDMARDs (*n*, %)	6 (33%)	5 (11%)	11 (17%)	–
bDMARDs (*n*, %)	1 (6%)	14 (30%)	15 (23%)	–
mSASSS ≥ 1/mSASSS = 0 (*n*)	0/18	22/24	22/42	–
Extra-articular features (*n*, %)	15 (83%)	25 (54%)	40 (62%)	–
Enthesitis/tendinitis (*n*, %)	11 (61%)	12 (26%)	23 (36%)	–
Peripheral arthritis (*n*, %)	14 (77%)	19 (41%)	33 (52%)	–
Uveitis (*n*, %)	7 (38%)	15 (32%)	22 (34%)	–
Psoriasis (*n*, %)	0 (0%)	2 (4%)	2 (3%)	–
Dactylitis (*n*, %)	2 (11%)	3 (7%)	5 (8%)	–

Data are presented as the median (interquartile range) or mean ± SD

axSpA axial spondyloarthritis; *nr-axSpA* non-radiographic axial spondyloarthritis; *r-axSpA* radiographic axial spondyloarthritis; *HC* healthy controls; *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index; *CRP* C-reactive protein; *csDMARDs* conventional synthetic disease-modifying antirheumatic drugs sulfasalazine (*n* = 8), methotrexate (*n* = 2), azathioprine (*n* = 1); *bDMARDs* biological disease-modifying antirheumatic drugs [adalimumab (*n* = 1), golimumab (*n* = 2), etanercept (*n* = 9), infliximab (*n* = 3)]

than the proportion of those with nr-axSpA ($p = 0.003$). Patients with r-axSpA had a significantly longer disease duration ($p < 0.001$) and higher CRP levels ($p = 0.013$) than those with nr-axSpA. Peripheral arthritis, extra-articular manifestations, enthesitis and/or tendinitis were significantly more frequent in patients with nr-axSpA compared with patients with r-axSpA ($p = 0.012$, $p = 0.044$ and $p = 0.019$, respectively). Age, BMI, BASDAI and HLA-B27 positivity were comparable between the two subgroups of patients. Out of all, one patient suffered from diabetes mellitus type 2 and another patient had insulin resistance. Both patients were from nr-axSpA group.

Serum visfatin levels are elevated in patients with axSpA

Serum visfatin levels were significantly higher in patients with axSpA than in healthy individuals [2.69 (1.09–4.27) vs. 1.62 (0.93–2.70) ng/mL; $p = 0.009$] (Fig. 1a). After dividing the patients into r-axSpA and nr-axSpA subgroups, the difference remained statistically significant only for patients with r-axSpA [2.77 (1.25–4.43) vs. 1.62 (0.93–2.70) ng/mL; $p = 0.005$], but not for those with nr-axSpA [2.25 (0.89–3.13) vs. 1.62 (0.93–2.70) ng/mL; $p = 0.390$]. The concentrations of visfatin were comparable between patients with nr-axSpA and r-axSpA ($p = 0.235$) (Fig. 1b). There was no difference in the visfatin levels between patients receiving biological therapy compared to patients not receiving biological therapy [2.45 (1.45–4.28) vs. 2.75 (1.00–4.28) ng/mL; $p = 0.918$]. In addition, excluding the two patients with diabetes mellitus and insulin resistance from nr-axSpA group did not affect statistical visfatin level differences among groups (Supplementary Figure 1).

Visfatin levels in relationship to radiographic damage and disease activity

In patients with axSpA, serum visfatin levels were significantly higher in individuals with mSASSS ≥ 1 than in those with mSASSS = 0 [4.12 (1.65–5.41) vs. 2.33 (0.87–3.49) ng/mL; $p = 0.025$] (Fig. 2a). Furthermore, a positive correlation between visfatin levels and mSASSS was confirmed ($r = 0.281$; $p = 0.026$) (Fig. 2b). In the r-axSpA subgroup, patients with mSASSS ≥ 1 showed non-significantly higher levels of visfatin than those with mSASSS = 0 [3.05 (1.92–5.53) vs. 2.26 (0.85–3.34) ng/mL; $p = 0.053$]. Visfatin levels remained unchanged even after excluding the

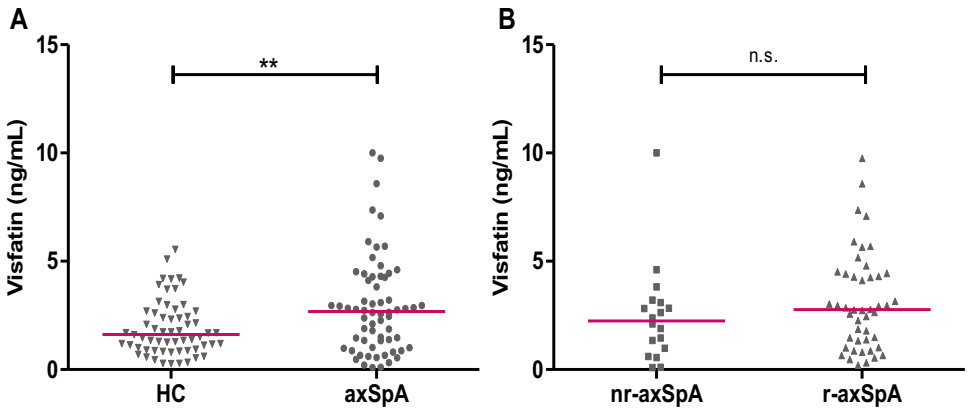

Fig. 1 Serum visfatin levels were significantly higher in patients with axial spondyloarthritis (axSpA) than in healthy controls (HC) (**a**), and they were comparable between patients with non-radiographic axial spondyloarthritis (nr-axSpA) and those with radiographic axSpA (r-axSpA) (**b**). The horizontal line represents the median. $^{**}p < 0.01$. *ns* non-significant

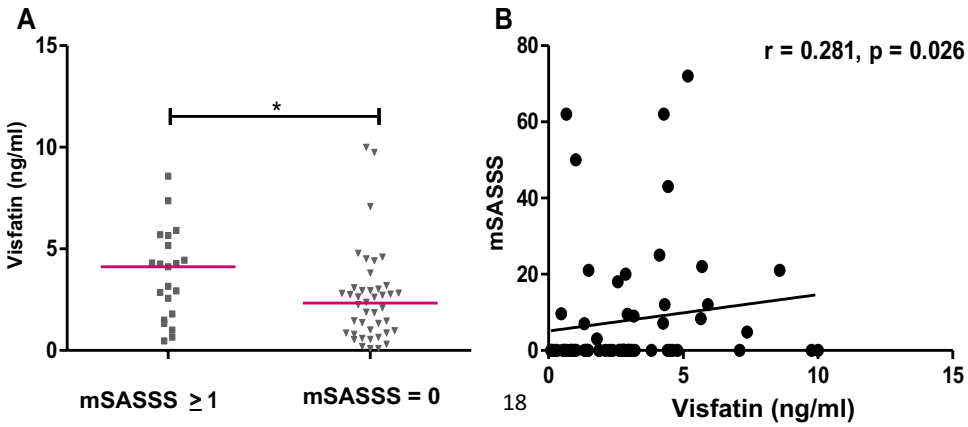

Fig. 2 Serum visfatin levels were significantly higher in axSpA patients with mSASSS ≥ 1 than in those with mSASSS = 0 (**a**) and were positively correlated with mSASSS (**b**). The horizontal line represents the median. $^{*}p < 0.05$. *mSASSS* modified Stoke Ankylosing Spondylitis Spine Score. *P* values are results of Mann–Whitney and Spearman correlation tests

two patients with diabetes mellitus and insulin resistance from nr-axSpA group.

The levels of visfatin did not differ between males and females or between patients with and without the presence of extra-articular manifestations, peripheral joint involvement or HLA-B27 antigen in any of the study groups. Similarly, there was no association of visfatin levels with age, disease duration, BMI or CRP levels. In patients with nr-axSpA, but not in patients with r-axSpA, visfatin levels were positively correlated with the BASDAI ($r=0.585$; $p=0.011$) (Fig. 3). After excluding the two patients with diabetes mellitus and insulin resistance, the correlation even improved ($r=0.732$, $p=0.001$). In the total group of axSpA patients, there was no difference in the visfatin levels between patients with BASDAI ≥ 4 compared to those with BASDAI < 4 [2.78 (1.33–4.28) vs. 2.11 (0.82–4.28) ng/mL; $p=0.395$].

Discussion

In the present study, we showed that higher serum visfatin levels are found in patients with axSpA than in healthy individuals and that there is an association between visfatin levels and radiographic spinal damage.

A number of studies have reported that visfatin regulates joint tissue metabolism and exerts proinflammatory effects [11, 13–15, 20]. However, the data regarding a possible link between visfatin, r-axSpA and nr-axSpA are limited. In the present study, we found higher levels of visfatin in patients with axSpA, and particularly in the subgroup of patients with r-axSpA, than in healthy controls. In line with our findings, higher serum visfatin levels were demonstrated in patients with r-axSpA than in healthy subjects in a previous study by Syrbe et al. [8]. As far as we know, visfatin levels in patients with nr-axSpA have not been studied to date. We showed that visfatin levels in patients with nr-axSpA are comparable to those in patients with r-axSpA but are not significantly higher than those in healthy controls. Interestingly, after dividing the patients into r-axSpA and nr-axSpA subsets, we observed significantly positive correlation between visfatin levels and the BASDAI in patients with nr-axSpA. However, this relationship was not found neither in patients with r-axSpA nor in the entire cohort of patients with axSpA. This discrepancy could be explained by lower number of patients with nr-axSpA and their slightly lower disease activity compared to those with r-axSpA. Since limited data on biomarkers in nr-axSpA exists, there is evidence that serum CRP levels are lower in patients with nr-axSpA compared to those with r-axSpA [21]. On the other side, Huang et al. [22] found that serum calprotectin levels were comparable between both subsets, correlated with inflammatory measures of axSpA, and were higher in both subsets compared to healthy subjects. In line with our study, calprotectin, however, did not contribute to discriminate between non-radiographic and radiographic form of the disease.

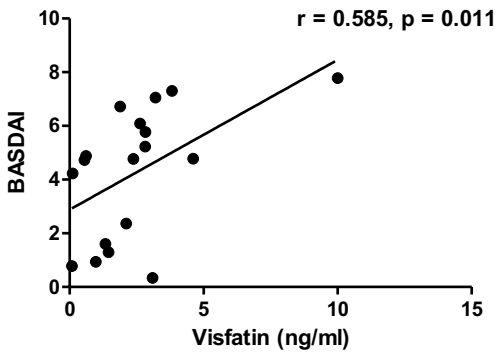

Fig. 3 Serum levels of visfatin were positively correlated with the BASDAI in patients with non-radiographic axial spondyloarthritis (nr-axSpA). *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index

In addition, we demonstrated comparable levels of visfatin between patients with axSpA with and without the presence of extra-articular manifestations, peripheral arthritis or the presence of HLA-B27 antigen. Furthermore, we did not observe any difference in visfatin levels due to sex, and there were no associations between visfatin levels and age, disease duration, BMI or CRP levels. These findings are consistent with those reported in other studies that evaluated patients with r-axSpA [8, 16, 17].

According to recent findings, MRI defined fatty lesions at anterior vertebral corners on spine are predictive of new bone formations [23]. Fatty lesions are thought to replace the active inflammatory lesions of bone marrow edema and from preliminary reports on biopsies performed from these sites, fatty lesions correspond to fat deposition in the bone marrow of patients with advanced r-axSpA. Since vertebral bone marrow is harbouring hematopoiesis, r-axSpA seems to lead to local disruption of hematopoiesis in the bone marrow microenvironment, which is followed by fat replacement [24]. Therefore, it can be speculated that adipokines might have some effects on the disease progression. Although increased visfatin is known to play a role in diabetes mellitus [25], excluding the two diabetic and prediabetic patients did not affect our results. As far as we know, there is no association between diabetes mellitus and radiographic progression of axSpA, which is worth to explore in future studies.

It has recently been demonstrated that visfatin induces osteoblast proliferation and type I collagen production in vitro [13]. Moreover, visfatin has been shown to inhibit osteoclast formation and differentiation [14, 15]. Therefore, we hypothesized that visfatin concentrations could be affected by bone changes and thus reflect syndesmophyte formation. In line with this, we found a positive correlation between visfatin levels and radiographic damage as

assessed by the mSASSS in patients with axSpA and significantly higher visfatin concentrations in patients with spinal involvement than in those without spinal radiographic changes. On the other hand, we did not find any relationship between visfatin levels and mSASSS in a subgroup of patients with r-axSpA. Previous studies examining the association between visfatin levels and radiographic changes in axSpA included only patients with radiographic form of the disease. Consistent with our findings, Syrbe et al. [8] also reported no significant association between visfatin levels and structural damage based on the mSASSS in patients with r-axSpA. Nevertheless, elevated baseline visfatin levels were found to be predictive of subsequent radiographic progression and new syndesmophyte formation after 2 years [8]. However, ENRADAS study did not reveal correlation between visfatin levels and radiographic spinal progression or new syndesmophyte formation [9]. Thus, the association between visfatin levels and radiographic spinal damage or progression in patients with r-axSpA is not consistent across all studies and needs to be further determined.

Our study has several limitations. First, design of the study was cross-sectional. Therefore, a longitudinal association between visfatin levels and disease activity/improvement could not be assessed. Long-term prospective study is needed to further investigate the role of visfatin in patients with axSpA. Second, the Ankylosing Spondylitis Disease Activity Score (ASDAS) was not calculated in majority of the patients, therefore, only the BASDAI was used for the evaluation of disease activity in this study. Third, low number of patients with nr-axSpA was included in our cohort.

Conclusion

In conclusion, we demonstrated elevated visfatin levels in patients with axSpA, particularly in those with r-axSpA. Furthermore, we found higher visfatin levels in patients with radiographic spinal involvement and a positive correlation between visfatin levels and radiographic spinal damage. These findings further support a possible role of visfatin in bone remodelling, the exact link between visfatin, new bone formation and a putative role of visfatin in early phases of axSpA pathogenesis need to be confirmed in a larger cohort of patients.

Acknowledgements This work was supported by the Ministry of Health of the Czech Republic project 17-33127A.

Author contributions LŠ and JV were responsible for the study concept and design. HH performed ELISA tests. KB, MF, HM, ŠF, JV and LŠ were involved in enrolling the patients and made clinical assessments. HH, TL and OK carried out the statistical analysis. HH, TL and LŠ were responsible for data interpretation and manuscript preparation. MT, JV, KP and LŠ revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standard of the local ethics committee of the Institute of Rheumatology in Prague, Czech Republic.

Informed consent Written informed consent was obtained from all participants.

Conflict of interest Hana Hulejová declares that she does not have competing interests. Tereza Kropáčková declares that she does not have competing interests. Kristýna Bubová declares that she does not have competing interests. Olga Kryštůfková declares that she does not have competing interests. Mária Filková declares that she does not have competing interests. Šárka Forejtová declares that she does not have competing interests. Heřman Mann declares that he does not have competing interests. Michal Tomčík declares that he does not have competing interests. Jiří Vencovský declares that he does not have competing interests. Karel Pavelka declares that he does not have competing interests. Ladislav Šenolt declares that he does not have competing interests.

References

1. Sieper J, Poddubnyy D (2017) Axial spondyloarthritis. Lancet 390(10089):73–84
2. Turina MC, Yeremenko N, van Gaalen F et al (2017) Serum inflammatory biomarkers fail to identify early axial spondyloarthritis: results from the SpondyloArthritis Caught Early (SPACE) cohort. RMD Open 3(1):e000319
3. Poddubnyy D, Conrad K, Haibel H et al (2014) Elevated serum level of the vascular endothelial growth factor predicts radiographic spinal progression in patients with axial spondyloarthritis. Ann Rheum Dis 73(12):2137–2143
4. Prajzlerova K, Grobelna K, Pavelka K, Senolt L, Filkova M (2016) An update on biomarkers in axial spondyloarthritis. Autoimmun Rev 15(6):501–509
5. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
6. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11(2):85–97. http://www.nature.com/articles/nri2921. Accessed 21 Jan 2011
7. Neumann E, Junker S, Schett G, Frommer K, Muller-Ladner U (2016) Adipokines in bone disease. Nat Rev Rheumatol 12(5):296–302. <Go to ISI>://WOS:000375276700006
8. Syrbe U, Callhoff J, Conrad K et al (2015) Serum adipokine levels in patients with ankylosing spondylitis and their relationship to clinical parameters and radiographic spinal progression. Arthritis Rheumatol 67(3):678–685
9. Hartl A, Sieper J, Syrbe U et al (2017) Serum levels of leptin and high molecular weight adiponectin are inversely associated with radiographic spinal progression in patients with ankylosing spondylitis: results from the ENRADAS trial. Arthritis Res Ther 19(1):140. https://doi.org/10.1186/s13075-017-1350-9 **(Published online first 2017/06/18)**

10. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I (1994) Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol 14(2):1431–1437
11. Moschen AR, Kaser A, Enrich B et al (2007) Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 178(3):1748–1758
12. Luk T, Malam Z, Marshall JC (2008) Pre-B cell colony-enhancing factor (PBEF)/visfatin: a novel mediator of innate immunity. J Leukoc Biol 83(4):804–816
13. Xie H, Tang SY, Luo XH et al (2007) Insulin-like effects of visfatin on human osteoblasts. Calcif Tissue Int 80(3):201–210
14. Moschen AR, Geiger S, Gerner R, Tilg H (2010) Pre-B cell colony enhancing factor/NAMPT/visfatin and its role in inflammation-related bone disease. Mutat Res 690(1–2):95–101
15. Baek JM, Ahn SJ, Cheon YH, Lee MS, Oh J, Kim JY (2017) Nicotinamide phosphoribosyltransferase inhibits receptor activator of nuclear factor-kappaB ligand-induced osteoclast differentiation in vitro. Mol Med Rep 15(2):784–792
16. Hulejova H, Levitova A, Kuklova M et al (2012) No effect of physiotherapy on the serum levels of adipocytokines in patients with ankylosing spondylitis. Clin Rheumatol 31(1):67–71. https://doi.org/10.1007/s10067-011-1773-7. <Go to ISI>://WOS:000299768200009
17. Miranda-Filloy JA, Lopez-Mejias R, Genre F et al (2013) Leptin and visfatin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-alpha antagonist therapy. Clin Exp Rheumatol 31(4):538–545
18. Rudwaleit M, van der Heijde D, Landewe R et al (2009) The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis 68(6):777–783
19. van der Linden S, Valkenburg HA, Cats A (1984) Evaluation of diagnostic-criteria for ankylosing-spondylitis—a proposal for modification of the New-York criteria. Arthritis Rheum 27(4):361–368. <Go to ISI>://WOS:A1984SN19400001
20. Brentano F, Schorr O, Ospelt C et al (2007) Pre-B cell colony-enhancing factor/visfatin, a new marker of inflammation in rheumatoid arthritis with proinflammatory and matrix-degrading activities. Arthritis Rheum 56(9):2829–2839
21. Baraliakos X, Braun J (2015) Non-radiographic axial spondyloarthritis and ankylosing spondylitis: what are the similarities and differences? RMD Open. https://doi.org/10.1136/rmdopen-2015-000053
22. Huang J, Yin Z, Song G et al (2017) Discriminating value of calprotectin in disease activity and progression of nonradiographic axial spondyloarthritis and ankylosing spondylitis. Dis Markers 2017:7574147. https://doi.org/10.1155/2017/7574147 **(Published online first 2017/06/21)**
23. Baraliakos X (2014) The contribution of imaging in the diagnosis and treatment of axial spondyloarthritis. Eur J Clin Investig 45(1):81–86. https://doi.org/10.1111/eci.12369 **(Published online first 2014/11/08)**
24. Baraliakos X, Boehm H, Samir Barakat A et al (2018) OP0242 Fatty lesions detected on MRI scans in patients with ankylosing spondylitis are based on the deposition of fat in the vertebral bone marrow. Ann Rheum Dis 77(Suppl 2):170. https://doi.org/10.1136/annrheumdis-2018-eular.7158
25. Esteghamati A, Alamdari A, Zandieh A et al (2011) Serum visfatin is associated with type 2 diabetes mellitus independent of insulin resistance and obesity. Diabetes Res Clin Pract 91(2):154–158. https://doi.org/10.1016/j.diabres.2010.11.003 **(Epub 2010 Nov 30)**

Affiliations

Hana Hulejová[1] · Tereza Kropáčková[1,2] · Kristýna Bubová[1,2] · Olga Kryštůfková[1,2] · Mária Filková[1,2] · Heřman Mann[1,2] · Šárka Forejtová[1,2] · Michal Tomčík[1,2] · Jiří Vencovský[1,2] · Karel Pavelka[1,2] · Ladislav Šenolt[1,2]

Tereza Kropáčková
kropackova@revma.cz

Kristýna Bubová
bubova@revma.cz

Olga Kryštůfková
krystufkova@revma.cz

Mária Filková
filkova@revma.cz

Heřman Mann
mann@revma.cz

Šárka Forejtová
forejtova@revma.cz

Michal Tomčík
tomcik@revma.cz

Jiří Vencovský
vencovsky@revma.cz

Karel Pavelka
pavelka@revma.cz

Ladislav Šenolt
senolt@revma.cz

[1] Institute of Rheumatology, Na Slupi 4, 12850 Prague 2, Czech Republic

[2] Department of Rheumatology, First Faculty of Medicine, Charles University, Prague, Czech Republic

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

A.S. Siebuhr[1], M. Hušáková[2], S. Forejtová[2], K. Zegzulková[2], M. Tomčik[2], M. Urbanová[2], K. Grobelná[2], J. Gatterová[2], A.-C. Bay-Jensen[1], K. Pavelka[2]

[1]Nordic Bioscience Biomarkers and Research, Herlev, Denmark; [2]Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

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

*Anne Sofie Siebuhr, PhD**
*Markéta Hušáková, PhD**
Šárka Forejtová, MD
Kateřina Zegzulková, MD
Michal Tomčík, PhD
Monika Urbanová, MD
Kristýna Grobelná, MD
Jindra Gatterová, MD
Anne-Christine Bay-Jensen, PhD
Karel Pavelka, PhD

**These authors contributed equally to this work.*

Please address correspondence to:
Dr Anne Sofie Siebuhr,
Herlev Hovedgade 205-207,
Herlev, Denmark.
E-mail: aso@nordicbio.com

Reprints will not be available from author.

Received on December 4, 2017; accepted in revised form on March 5, 2018.

Funding: this study was supported by the Project for Conceptual Development for the institution of the Ministry of Health Czech Republic (MHCR), Institute of Rheumatology (no. 023728) - recipient K. Pavelka, by The Specific University Research (SVV no. 260373) - recipient: K. Grobelná and by grant MHCR no. 17-33127A - recipient: K. Pavelka, M. Hušáková, S. Forejtová, K. Grobelná, M. Urbanová, and K. Zegzulková.

Competing interests: A.S. Siebuhr and A.C. Bay-Jensen are full-time employees of Nordic Bioscience, a privately owned SME developing biomarkers of fibrosis. A.S. Siebuhr and A.C. Bay-Jensen have no other relevant affiliation or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. M. Hušáková, Š. Forejtová, K. Zegzulková, M. Urbanová, K. Grobelná, J. Gatterován, M. Tomčík and K. Pavelka declare that they have no financial or non-financial competing interests.

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.

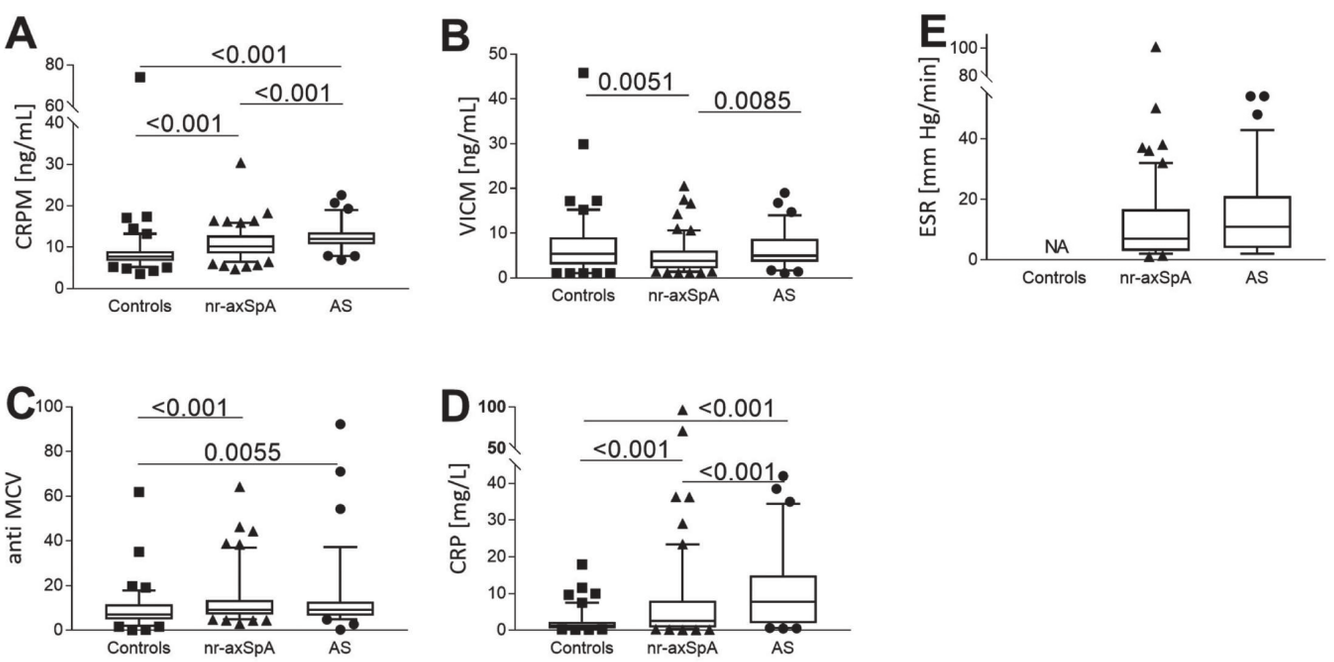

Fig. 1. Biomarker levels in the AS, nr-axSpA group and asymptomatic controls.
A: Tissue inflammation - MMP-degraded C-reactive protein, **B**: Macrophage activation - Citrullinated and MMP-degraded vimentin; **C**: Anti-mutated citrullinated vimentin; **D**: Systemic inflammation - C-reactive protein; **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.

Acknowledgements

The authors would like to thank MUDr Jana Horinkova for her professional care of axSpA patients, RNDr. Ivana Putova for the anti-MCV analysis and Mrs Jitka Smekalova for her technical assistance during samples storing and shipment.

References

1. SIEPER J, RUDWALEIT M, BARALIAKOS X *et al*.: The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009; 68: ii1-ii44.
2. DE WINTER JJ, VAN MENS LJ, VAN DER HEIJDE D *et al*.: Prevalence of peripheral and extra-articular disease in ankylosing spondylitis versus non-radiographic axial spondyloarthritis: a meta-analysis. *Arthritis Res Ther* 2016; 18: 196.
3. WALLMAN JK, KAPETANOVIC MC, PETERSSON IF, GEBOREK P, KRISTENSEN LE: Comparison of non-radiographic axial spondyloarthritis and ankylosing spondylitis patients – baseline characteristics, treatment adherence, and development of clinical variables during three years of anti-TNF therapy in clinical practice. *Arthritis Res Ther* 2015; 17: 378.
4. RUDWALEIT M, HAIBEL H, BARALIAKOS X *et al*.: The early disease stage in axial spondylarthritis: Results from the German spondyloarthritis inception cohort. *Arthritis Rheum* 2009; 60: 717-27.
5. PODDUBNYY D, RUDWALEIT M, HAIBEL H *et al*.: Rates and predictors of radiographic sacroiliitis progression over 2 years in patients with axial spondyloarthritis. *Ann Rheum Dis* 2011; 70: 1369-74.
6. WANG R, GABRIEL SE, WARD MM: Progression of nonradiographic axial spondyloarthritis to ankylosing spondylitis: a population-based cohort study. *Arthritis Rheumatol* 2016; 68: 1415-21.
7. VAN DER LINDEN S, VALKENBURG HA, CATS A: Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984; 27: 361-8.
8. DOUGADOS M, DEMATTEI C, VAN DEN BERG R *et al*.: Rate and predisposing factors for sacroiliac joint radiographic progression after a two-year follow-up period in recent-onset spondyloarthritis. *Arthritis Rheumatol* 2016; 68: 1904-13.
9. BENHAMOU M, GOSSEC L, DOUGADOS M: Clinical relevance of C-reactive protein in ankylosing spondylitis and evaluation of the NSAIDs/coxibs' treatment effect on C-reactive protein. *Rheumatology* 2009; 49: 536-41.
10. LAMBERT RGW, SALONEN D, RAHMAN P *et al*.: Adalimumab significantly reduces both spinal and sacroiliac joint inflammation in patients with ankylosing spondylitis: a multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2007; 56: 4005-14.
11. VANDOOREN B, KRUITHOF E, YU DTY *et al*.: Involvement of matrix metalloproteinases and their inhibitors in peripheral synovitis and down-regulation by tumor necrosis factor ?? blockade in spondylarthropathy. *Arthritis Rheum* 2004; 50: 2942-53.
12. MATTEY DL, PACKHAM JC, NIXON NB *et al*.: Association of cytokine and matrix metalloproteinase profiles with disease activity and function in ankylosing spondylitis. *Arthritis Res Ther* 2012; 14: R127.
13. 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.
14. SIEBUHR AS, PETERSEN KK, ARENDT-NIELSEN L *et al*.: Identification and characterisation of osteoarthritis patients with inflammation derived tissue turnover. *Osteoarthritis Cartilage* 2014; 22: 44-50.
15. SKJØT-ARKIL H, SCHETT G, ZHANG C *et al*.: Investigation of two novel biochemical markers of inflammation, matrix metalloproteinase and cathepsin generated fragments of C-reactive protein, in patients with ankylosing spondylitis. *Clin Exp Rheumatol* 2012; 30: 371-9.
16. BAY-JENSEN AC, KARSDAL MA, VASSILIADIS E *et al*.: Circulating citrullinated vimentin fragments reflect disease burden in ankylosing spondylitis and have prognostic capacity for radiographic progression. *Arthritis Rheum* 2013; 65: 972-80.
17. MOR-VAKNIN N, PUNTURIERI A, SITWALA K, MARKOVITZ DM: Vimentin is secreted by activated macrophages. *Nat Cell Biol* 2003; 5: 59-63.
18. HSU P-C, LIAO Y-F, LIN C-L, LIN W-H, LIU G-Y, HUNG H-C: Vimentin is involved in peptidylarginine deiminase 2-induced apoptosis of activated jurkat cells. *Mol Cells* 2014; 37: 426-34.
19. VAN DER HEIJDE D, LIE E, KVIEN TK *et al*.: ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009; 68: 1811-18.
20. GARRETT S, JENKINSON T, KENNEDY LG, WHITELOCK H, GAISFORD P, CALIN A: A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994; 21: 2286-91.

21. CALIN A, GARRETT S, WHITELOCK H, O'HEA J, MALLORIE P, JENKINSON T: A new approach to defining functional ability in ankylosing spondylitis: The development of the bath ankylosing spondylitis functional index. *J Rheumatol* 1994; 21: 2281-5.
22. MAKSYMOWYCH WP, INMAN RD, SALONEN D *et al*.: Spondyloarthritis Research Consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. *Arthritis Care Res* 2005; 53: 703-9.
23. HERMANN KG, BRAUN J, FISCHER T, REISSHAUER H, BOLLOW M: [Magnetic resonance tomography of sacroiliitis: anatomy, histological pathology, MR-morphology, and grading]. *Radiologe* 2004; 44: 217-28.
24. WANDERS AJ, LANDEWÉ RB, SPOORENBERG A *et al*.: What is the most appropriate radiologic scoring method for ankylosing spondylitis? A comparison of the available methods based on the Outcome Measures in Rheumatology Clinical Trials filter. *Arthritis Rheum* 2004; 50: 2622-32.
25. DOWARD LC, SPOORENBERG A, COOK SA *et al*.: Development of the ASQoL: a quality of life instrument specific to ankylosing spondylitis. *Ann Rheum Dis* 2003; 62: 20-6.
26. RABIN R, CHARRO F DE: EQ-5D: a measure of health status from the EuroQol Group. *Ann Med* 2001; 33: 337-43.
27. VASSILIADIS E, OLIVEIRA CP, ALVARES-DA-SILVA MR *et al*.: Circulating levels of citrullinated and MMP-degraded vimentin (VICM) in liver fibrosis related pathology. *Am J Transl Res* 2012; 4: 403-14.
28. SKJØT-ARKIL H, SCHETT G, ZHANG C *et al*.: Investigation of two novel biochemical markers of inflammation, matrix metalloproteinase and cathepsin generated fragments of C-reactive protein, in patients with ankylosing spondylitis. *Clin Exp Rheumatol* 2012; 30: 371-9.
29. TURINA MC, YEREMENKO N, VAN GAALEN F *et al*.: Serum inflammatory biomarkers fail to identify early axial spondyloarthritis: results from the SpondyloArthritis Caught Early (SPACE) cohort. *RMD Open* 2017; 3: e000319.
30. SIEBUHR AS, BAY-JENSEN AC, KARSDAL MA, LORIES RJ, DE VLAM K: CRP and a biomarker of type I collagen degradation, C1M, can differentiate anti-inflammatory treatment response in ankylosing spondylitis. *Biomark Med* 2016; 10: 197-208.
31. BAY-JENSEN AC, LEEMING DJ, KLEYER A, VEIDAL SS, SCHETT G, KARSDAL MA: Ankylosing spondylitis is characterized by an increased turnover of several different metalloproteinase-derived collagen species: A cross-sectional study. *Rheumatol Int* 2012; 32: 3565-72.
32. SABERI HOSNIJEH F, SIEBUHR AS, UITTERLINDEN AG *et al*.: Association between biomarkers of tissue inflammation and progression of osteoarthritis: evidence from the Rotterdam study cohort. *Arthritis Res Ther* 2016; 18: 1-10.
33. MAIJER KI, GUDMANN NS, KARSDAL MA, GERLAG DM, TAK PP, BAY-JENSEN AC: Neoepitopes-fragments of cartilage and connective tissue degradation in early rheumatoid arthritis and unclassified arthritis. *PLoS One* 2016; 11: e0149329.
34. BAY-JENSEN AC, PLATT A, BYRJALSEN I, VERGNOUD P, CHRISTIANSEN C, KARSDAL MA: Effect of tocilizumab combined with methotrexate on circulating biomarkers of synovium, cartilage, and bone in the LITHE study. *Semin Arthritis Rheum* 2014; 43: 470-8.
35. BAY-JENSEN AC, PLATT A, SIEBUHR AS, CHRISTIANSEN C, BYRJALSEN I, KARSDAL MA: Early changes in blood-based joint tissue destruction biomarkers are predictive of response to tocilizumab in the LITHE study. *Arthritis Res Ther* 2016; 18: 13.
36. KILIC E, KILIC G, AKGUL O, OZGOCMEN S: Discriminant validity of the Ankylosing Spondylitis Disease Activity Score (ASDAS) in patients with non-radiographic axial spondyloarthritis and ankylosing spondylitis: A cohort study. *Rheumatol Int* 2014; 35: 981-9.
37. REVEILLE JD: Biomarkers for diagnosis, monitoring of progression, and treatment responses in ankylosing spondylitis and axial spondyloarthritis. *Clin Rheumatol* 2015; 34: 1009-18.
38. BARALIAKOS X, SIEPER J, CHEN S, PANGAN AL, ANDERSON JK: Non-radiographic axial spondyloarthritis patients without initial evidence of inflammation may develop objective inflammation over time. *Rheumatology* (Oxford) 2017; 6: 1162-66.
39. ALTHOFF CE, SIEPER J, SONG I-H *et al*.: Active inflammation and structural change in early active axial spondyloarthritis as detected by whole-body MRI. *Ann Rheum Dis* 2013;72:967–73.
40. QUADEN DHF, DE WINTER LM, SOMERS V: Detection of novel diagnostic antibodies in ankylosing spondylitis: An overview. *Autoimmun Rev* 2016; 15: 820-32.
41. BODNÁR N, SZEKANECZ Z, PROHÁSZKA Z *et al*.: Anti-mutated citrullinated vimentin (anti-MCV) and anti-65kDa heat shock protein (anti-hsp65): New biomarkers in ankylosing spondylitis. *Joint Bone Spine* 2011; 79: 63-6.
42. BELTRAMI A, ROSSMANN M, FIORILLO MT *et al*.: Citrullination-dependent differential presentation of a self-peptide by HLA-B27 subtypes. *J Biol Chem* 2008; 283: 27189-99.
43. SZODORAY P, SZABÓ Z, KAPITÁNY A *et al*.: Anti-citrullinated protein/peptide autoantibodies in association with genetic and environmental factors as indicators of disease outcome in rheumatoid arthritis. *Autoimmun Rev* 2010; 9: 140-3.

SCIENTIFIC REPORTS
nature research

OPEN

Metabolites of type I, II, III, and IV collagen may serve as markers of disease activity in axial spondyloarthritis

Received: 23 November 2018
Accepted: 12 July 2019
Published online: 02 August 2019

Markéta Hušáková[1], Anne-C. Bay-Jensen[2], Šárka Forejtová[1], Kateřina Zegzulková[1], Michal Tomčík[1], Monika Gregová[1,2], Kristýna Bubová[1,2], Jana Hořínková[1,2], Jindra Gatterová[1], Karel Pavelka[1] & Anne Sofie Siebuhr[2]

Local inflammation in axial spondyloarthritis (axSpA) leads to the release of collagen metabolites from the disease-affected tissue. We investigated whether collagen metabolites were associated with disease activity and could distinguish non-radiographic(nr)-axSpA from ankylosing spondylitis (AS). A total of 193 axSpA patients (nr-axSpA, n = 121 and AS, n = 72) and asymptomatic controls (n = 100) were included. Serum levels of metalloproteinase (MMP)-degraded collagen type I (C1M), type II (C2M), type III (C3M) and type IV (C4M2) were quantified by enzyme-linked immunosorbent assay (ELISA). All metabolites were higher in axSpA than in controls (all $p < 0.001$). Serum levels of C1M, C3M, and C4M2 were increased in AS compared to nr-axSpA (43.4 ng/mL vs. 34.6; $p < 0.001$, 15.4 vs. 12.8; $p = 0.001$, and 27.8 vs. 22.4; $p < 0.001$). The best metabolite to differentiate between axSpA and controls was C3M (AUC 0.95; specificity 92.0, sensitivity 83.4). C1M correlated with ASDAS-CRP in nr-axSpA ($\rho = 0.37$; $p < 0.001$) and AS ($\rho = 0.57$; $p < 0.001$). C1M, C3M, and C4M2 were associated with ASDAS-CRP in AS and nr-axSpA after adjustment for age, gender, and disease duration. Serum levels of collagen metabolites were significantly higher in AS and nr-axSpA than in controls. Moreover, the present study indicates that collagen metabolites reflect disease activity and are useful biomarkers of axSpA.

Axial spondyloarthritis (axSpA) is a chronic inflammatory disorder that is characterised by sacroiliitis, inflammation of the spine and extra-musculoskeletal involvement. AxSpA generally manifests in early adulthood and men are more frequently affected than women. Most patients are also HLA-B27 positive. Two pathogenic events are the main contributors to disease burden, namely, disease-related inflammation and osteoproliferation in the axial skeleton[1]. Patients are divided into two sub-populations according to imaging features by the Assessment of SpondyloArthritis International Society (ASAS)[2]. Ankylosing spondylitis (AS) fulfils the modified New York criteria, including determination of radiographic sacroiliitis[3], whereas the non-radiographic (nr)-axSpA does not meet these criteria. However, the inflammation-related pattern assessed by magnetic resonance imaging (MRI), such as bone marrow oedema with or without structural changes within the sacroiliac joints (SIJ), may be present in some nr-axSpA patients[4]. The clinical features of both axSpA subtypes include all, axial, peripheral and extra-articular symptoms. Back pain and peripheral arthritis or enthesitis are equally distributed between the axSpA subtypes, but uveitis occurs more frequently in AS[5]. Conventional synthetic disease-modifying drugs and biologics are currently recommended for the pharmacological treatment of the axSpA subtypes, but the monoclonal antibody tumour necrosis factor (TNF)-α inhibitor, infliximab and the inhibitor of interleukin (IL)-17 secukinumab are reserved only for AS[6]. Nr-axSpA was suggested as an early phase of AS because 4.9–11.6% of nr-axSpA patients develop radiographic sacroiliitis over two years[7,8] and 19% develop radiographic sacroiliitis during a follow-up of 10 years[9]. Serological markers of disease activity that identify patients who structurally progress rapidly are highly needed for clinical trial enrichment and personalised health care[10,11].

[1]Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine Charles University in Prague, Na Slupi 4, Prague, 128 50, Czech Republic. [2]Department of Rheumatology, Nordic Bioscience, Biomarkers and Research, Herlev, Denmark. Correspondence and requests for materials should be addressed to M.H. (email: fojtikova05@gmail.com)

Inflammation is currently assessed by C-reactive protein (CRP), which is elevated in AS patients compared to nr-axSpA patients[8,12,13]. An increase in CRP may arise from many pathological events, such as a common cold or chronic inflammation. Therefore, CRP may not be able to identify the pathogenic-related inflammation occurring locally within the affected joints. The extracellular matrix (ECM) is a network of collagens, glycosaminoglycans and other molecules and fibrils filling the intercellular spaces that undergo substantial changes during inflammation and reflect pathological events, such as inflammatory cell influx. ECM proteins are prone to degradation by proteases, such as matrix metalloproteinases (MMPs), which result in the release of protease-specific metabolites. These MMP-degraded ECM metabolites that are produced locally during inflammatory escalated ECM turnover may be detected as serum biomarkers of ECM tissue turnover, which would reflect local pathogenic processes[14]. Serological levels of MMP-3, MMP-8, and MMP-9 are elevated in AS, especially in patients with higher disease activity and structural progression[15,16].

Type I, II, III, and IV collagens are expressed in the ECM of different joint tissues (articular and hyaline cartilage, tendons, bone, and connective tissue) and are prone to degradation by proteases. The MMP-mediated metabolite of type I collagen (C1M) reflects soft tissue destruction, and it is elevated in AS[17] and rheumatoid arthritis (RA)[18,19]. C2M is an MMP-mediated metabolite of type II collagen, and it reflects cartilage destruction[20]. An MMP-mediated metabolite of type III collagen (C3M) reflects soft tissue degradation. C2M and C3M are higher in AS patients compared to controls[17,21], and C3M is found in the inflamed tissue of liver fibrosis[22]. The main collagen of the basement membrane is type IV collagen, and an MMP-mediated metabolite of type IV collagen (C4M2) is higher with soft tissue destruction[23]. C1M, C2M, and C3M were recently associated with the response to biologic therapy in AS[21] and were correlated with CRP, erythrocyte sedimentation rate (ESR) and radiographic severity[24]. Therefore, higher ECM turnover might be a common pathogenic event in axSpA, and products of MMP-degraded collagens may be biomarkers of disease activity in axSpA. However, whether the levels of MMP-degraded collagen products are different between radiographic and non-radiographic axSpA and whether they are associated with disease activity is unknown.

In our study, we investigated the following factors: (1) the profile of serological MMP-mediated products of type I, II, III, and IV collagens (C1M, C2M, C3M and C4M2) in AS and nr-axSpA; (2) whether ECM metabolites could separate the two axSpA forms; and (3) whether these metabolites were associated with disease activity.

Results

Demographic description. The demographics of AS and nr-axSpA patients are characterised in Table 1. AS and nr-axSpA patients differed significantly in the following variables: disease duration since the first symptoms, CRP levels, and gender (all $p < 0.001$) and peripheral arthritis ($p < 0.01$). AS patients exhibited higher structural scores (modified Stoke ankylosing spondylitis spine score [mSASSS], $p < 0.05$, Spondyloarthritis research consortium of Canada [SPARCC] MRI and Berlin MRI, both $p < 0.01$) compared to nr-axSpA patients, but this difference was due to the group inclusion criteria. Both subgroups of axSpA were comparable in current or previous medications, disease activity and quality of life (Table 1 and Supplementary Table S1).

Asymptomatic controls were sex and age matched to nr-axSpA patients, but in comparison to AS were older (age at the time of analysis: 38.1; 95% CI 36.3–39.9), had different gender distribution (women predominance 51%) and higher body mass indexes (BMIs) (mean 35.4; 95% CI 24.7–26.1); all $p < 0.05$. As expected, both AS and nr-axSpA had significantly higher CRP levels than asymptomatic controls (mean 1.22; 95% CI 0.88–1.61), all $p < 0.001$.

ECM metabolism is accelerated in axSpA patients particularly in the AS subgroup. MMP-degraded collagen type I, II, III and IV products were significantly increased in axSpA patients compared to asymptomatic controls (Supplementary Table S2). We looked for the relationships between all four biomarkers. C1M, C3M, and C4M2 were moderately to highly correlated to each other (all Spearman's $\rho > 0.53$; all $p < 0.001$) and C2M was only weakly correlated to C1M ($\rho = 0.25$; $p < 0.001$), but not to the others biomarkers (Supplementary Table S3).

Patients of both axSpA subtypes, namely, AS and nr-axSpA, expressed significantly higher levels of all four collagen metabolites than the asymptomatic controls: C1M 43.4 (95% CI 38.0–51.1) and 34.6 (29.3–36.5) vs. 24.5 (20.4–24.3) respectively; C2M 0.35 (0.32–0.37) and 0.36 (0.34–0.38) vs. 0.26 (0.24–0.28) respectively; C3M 15.4 (14.3–16.2) and 12.8 (12.3–13.8) vs. 7.8 (7.1–8.3), respectively; and C4M2 27.8 (25.4–30.8) and 22.4 (21.0–24.8) vs. 15.2 (14.7–15.7) respectively (Fig. 1a–d, all $p < 0.001$). However, the serum levels of the MMP-degraded products of collagen C1M, C3M, C4M2 were significantly higher in AS patients compared to nr-axSpA patients ($p < 0.001$, $p = 0.001$, $p < 0.001$, respectively), but the levels of C2M were comparable between AS and nr-axSpA patients (Fig. 1a–d, Table 1). In both subgroups, the three biomarkers: C1M, C3M and C4M2 correlated significantly to each other (for all analyses, Spearman's $\rho > 0.40$; all $p < 0.001$), Table 2. The biomarker C2M, however, correlated only modestly with C1M (for AS Spearman's $\rho = 0.36$; $p < 0.01$ and for nr-axSpA Spearman's $\rho = 0.19$; $p < 0.05$), but not with C3M and C4M2 (Table 2).

The products of ECM turnover differentiated AS and nr-axSpA from asymptomatic controls. While serum levels of all four tested MMP-degraded collagen products and CRP levels were higher in AS and nr-axSpA than in controls we investigated their abilities to identify axSpA patients (AS and nr-axSpA) from asymptomatic controls using Area under receiver operator characteristics curve (AUC ROC). C3M had the highest AUC to differentiate between axSpA and asymptomatic controls. The AUC of C3M was 0.95, with a specificity and sensitivity of 92.0 and 83.4, respectively, and an odds ratio of 30.9 (95% CI 4.0–236.7), see Table 3. C3M was also the best metabolite for the identification of nr-axSpA and AS patients from asymptomatic controls. The AUC for nr-axSpA was 0.93 [specificity 80.0 and sensitivity 92.6, odds ratio of 22.9 (3.0–175.7)], and the AUC for AS was 0.98 [specificity 92.0, sensitivity 91.7, odds ratio of 111.2 (37.7–327.7)], see Table 3. An AUC higher than

	nr-axSpA (n = 121)		AS (n = 72)		nr-axSpA vs. AS
	Mean	95% CI	Mean	95% CI	P-value
Age at the time of study (years)	37.5	35.5–39.6	34.5	32.3–36.8	*0.110*
Disease duration since first symptom (years)	7.9	6.3–9.5	10.0	7.9–12.1	*<0.001*
Gender (% female)	59		32		*<0.001*
BMI	25.1	24.2–26.0	24.4	23.5–25.4	*0.390*
ASDAS-CRP	2.0	1.8–2.2	2.2	2.0–2.4	*0.130*
ASQoL	5.3	4.4–6.2	5.7	4.5–6.8	*0.450*
BASDAI	3.0	2.6–3.4	2.6	2.1–3.0	*0.410*
BASFI#	1.0	0.7–1.4	1.3	1.0–1.9	*0.190*
EQ-5D	0.66	0.61–0.71	0.66	0.60–0.72	*0.400*
Swollen joint count	0.43	0.24–0.62	0.17	−0.014–0.35	*0.009*
Current therapy: NSA (%)	31		33		*0.749*
Current therapy: csDMARD/bDMARD (%)	27/1		11/4		*0.299/0.142*
Berlin MRI#	2.0	1.0–2.0	4.5	2.4–7.0	*0.003*
mSASSS	0.74	0.17–1.31	4.83	1.47–8.19	*0.018*
SPARCC MRI	6.8	5.2–8.5	14.7	10.2–19.3	*0.003*
Biomarker's levels					
CRP (mg/l)#	2.7	1.7–4.0	7.7	4.4–12.0	*<0.001*
ESR (mm/h)	11.3	9.0–13.5	14.3	11.3–17.3	*0.073*
C1M (ng/ml)	34.6	29.3–36.5	43.4	38.0–51.1	*<0.001*
C2M (ng/ml)	0.36	0.34–0.38	0.35	0.32–0.37	*0.856*
C3M (ng/ml)	12.8	12.3–13.8	15.4	14.3–16.2	*0.001*
C4M2 (ng/ml)	22.4	21.0–24.8	27.8	25.4–30.8	*<0.001*

Table 1. Demographic and Biomarker's Table of the study. Mean or median with 95% CI - confidence interval is presented. Characteristics marked with # indicate where the median was used. Mann-Whitney T-test and Fisher's exact test were used. Abbreviations: AS: Ankylosing spondylitis, nr-axSpA: non-radiographic axial spondyloarthritis, BMI: body mass index, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, ASDAS-CRP: AS disease activity score CRP, ASQoL: AS quality of life, BASDAI: Bath AS disease activity score, BASFI: Bath AS functional index, EQ-5D: EuroQol five dimension scale, NSA: non-steroidal antirheumatic drugs, csDMARD: conventional synthetic disease-modifying drugs, bDMARD: biological disease-modifying drugs, MRI: magnetic resonance imagining, mSASSS: modified Stoke ankylosing spondylitis spine score, SPARCC: Spondyloarthritis research consortium of Canada, C1M: metalloproteinase (MMP)-degraded type I collagen, C2M: MMP-degraded type II collagen, C3M: MMP-degraded type III collagen, C4M2: MMP-degraded type IV collagen alpha 3.

0.90 was also found for C1M and C4M2 to discriminate AS and asymptomatic controls, but with lower sensitivity and specificity.

Moreover, for identifying AS or nr-axSpA from asymptomatic controls, all tested MMP-degraded collagen products, except C2M in the case of AS, had higher AUCs than CRP (see Table 3). The AUC of CRP levels was lowest of all serum biomarkers for the separation of nr-axSpA from asymptomatic controls (see Table 3). On the other hand, however, C1M, C3M, C4M2, and CRP had significant, albeit weak, AUCs for differentiating AS from nr-axSpA (AUC 0.65, 0.64, 0.67 and 0.66, respectively), Table 3.

We also investigated the difference in biomarker levels between groups when adjusted for age, gender, BMI, disease duration and CRP using logistic regression. The C1M, C3M and C4M2 remain significant after adjustment for age, gender, disease duration and BMI and only C4M2, when additionally adjusted for CRP (Supplementary material, Table S4).

The C1M levels were higher in females than in males (median 38.0 [95% CI 34.8–45.7] vs. 52.6 [42.6–63.55], p = 0.025) in AS, but not in nr-axSpA (32.7 [26.8–38.7] vs. 34.8 [28.8–39.0], p = 0.77; data not shown). C3M levels in nr-axSpA patients were higher in patients with the extra-articular manifestations than in patients without this symptom (15.0 [13.8–16.8] vs. 12.2 [11.0–12.7], p = 0.0003; data not shown). No differences in all MMP-degraded collagen type I, II, III and IV products serum levels were observed within the AS and nr-axSpA groups with different disease duration, involvement of peripheral joint, presence of uveitis, smoking status, current or previous pharmacological therapy, or HLA-B27 status (data not shown).

The MMP-degraded collagen type I, II, III and IV products are related to disease activity of AS and nr-axSpA. We next investigated the correlation between ECM turnover metabolites and clinical assessments. Due to correction for multiple comparisons, the alpha value was 0.00625 for this analysis. The ECM metabolites were not correlated with age, disease duration, or presence of peripheral arthritis reflected in the swollen joints count (SJC) (Table 2). C1M, C3M, and C4M2 were moderately to strongly correlated to CRP (for

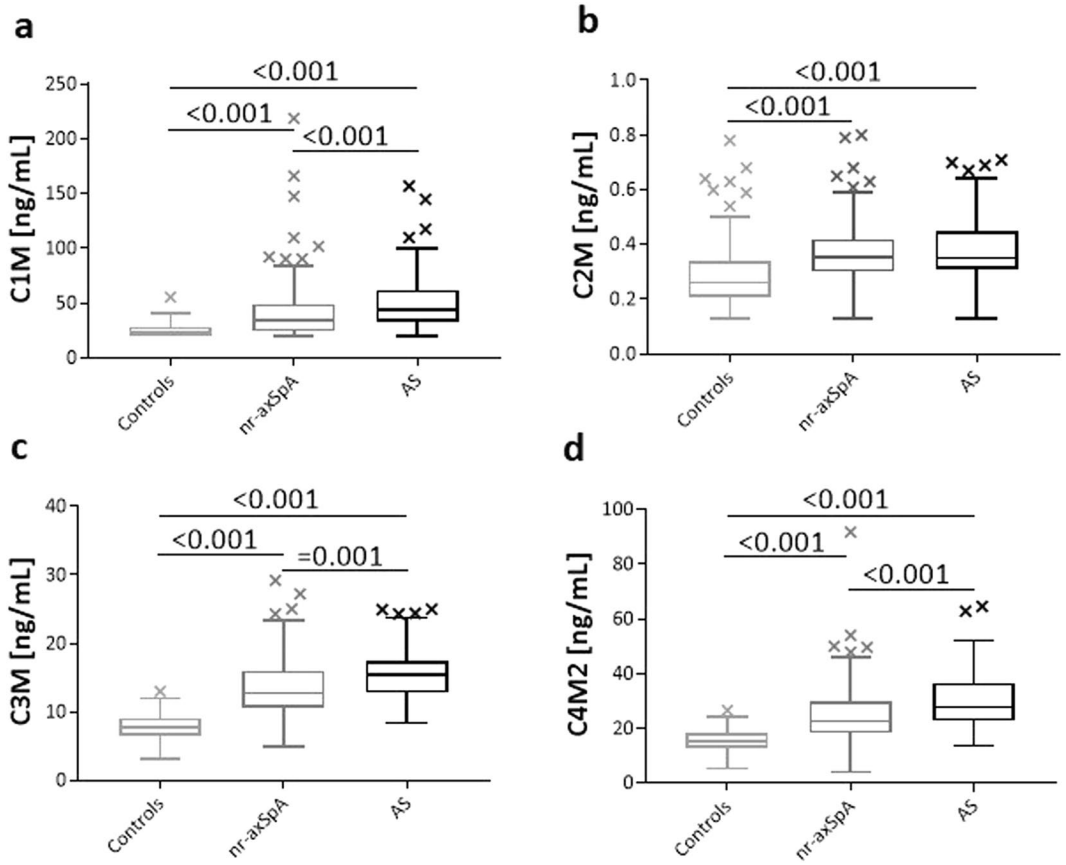

Figure 1. Metabolite levels in the AS group, nr-axSpA group and asymptomatic controls. (**a**) Type I collagen degraded by MMPs. (**b**) Type II collagen degraded by MMPs. (**c**) Type III collagen degraded by MMPs. (**d**) Type IV collagen degraded by MMPs. Kruskal-Wallis was used for differences between the three groups with Dunn's multi-comparisons test. Statistical significant differences between groups are reported ($P < 0.001$ or $P = 0.001$). Data are shown as Tukey's box plots.

all analyses, Spearman's $\rho > 0.36$; all $p < 0.001$) and ESR (for all analyses, Spearman's $\rho > 0.30$; all $p < 0.01$) in the AS and nr-axSpA groups (Table 2). C2M moderately correlated to ESR only in AS patients ($\rho = 0.39$; $p < 0.001$), Table 2.

The values of the Bath AS function index (BASFI) and the quality of life questionnaires [EuroQol five dimension scale (EQ-5D), and AS quality of life (ASQoL)] were similar in the AS and nr-axSpA patients, but the correlations differed between the groups. C1M in the AS patients correlated to the BASFI ($\rho = 0.38$; $p < 0.001$), and C1M and C2M correlated with the ASQoL ($\rho = 0.33$ and $\rho = 0.38$, respectively; both $p < 0.01$), Table 2. There were no correlations of ECM turnover metabolite levels to the scores of radiographic severities, albeit C1M and C4M2 tended to correlate to mSASSS in the AS subgroup, Table 2.

Although the AS disease activity score (ASDAS)-CRP level was not different between AS and nr-axSpA patients, there were differences in correlations to the MMP-degraded collagen products. The C1M levels correlated with the ASDAS-CRP in the nr-axSpA ($\rho = 0.37$; $p < 0.001$) and AS ($\rho = 0.57$; $p < 0.001$) subgroups (Table 2). C2M and C4M2 correlated to ASDAS-CRP only in the AS patients ($\rho = 0.34$; $p < 0.05$ and $\rho = 0.38$; $p = 0.01$, respectively), and C3M correlated weakly to ASDAS-CRP only in the nr-axSpA patients ($\rho = 0.27$; $p < 0.01$), Table 2. However, disease activity assessed by the Bath disease activity index (BASDAI) did not correlate to the four products of collagen degradation. The differences in these results may be the correlation of the metabolites to CRP, which is part of the ASDAS-CRP score, but not the BASDAI.

The multiple regression model was used to investigate the relationship of MMP-degraded collagen products to disease activity (ASDAS-CRP) with adjustment for confounders. Notably, when metabolites were adjusted for age, gender, BMI, and disease duration, the relationship of all four biomarkers to ASDAS-CRP remained at the same level as the unadjusted model (Table 4). C2M reflected the disease activity only in AS. C1M, C3M and C4M2 reflected the changes of disease activity in both subgroups, but somewhat more clearly in AS. For example, if ASDAS-CRP was altered one unit, then C1M showed a change of 0.57 in AS and 0.40 in nr-axSpA patients (Table 4). However, for these analyses, the effect-size (beta) was too low, and the association was not considered clinically relevant.

	C1M				*C2M*				*C3M*				*C4M2*			
	nr-axSpA		**AS**		**nr-axSpA**		**AS**		**nr-axSpA**		**AS**		**nr-axSpA**		**AS**	
	ρ	***P value***	**ρ**	***P value***	**ρ**	***P value***	**ρ**	***P value***	**ρ**	***P value***	**ρ**	***P value***	**ρ**	***P value***	**ρ**	***P value***
Age at diagnosis	−0.05	*0.59*	0.17	*0.16*	−0.02	*0.79*	−0.08	*0.045*	0.03	*0.78*	0.19	*0.12*	−0.05	*0.57*	0.20	*0.099*
Disease duration	−0.03	*0.76*	0.12	*0.32*	−0.04	*0.57*	−0.02	*0.76*	−0.00	*0.99*	0.23	*0.057*	−0.11	*0.24*	0.14	*0.24*
SJC	0.10	*0.27*	0.06	*0.63*	0.21	*0.035*	0.16	*0.21*	−0.01	*0.90*	−0.03	*0.83*	0.03	*0.78*	0.12	*0.33*
CRP	**0.57**	***<0.001***	**0.71**	***<0.001***	0.12	*0.19*	0.21	*0.075*	**0.36**	***<0.001***	**0.44**	***<0.001***	**0.42**	***<0.001***	**0.59**	***<0.001***
ESR	**0.38**	***<0.001***	**0.66**	***<0.001***	0.01	*0.94*	**0.39**	***<0.001***	**0.30**	***<0.001***	**0.36**	***0.0019***	**0.35**	***<0.001***	**0.53**	***<0.001***
ASDAS-CRP	**0.37**	***<0.001***	**0.57**	***<0.001***	0.06	*0.62*	**0.34**	***0.016***	**0.27**	***0.0029***	0.28	*0.07*	0.24	*0.0079*	**0.38**	***0.010***
BASDAI	0.11	*0.23*	0.25	*0.038*	0.05	*0.83*	0.23	*0.13*	0.10	*0.30*	0.14	*0.023*	0.02	*0.86*	0.13	*0.28*
ASQoL	−0.05	*0.56*	**0.33**	***<0.01***	0.02	*0.77*	**0.38**	***0.0046***	0.07	*0.42*	0.07	*0.58*	−0.11	*0.23*	0.17	*0.15*
EQ-5D	0.01	*0.88*	−0.31	*0.009*	−0.08	*0.59*	−0.32	*0.007*	−0.01	*0.93*	−0.15	*0.22*	0.11	*0.22*	−0.23	*0.052*
BASFI	0.01	*0.94*	**0.38**	***<0.001***	−0.05	*0.41*	0.29	*0.016*	−0.04	*0.64*	0.20	*0.091*	−0.06	*0.55*	0.29	*0.012*
Berlin MRI	0.03	*0.80*	0.26	*0.18*	−0.11	*0.30*	0.32	*0.071*	−0.10	*0.35*	0.02	*0.95*	−0.06	*0.55*	0.11	*0.58*
mSASSS	−0.02	*0.87*	0.33	*0.009*	0.07	*0.52*	0.02	*0.95*	−0.08	*0.47*	0.23	*0.77*	−0.09	*0.42*	0.29	*0.026*
SPARCC MRI	0.06	*0.56*	0.29	*0.14*	−0.09	*0.41*	0.34	*0.075*	−0.09	*0.42*	0.02	*0.94*	−0.04	*0.72*	0.11	*0.58*
C1M	—	—	—	—	**0.19**	***0.034***	**0.36**	***0.002***	**0.52**	***<0.001***	**0.40**	***<0.001***	**0.61**	***<0.001***	**0.58**	***<0.001***
C2M	—	—	—	—	—	—	—	—	0.10	*0.28*	0.17	*0.16*	0.02	*0.87*	0.20	*0.087*
C3M	—	—	—	—	—	—	—	—	—	—	—	—	**0.71**	***<0.001***	**0.63**	***<0.001***

Table 2. Correlation of the MMP-degraded collagen type I, II, III and IV serum products with clinical variables and biomarkers. Spearman's correlation between serological metabolites and clinical assessments were done. After Bonferroni correction, the alpha value was 0.00625 (0.05/8). Spearman's rho (ρ) and p- values are presented. The italic P value demonstrates the exact value before correction, the bolt italic P value determines significance on $p < 0.05$; $p < 0.01$ and $p < 0.001$ after correction. The recurring values (ρ and P value) of the correlation between C1M, C2M, C3M and C4M2 are not shown in the Table. Abbreviations: AS: Ankylosing spondylitis, nr-axSpA: non-radiographic axial spondyloarthritis, SJC: swollen joint count, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, ASDAS-CRP: AS disease activity score CRP, BASDAI: Bath AS disease activity score, ASQoL: AS quality of life, EQ-5D: EuroQol five-dimension scale, BASFI: Bath AS functional index, MRI: magnetic resonance imagining, mSASSS: modified Stoke ankylosing spondylitis spine score, SPARCC: Spondyloarthritis research consortium of Canada, C1M: metalloproteinase (MMP)-degraded type I collagen, C2M: MMP-degraded type II collagen, C3M: MMP-degraded type III collagen, C4M2: MMP-degraded type IV collagen alpha 3.

Discussion

This cross-sectional study investigated the level of ECM tissue turnover metabolites (C1M, C2M, C3M, and C4M2) in axSpA. We investigated whether MMP-degraded collagen metabolite levels, measured in serum, were different in radiographic axSpA (AS) compared to non-radiographic axSpA, and whether these metabolites were associated with disease activity. We found that the metabolite levels were higher in axSpA (both nr-axSpA and AS) compared to asymptomatic controls and all metabolites, except C2M, were higher in AS compared to nr-axSpA. Next, the ECM metabolites, C3M in particular, differentiated axSpA from asymptomatic controls, but not AS from nr-axSpA. Finally, C1M, C3M, and C4M2 were associated with quality of life, function index, and disease activity on the ASDAS-CRP in nr-axSpA and AS patients. All the tested biomarkers, except C2M in nr-axSpA patients, had weak to moderate associations to ASDAS-CRP. Our results indicate that collagen degradation metabolites are higher in axSpA and these metabolites may be disease activity biomarkers of axSpA.

Although numerous similarities, such as disease activity and response to pharmacological therapy, including TNF-α inhibitors, between nr-axSpA and AS were observed in longitudinal studies[5,8,13,25], both forms of axSpA generally differ in the severity of inflammatory changes evaluated in serum using CRP assessment or imaging methods within the sacroiliac joints and spine[25,26]. As expected, features, such as predominance in women, shorter disease duration, milder radiographic status and lower CRP level, were more common in nr-axSpA than AS in our study. However, a higher occurrence of peripheral arthritis was found in the nr-axSpA subgroup compared to the AS subgroup. A recent meta-analysis revealed that the occurrence of peripheral arthritis tended to be higher in nr-axSpA than in AS[5]. More recently, de Winter *et al.*[27] suggested that half of axSpA patients suffered from a combination of axial and peripheral symptoms, but these patients did not differ in the presence of radiographic sacroiliitis from patients with only pure axial symptoms.

All of the MMP-degraded collagen products assessed in this study were higher in nr-axSpA and AS patients than asymptomatic controls and correlated with CRP. All of the analysed products indicate an accelerated ECM turnover and reflect different events in the immune-musculoskeletal pathology of axSpA because collagen types I and III are expressed in the bone, tendons and ligaments, collagen type II is primarily expressed in the entheses and cartilages, and type IV is part of the basement membrane. All these collagens are naturally substrates for several metalloproteinases, including the MMPs that are highly expressed during inflammation. Serum levels of MMP-1, 2, 3, 8 and -9 reflect disease activity and progression[15,16,28] and MMP-3 expressed locally within in the

Biomarker	*Criterion (cutt-off)*	*AUC*	*Specificity*	*Sensitivity*	*Odds ratio (95% CI)*	
AS vs. asymptomatic controls						
C1M	>29.0	0.90*	79.0	87.5	24.8	(10.7–57.7)
C2M	>0.28	0.71*	57.0	82.9	6.6	(3.2–13.8)
C3M	>10.5	0.98*	92.0	91.7	111.2	(37.7–327.7)
C4M2	>20.6	0.96*	91.0	87.5	70.8	(26.6–188.2)
CRP	>4.2	0.82*	88.0	62.5	10.2	(4.9–21.4)
nr-axSpA vs. asymptomatic controls						
C1M	>32.3	0.77*	90.0	54.5	9.1	(4.5–18.4)
C2M	>0.29	0.72*	61.0	78.2	5.2	(2.9–9.3)
C3M	>6.3	0.93*	80.0	92.6	22.9	(3.0–175.7)
C4M2	>16.9	0.84*	72.0	84.3	13.8	(7.2–26.6)
CRP	>2.3	0.66*	76.0	53.7	3.3	(1.9–5.9)
axSpA vs. asymptomatic controls						
C1M	>32.3	0.82*	90.0	62.7	12.6	(6.4–24.6)
C2M	>0.29	0.71*	61.0	77.2	5.3	(3.1–8.9)
C3M	>10.5	0.95*	92.0	83.4	30.9	(4.0–236.7)
C4M2	>20.5	0.89*	90.0	73.6	7.2	(4.2–12.3)
CRP	>3.8	0.72*	85.0	51.3	3.0	(1.8–5.1)
AS vs. nr-axSpA						
C1M	>29.2	0.65*	41.3	87.5	4.6	(2.1–10.1)
C2M	>0.42	0.51	76.0	31.9	1.4	(0.8–2.8)
C3M	>13.3	0.64*	57.0	73.6	7.6	(3.4–16.7)
C4M2	>23.3	0.67*	55.4	73.6	3.5	(1.8–6.5)
CRP	>10.7	0.66*	82.6	44.4	3.6	(1.9–6.9)

Table 3. Diagnostic utility values of the collagen metabolite levels for axial spondyloarthritis. The analyses were performed using AUC ROC, and p-values, specificity and sensitivity are provided. Criterion (cut-off) determined the serum levels of each biomarker to differentiate between groups. *Means statistical significance of $p < 0.001$. Abbreviations: AS: ankylosing spondylitis, nr-axSpA: non-radiographic axial spondyloarthritis, C1M: metalloproteinase (MMP)-degraded type I collagen, C2M: MMP-degraded type II collagen, C3M: MMP-degraded type III collagen, C4M2: MMP-degraded type IV collagen alpha 3, AUC: Area under receiver operator characteristics curve.

		Unadjusted			*Adjusted for age, gender, BMI, disease duration*		
		Beta (SD)	**r-partial**	***P value***	**Beta (SD)**	**r-partial**	***P value***
C1M	**AS**	0.42 (0.10)	0.46	*<0.0001*	0.57 (0.11)	0.47	*0.0001*
	nr-axSpA	0.39 (0.07)	0.46	*<0.0001*	0.40 (0.07)	0.46	*<0.0001*
C2M	**AS**	0.31 (0.11)	0.32	*0.0063*	0.30 (0.12)	0.30	*0.015*
	nr-axSpA	0.03 (0.10)	0.03	*0.76*	0.04 (0.10)	0.04	*0.66*
C3M	**AS**	0.36 (0.13)	0.31	*0.0086*	0.43 (0.14)	0.35	*0.0041*
	nr-axSpA	0.37 (0.09)	0.33	*0.0002*	0.34 (0.10)	0.32	*0.0005*
C4M2	**AS**	0.42 (0.11)	0.42	*0.0003*	0.44 (0.12)	0.43	*0.0004*
	nr-axSpA	0.40 (0.08)	0.41	*<0.0001*	0.41 (0.08)	0.43	*<0.0001*

Table 4. Multiple regression analyses of the relationship between MMP-degraded collagen type I, II, III and IV products and ASDAS-CRP. Beta with SD and r-partial are shown, and the analyses were performed on z-scores. The dependent variables were C1M, C3M, C3M and C4M2, and the independent variable was ASDAS-CRP. The following covariates were tested: Age gender, BMI and disease duration. Abbreviations: AS: ankylosing spondylitis, nr-axSpA: non-radiographic axial spondyloarthritis, BMI: body mass index, SD: standard deviation, C1M: metalloproteinase (MMP)-degraded type I collagen, C2M: MMP-degraded type II collagen, C3M: MMP-degraded type III collagen, C4M2: MMP-degraded type IV collagen alpha 3.

inflamed tissue[29] participates in the activation of other MMPs and the changes that lead to osteogenesis[29]. The sources of MMPs may be fibroblasts and immune cells, such as macrophages. The serum levels of C1M, C3M and C4M2 in our study indicated an accelerated ECM turnover of soft tissue and joint structures. All three of these biomarkers tightly correlated with CRP and were higher in AS patients than in nr-axSpA patients, which may reflect the different degrees of inflammation of these two entities. An increased number of inflammatory spinal lesions in AS patients than in nr-axSpA patients was found previously[26,30] and a recent study demonstrated

increased entheseal abnormalities in axSpA patients with worse structural damage[31]. We did not find correlations between the severity of MRI lesions in the SIJ and biomarkers levels, but the mSASSS tended to correlate with C1M and C4M2 in AS. A recent study did not confirm C1M as a prognostic marker for the structural progression for AS patients with longstanding disease[32]. Bay-Jensen *et al.*[24] demonstrated a correlation between C3M serum levels and disease activity and structural damage characterised by mSASSS in AS patients, and C3M showed a prognostic capacity for disease structural burden. However, the disease duration of our patients with AS and the scores of mSASSS were lower than in previous studies. Therefore, further work with axSpA patients at follow-up evaluations to monitor structural changes in disease is necessary to determine if the MMP-degraded collagen products may act as prognostic biomarkers, particularly at different stages of the disease. On the other hand, we detected increased levels of C2M in nr-axSpA and AS patients. The C2M biomarker was similar in both groups and it did not correlate with CRP or radiographic scores. This finding is consistent with Bay-Jensen's study in which C2M was not associated with mSASSS or CRP in AS patients with longstanding disease, but high serum levels of C2M together with C3M predicted structural progression[24]. The main tissues with collagen type II are cartilage and entheses and our findings suggest similar pathogenetic events in the extracellular matrix in AS and nr-axSpA. However, a follow-up of nr-axSpA is necessary to determine whether C2M will have additional effects on the prognostic capacity of the other MMP-degraded collagen biomarkers. C1M and C3M were recently associated with disease activity in psoriatic arthritis[33], and C1M serves as a metabolite of rapid structural progression in RA[18]. Kang *et al.*[34] found that another serological type I collagen degradation metabolite, the C-terminal telopeptide of type I collagen (sCTX-I), reflected the intensity of MRI-established bone marrow oedema within the SIJ in AS, but not nr-axSpA, patients. In a SpA model, a lowered resistance to mechanical stress maintained the entheseal inflammation, which may induce new bone formation[35]. Increased degradation of type I, II, and IV collagens (C1M, C2M, C4M2) may result in the insufficient formation of the structures of the joints, enthesis, and adjacent structures and participate in their biomechanical insufficiency.

The overlap between AS and nr-axSpA may be associated with disease activity because patients with higher biomarker levels may be the patients who progress rapidly, and patients with lower levels may be stable patients. Although, the positive correlation between ASDAS-CRP and biomarkers in AS and nr-axSpA patients are supportive, this hypothesis should be tested in longitudinal studies. A recent work demonstrated the efficacy of C1M to reflect improvement of disease activity after TNF-α inhibitor therapy in AS patients[21]. The weak association between C2M and ASDAS-CRP in AS patients, but not in nr-axSpA patients, may reflect the severity of cartilage destruction in the fully blunted disease. This association indicates a relationship between disease activity and disease burden, but this connection must be confirmed. Based on previous results from AS and other pathologies, this type of biomarker could aid in the identification of patients with rapid progression, who are most in need of immediate treatment.

Although the mean ECM metabolites levels were higher in AS compared to nr-axSpA patients, there was too great of an overlap (large variation) in metabolite levels for the metabolites to provide good separation of the axSpA groups. C3M was the best metabolite for segregating AS from nr-axSpA and asymptomatic controls, which is consistent with a previous study that found that C3M was better than C2M[17]. C1M, C3M and C4M2 performed better in differentiating AS and nr-axSpA from asymptomatic controls than CRP. However, for true diagnostic purposes, the metabolites should be included in a diagnostic panel to ensure good specificity and sensitivity in the diagnosis and identification of disease subgroups. In our study, C3M has very high sensitivity for AS and nr-axSpA to discriminate from healthy individuals (91.7 and 92.6, respectively), but the specificity remain higher in AS (92.0) than in nr-axSpA (80.0). As the commonly used CRP has the lower specificity and sensitivity for AS (88.0 and 62.5, respectively) and nr-axSpA (76.0 and 53.7, respectively) in our study, the findings of C3M might suggest the new potential serological biomarker for both axSpA subtypes. Although these results show potential, larger studies are essential for the application of C3M as potential diagnostic choice for AS and nr-axSpA particularly if C3M will accomplish enough strength for prediction of the disease. However, whether a serological diagnostic criteria or method is needed is debatable, because these biomarkers would be assessed alongside the already used CRP. On the other hand, additional information about the C3M (and others MMP-degraded metabolites) in relation to disease phenotype and pathogenesis could be useful for therapeutic decisions in individual patient.

Axial spondyloarthritis is a longstanding disorder, and epidemiological studies suggest an increased cardiovascular disease risk[36]. Systemic cardiovascular impairment, such as atherosclerotic changes, were found in AS patients, but not nr-axSpA patients, but the number of atherosclerotic plaques was increased with extra-articular manifestations in nr-axSpA patients[37]. A large epidemiologic cohort of postmenopausal women found higher levels of C1M as an independent mortality risk factor for several diseases, including cancer and cardiovascular disorders[38]. Degradation of type I collagen assessed with C1M was higher in women with AS and nr-axSpA in the current study. The cardiovascular complications are estimated as most frequent in men with AS compared to the general population[39], but an equal increased risk of cancer is estimated for both sexes[40]. Data in a recent meta-analysis showed that nr-axSpA and AS patients do not differ in extra-articular manifestations[5], which was confirmed in the current study. Notably, C3M levels were higher in nr-axSpA patients with a prevalence of extra-articular manifestation compared to nr-axSpA patients with no extra-articular manifestation. This result illustrates that C3M is not exclusively originating from tissues of the joint but may originate from the tissues of the extra-articular manifestations. C1M and C3M reflect fibro-proliferative and cardiovascular changes, and future studies should elucidate the relationships between systemic involvement and collagen tissue turnover in axSpA. Understanding this relationship may be useful to distinguish patients with a higher risk for systemic complications for earlier and personalised treatment of these patients and provide knowledge of metabolites levels in patients with several pathologies. Each pathology may add to the total pool of metabolites and be a false positive for another pathology, if the pathology is undiagnosed.

Some limitations should be considered during the interpretation of the current work. First, this study was a cross-sectional study, and we were not able to investigate changes in biomarker levels with changes in disease activity over time. However, the disease duration since the first symptoms was long, and the disease may be well established with stable disease activity. Furthermore, the disease duration was significantly longer in AS patients compared to nr-axSpA patients. However, this difference was tested in the multiple regression analysis, and adjustment for disease duration did not change the results significantly. Second, another limitation is the lack of clinical information about cardiovascular and other systemic complications. These complications may affect the MMP-degraded collagen products levels and undermine the signals related to axSpA. Finally, our control group was asymptomatic individuals without personally reported clinical symptoms of back pain, but they did not undergo clinical or radiographic examination. Therefore, any underlying non-diagnosed diseases could have influenced the metabolite levels.

Conclusion

This study demonstrated elevated serum levels of ECM metabolites (C1M, C2M, C3M, and C4M2) in radiographically determined forms of axSpA compared to controls. Our results support the distinct tissue degenerative events in different axSpA population (i.e. nr-axSpA and AS). The serum levels of products of MMP-degraded collagen, especially C1M, C3M and C4M2, was significantly associated with disease activity, linking tissue turnover with clinical activity of disease. C3M was the best biomarker at separating AS and nr-axSpA, but investigation of C3M as a true diagnostic tool within axSpA, must be studied in more and larger studies. However, this current study illustrates the potential of serological metabolites of tissue destruction as novel disease activity biomarkers in axSpA.

Methods

Patients. Patients (n = 193) with recently diagnosed axSpA were included into the Prague Axial SPondyloArthritis Cohort (PRASPAC)[41]. The inclusion criteria were a maximum of three years since the diagnosis of axSpA, characteristics of the first symptoms, and the disease course of axSpA.

The following set of clinical assessments were available for all patients: personal and family history; current and previous therapy; smoking history; BMI; clinical manifestation of axSpA; clinical determination of peripheral arthritis with SJC; evaluation enthesis involvement using the Maastrich Enthesitis Score (MASES)[2]; disease activity according to the ASDAS-CRP[42]; the BASDAI[43]; and the BASFI[43]. Patient-reported outcomes evaluating the quality of life, the ASQoL, and the EQ-5D[44,45] were included. Imaging examinations consisted of X-rays of the SIJ and spine evaluated by two independent radiologists and one rheumatologist trained in the evaluation of X-rays in axSpA. Patients with radiographic sacroiliitis, according to the modified New York criteria[3], were characterised as AS (n = 72). MRI of the SIJ was performed in cases of negative findings of the SIJ on X-ray and analysed according the ASAS[2] independently by one radiologist and one rheumatologist with training in MRI assessment of axSpA. Patients without radiographic changes in the SIJ, but who fulfilled the ASAS criteria of MRI findings or HLA-B27 positivity together with clinical findings, were classified as nr-axSpA (n = 121). The following radiologic scoring systems were used to establish the severity of MRI or X-ray findings of the SIJ or spine: the SPARCC[46] and the Berlin MRI grading system[47] or the mSASSS[48]. Fasting blood samples (serum) for metabolite evaluation were collected from all patients at the first visit to the PRASPAC and stored at −70 °C until assayed. However, all patients had assessments of CRP and ESR at the time of blood draw.

One hundred asymptomatic individuals without any autoimmune or other inflammatory disorder, current infection, or surgery were used as a reference group for metabolite analyses.

All patients signed informed consent for inclusion into the clinical and laboratory database. The local Ethical Committee of the Institute of Rheumatology in Prague approved the consent form (reference number 959/2014 and 960/2014), and the Scientific Board of the Institute of Rheumatology in Prague authorised the study design and the database creation. The study was performed in compliance with the Declaration of Helsinki.

Products of MMP-degraded collagen assessments. A panel of MMP-mediated products of collagen was measured in fasting serum using validated enzyme linked immunosorbent assays (ELISAs); the collagens types measured were MMP-degraded types I (C1M)[19], II (C2M)[20], III (C3M)[49], and type IV collagen alpha 3 (C4M2)[50] (Nordic Bioscience, Herlev, Denmark). All analyses were quality controlled with 2 kit controls and 3 in house quality controls. The inter- and intra-assay variations were below 15% and 10%, respectively. An acceptable linearity of <20% was observed, and no interference of biotin, haemoglobin or intralipid 20 was found. Sample measurements were accepted if the standard curve had an acceptable recovery of <20% in 75% individual standard curve assessments within the measurement range, and if 3 of the 5 control samples were accepted.

Statistics. The summary statistics are shown in Table 1. Fisher's exact test was used to identify differences in binary variables, and the Mann-Whitney U-test examined differences between levels in continuous variables because some assessments were not normally distributed. Kruskal-Wallis with Dunn's multiple comparisons test was used for examining differences between product levels between the three groups (AS, nr-axSpA, and controls). Spearman's correlation test was used for correlation analysis between the products and clinical assessments. Multiple regression and logistic regression analyses were used to test for correlations with adjustments for age, gender, BMI, and disease duration. Data for multiple regression were standardised by z-scores. An area under the receiver operating characteristics curve (AUC ROC) was used for examining the separation potential of the metabolites. Odds ratios were calculated from the cut-off values identified in the AUC ROC. Data analyses were performed using MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). Graphical illustrations were created using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com).

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Sieper, J. & Poddubnyy, D. Axial spondyloarthritis. *Lancet* **390**, 73–84 (2017).
2. Sieper, J. *et al*. The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. *Ann. Rheum. Dis.* **68**, ii1–ii44 (2009).
3. van der Linden, S., Valkenburg, H. A. & Cats, A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheumatol.* **27**, 361–368 (1984).
4. Maksymowych, W. P. *et al*. MRI evidence of structural changes in the sacroiliac joints of patients with non-radiographic axial spondyloarthritis even in the absence of MRI inflammation. *Arthritis Res. Ther.* **19**, 126 (2017).
5. de Winter, J. J., van Mens, L. J., van der Heijde, D., Landewe, R. & Baeten, D. L. Prevalence of peripheral and extra-articular disease in ankylosing spondylitis versus non-radiographic axial spondyloarthritis: a meta-analysis. *Arthritis Res. Ther.* **18**, 196 (2016).
6. van der Heijde, D. *et al*. 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann. Rheum. Dis.* **76**, 978–991 (2017).
7. Dougados, M. *et al*. Rate and predisposing factors for sacroiliac joint radiographic progression after a two-year follow-up period in recent-onset spondyloarthritis. *Arthritis Rheumatol.* **68**, 1904–1913 (2016).
8. Poddubnyy, D. *et al*. Rates and predictors of radiographic sacroiliitis progression over 2 years in patients with axial spondyloarthritis. *Ann. Rheum. Dis.* **70**, 1369–1374 (2011).
9. Wang, R., Gabriel, S. E. & Ward, M. M. Progression of nonradiographic axial spondyloarthritis to ankylosing spondylitis: a population-based cohort study. *Arthritis Rheumatol.* **68**, 1415–1421 (2016).
10. Anaya, J. M. *et al*. Personalized medicine. Closing the gap between knowledge and clinical practice. *Autoimmun. Rev.* **15**, 833–842 (2016).
11. Marzo-Ortega, H., Gaffney, K. M. & Gaffney, K. Defining the target: clinical aims in axial spondyloarthritis. *Rheumatology* **57**, vi18–vi22 (2018).
12. Maksymowych, W. P. Biomarkers for diagnosis of axial spondyloarthritis, disease activity, prognosis, and prediction of response to therapy. *Front. Immunol.* **10**, 305 (2019).
13. Baraliakos, X. & Braun, J. Non-radiographic axial spondyloarthritis and ankylosing spondylitis: what are the similarities and differences? *RMD Open* **1**, e000053 (2015).
14. Kjelgaard-Petersen, C. *et al*. Synovitis biomarkers: *ex vivo* characterization of three biomarkers for identification of inflammatory osteoarthritis. *Biomarkers* **20**, 547–556 (2015).
15. Mattey, D. L. *et al*. Association of cytokine and matrix metalloproteinase profiles with disease activity and function in ankylosing spondylitis. *Arthritis Res. Ther.* **14**, R127 (2012).
16. Maksymowych, W. P. *et al*. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum.* **56**, 1846–1853 (2007).
17. Bay-Jensen, A. C. *et al*. Ankylosing spondylitis is characterized by an increased turnover of several different metalloproteinase-derived collagen species: a cross-sectional study. *Rheumatol. Int.* **32**, 3565–3572 (2012).
18. Siebuhr, A. S. *et al*. Serological identification of fast progressors of structural damage with rheumatoid arthritis. *Arthritis Res. Ther.* **15**, R86 (2013).
19. Leeming, D. *et al*. A novel marker for assessment of liver matrix remodeling: an enzyme-linked immunosorbent assay (ELISA) detecting a MMP generated type I collagen neo-epitope (C1M). *Biomarkers* **16**, 616–628 (2011).
20. Bay-Jensen, A. C. *et al*. Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIM–increased serum CIIM in subjects with severe radiographic osteoarthritis. *Clin. Biochem.* **44**, 423–429 (2011).
21. Siebuhr, A. S., Bay-Jensen, A. C., Karsdal, M. A., Lories, R. J. & de Vlam, K. CRP and a biomarker of type I collagen degradation, C1M, can differentiate anti-inflammatory treatment response in ankylosing spondylitis. *Biomark. Med.* **10**, 197–208 (2016).
22. Vassiliadis, E. *et al*. Measurement of CO3-610, a potential liver biomarker derived from matrix metalloproteinase-9 degradation of collagen type III, in a rat model of reversible carbon-tetrachloride-induced fibrosis. *Biomark. Insights* **6**, 49–58 (2011).
23. Sand, J. M. *et al*. MMP mediated degradation of type IV collagen alpha 1 and alpha 3 chains reflects basement membrane remodeling in experimental and clinical fibrosis–validation of two novel biomarker assays. *PLoS One* **8**, e84934 (2013).
24. Bay-Jensen, A. C. *et al*. Circulating protein fragments of cartilage and connective tissue degradation are diagnostic and prognostic markers of rheumatoid arthritis and ankylosing spondylitis. *PLoS One* **8**, e54504 (2013).
25. Rudwaleit, M. *et al*. The early disease stage in axial spondylarthritis: results from the German Spondyloarthritis Inception Cohort. *Arthritis Rheum.* **60**, 717–727 (2009).
26. Althoff, C. E. *et al*. Active inflammation and structural change in early active axial spondyloarthritis as detected by whole-body MRI. *Ann. Rheum. Dis.* **72**, 967–973 (2013).
27. de Winter, J. J., Paramarta, J. E., de Jong, H. M., van de Sande, M. G. & Baeten, D. L. Peripheral disease contributes significantly to the level of disease activity in axial spondyloarthritis. *RMD Open* **5**, e000802 (2019).
28. Sun, S. *et al*. The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. *BMC Musculoskelet. Disord.* **15**, 93 (2014).
29. Liu, K. G., He, Q. H., Tan, J. W. & Liao, G. J. Expression of TNF-alpha, VEGF, and MMP-3 mRNAs in synovial tissues and their roles in fibroblast-mediated osteogenesis in ankylosing spondylitis. *Genet. Mol. Res.* **14**, 6852–6858 (2015).
30. Kiltz, U. *et al*. The degree of spinal inflammation is similar in patients with axial spondyloarthritis who report high or low levels of disease activity: a cohort study. *Ann. Rheum. Dis.* **71**, 1207–1211 (2012).
31. Ruyssen-Witrand, A. *et al*. Relationships between ultrasound enthesitis, disease activity and axial radiographic structural changes in patients with early spondyloarthritis: data from DESIR cohort. *RMD Open* **3**, e000482 (2017).
32. Siebuhr, A. S. *et al*. Is radiographic progression in radiographic axial spondyloarthritis related to matrix metalloproteinase degradation of extracellular matrix? *RMD Open* **4**, e000648 (2018).
33. Gudmann, N. S. *et al*. Type I and III collagen turnover is increased in axial spondyloarthritis and psoriatic arthritis. Associations with disease activity and diagnostic capacity. *Clin. Exp. Rheumatol.* **35**, 653–659 (2017).
34. Kang, K. Y., Jung, J. Y., Hong, Y. S., Ju, J. H. & Park, S. H. Positive correlation between inflammation on sacroiliac joint MRI and serum C-terminal telopeptide of type-I collagen in ankylosing spondylitis but not in non-radiographic axial spondyloarthritis. *Clin. Exp. Rheumatol.* **35**, 415–422 (2017).
35. Jacques, P. *et al*. Proof of concept: enthesitis and new bone formation in spondyloarthritis are driven by mechanical strain and stromal cells. *Ann. Rheum. Dis.* **73**, 437–445 (2014).
36. Wang, R. & Ward, M. M. Epidemiology of axial spondyloarthritis: an update. *Curr. Opin. Rheumatol.* **30**, 137–143 (2018).
37. Rueda-Gotor, J. *et al*. Subclinical atherosclerosis is not increased in patients with non-radiographic axial spondyloarthritis. *Clin. Exp. Rheumatol.* **34**, 159–160 (2016).
38. Dragsbaek, K. *et al*. Matrix metalloproteinase mediated type I collagen degradation - an independent risk factor for mortality in women. *EBioMedicine* **2**, 723–729 (2015).

39. Haroon, N. N., Paterson, J. M., Li, P., Inman, R. D. & Haroon, N. Patients with ankylosing spondylitis have increased cardiovascular and cerebrovascular mortality: a population-based study. *Ann. Intern. Med.* **163**, 409–416 (2015).
40. Sun, L. M. *et al*. Increased risk of cancer for patients with ankylosing spondylitis: a nationwide population-based retrospective cohort study. *Scand. J. Rheumatol.* **43**, 301–306 (2014).
41. Siebuhr, A. S. *et al*. Metabolites of C-reactive protein and vimentin are associated with disease activity of axial spondyloarthritis. *Clin. Exp. Rheumatol.* Feb 11 Epub ahead of print (2019).
42. van der Heijde, D. *et al*. ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. *Ann. Rheum. Dis.* **68**, 1811–1818 (2009).
43. Calin, A. *et al*. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J. Rheumatol.* **21**, 2281–2285 (1994).
44. Doward, L. C. *et al*. Development of the ASQoL: a quality of life instrument specific to ankylosing spondylitis. *Ann. Rheum. Dis.* **62**, 20–26 (2003).
45. Rabin, R. & de Charro, F. EQ-5D: a measure of health status from the EuroQol group. *Ann. Med.* **33**, 337–343 (2001).
46. Maksymowych, W. P. *et al*. Spondyloarthritis research Consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. *Arthritis Rheum.* **53**, 703–709 (2005).
47. Hermann, K. G., Braun, J., Fischer, T., Reisshauer, H. & Bollow, M. Magnetic resonance tomography of sacroiliitis: anatomy, histological pathology, MR-morphology, and grading. *Radiologe* **44**, 217–228 (2004).
48. Wanders, A. J. *et al*. What is the most appropriate radiologic scoring method for ankylosing spondylitis? A comparison of the available methods based on the outcome measures in rheumatology clinical trials filter. *Arthritis Rheum.* **50**, 2622–2632 (2004).
49. Barascuk, N. *et al*. Development and validation of an enzyme-linked immunosorbent assay for the quantification of a specific MMP-9 mediated degradation fragment of type III collagen–A novel biomarker of atherosclerotic plaque remodeling. *Clin. Biochem.* **44**, 900–906 (2011).
50. Veidal, S. S. *et al*. Assessment of proteolytic degradation of the basement membrane: a fragment of type IV collagen as a biochemical marker for liver fibrosis. *Fibrogenesis Tissue Repair* **4**, 22 (2011).

Acknowledgements

The authors thank Ms. Jitka Smekalova for the sample administration in the Institute of Rheumatology and technicians at Nordic Bioscience who measured the metabolites in the study samples. This work was supported by the project (Ministry of Health Czech Republic) for conceptual development of research organization 00023728 (Institute of Rheumatology).

Author Contributions

A.S.S. designed the metabolite study, performed the data analyses, and drafted the manuscript. M.H. designed the metabolite study, provided the study samples, performed the data analyses, drafted the manuscript, prepared the clinical database and provided clinical care to the axSpA patients in the PRASPAC cohort. S.F. prepared the clinical database and provided clinical care for the axSpA patients in the PRASPAC cohort, and contributed to, read, and approved the final manuscript. K.Z. prepared the clinical database and provided clinical care for the axSpA patients in the PRASPAC cohort, and contributed to, read, and approved the final manuscript. M.T. provided the control group, and contributed to, read, and approved the final manuscript. M.G. prepared the clinical database and provided clinical care for the axSpA patients in the PRASPAC cohort, and contributed to, read, and approved the final manuscript. K.B. determined the radiographic and MRI scores, and contributed to, read, and approved the final manuscript. J.H. prepared the clinical database and provided clinical care for the axSpA patients in the PRASPAC cohort, and contributed to, read, and approved the final manuscript. J.G. determined the radiographic and MRI scores and contributed to, read, and approved the final manuscript. K.P. prepared the clinical database and provided clinical care for the axSpA patients in the PRASPAC cohort, and contributed to, read, and approved the final manuscript. A.C.B.J. contributed to, read, and approved the final manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-47502-z.

Competing Interests: A.S.S. and A.C.B.J. are full-time employees of Nordic Bioscience, which is a privately-owned SME that develops biomarkers of fibrosis. A.C.B.J. is also a stockholder of Nordic Bioscience. The authors have no other relevant affiliation or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those previously disclosed. No writing assistance was used in the production of this manuscript. All authors of the Institute of Rheumatology (M.H., S.F., K.Z., M.T., M.G., K.B., J.H., J.G. and K.P.) declare no competing interest in this study. All authors declare no non-financial conflicts regarding to this study.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

PLOS ONE

RESEARCH ARTICLE

Association between circulating miRNAs and spinal involvement in patients with axial spondyloarthritis

Klára Prajzlerová[1]*, Kristýna Grobelná[1], Markéta Hušáková[1], Šárka Forejtová[1], Astrid Jüngel[2], Steffen Gay[2], Jiří Vencovský[1], Karel Pavelka[1], Ladislav Šenolt[1], Mária Filková[1]

1 Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic, **2** Center of Experimental Rheumatology, University Hospital Zurich, Zurich, Switzerland

* prajzlerova@revma.cz

Citation: Prajzlerová K, Grobelná K, Hušáková M, Forejtová Š, Jüngel A, Gay S, et al. (2017) Association between circulating miRNAs and spinal involvement in patients with axial spondyloarthritis. PLoS ONE 12(9): e0185323. https://doi.org/10.1371/journal.pone.0185323

Editor: Yun Zheng, Kunming University of Science and Technology, CHINA

Received: April 7, 2017

Accepted: September 11, 2017

Published: September 22, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was financially supported by a project of the Ministry of Health of the Czech Republic for conceptual research development number 023728 and grant project 17-33127A. There is no financial support or other benefits from commercial sources for the work reported on in the manuscript.

Abstract

Objectives

Dysregulation of miRNAs and their target genes contributes to the pathophysiology of autoimmune diseases. Circulating miRNAs may serve as diagnostic/prognostic biomarkers. We aimed to investigate the association between circulating miRNAs, disease activity and spinal involvement in patients with axial spondyloarthritis (AxSpA).

Methods

Total RNA was isolated from the plasma of patients with non-radiographic (nr)AxSpA, patients with ankylosing spondylitis (AS) and healthy controls (HC) via phenol-chloroform extraction. A total of 760 miRNAs were analysed with TaqMan® Low Density Arrays, and the expression of 21 miRNAs was assessed using single assays.

Results

Comprehensive analysis demonstrated the differential expression of miRNAs among patients with progressive spinal disease. Of the 21 miRNAs selected according to their expression patterns, the levels of miR-625-3p were significantly different between nr-AxSpA patients and HCs. We found no correlation between miRNA levels and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) in nr-AxSpA patients. Selected miRNAs, such as miR-29a-3p, miR-146a-5p or miR-222-3p with an established role in extracellular matrix formation and inflammation were associated with spinal changes and/or disease activity assessed by BASDAI in AS patients, including miR-625-3p reflecting disease activity in AS with spinal involvement.

Conclusions

Our data indicate that circulating miRNAs play a role in the pathogenesis of AxSpA and are also suggestive of their potential as biomarkers of disease progression. We hypothesize

Competing interests: The authors have declared that no competing interests exist.

that differential systemic levels of miRNA expression reflect miRNA dysregulation at sites of spinal inflammation or bone formation where these molecules contribute to the development of pathophysiological features typical of AxSpA.

Introduction

Axial spondyloarthritis (AxSpA) is a chronic inflammatory disease that mainly affects the axial skeleton, sacroiliac joints and entheseal spinal structures. It encompasses patients with ankylosing spondylitis (AS) with radiographic sacroiliitis and syndesmophytes, as well as patients with early or abortive forms of spondyloarthritis (SpA) characterized by the presence of sacroiliac inflammation detected by magnetic resonance imaging (MRI) or the presence of HLA-B27 in combination with features characteristic of SpA [1, 2]. AS is an inflammatory disease characterized by new bone formation. Mononuclear cells and osteoclasts initiate local osteitis, which leads to cartilage erosion and bone destruction, as well as osteoblast differentiation and subsequent syndesmophyte formation [3, 4].

Inflammation develops several years before structural damage becomes visible on plain radiographs. Although patients may have longstanding symptoms, the diagnosis of AS based on the modified New York criteria delays early treatment, as radiographic sacroiliitis represents a late sign of disease. Therefore, the Assessment of SpondyloArthritis International Society (ASAS) developed new classification criteria for the diagnosis of AxSpA that takes non-radiographic (nr-AxSpA) findings into account [5]. Shorter disease duration, younger age, elevated baseline C-reactive protein (CRP) levels and active inflammatory changes involving the sacroiliac joint are associated with better responses to anti-TNF therapy in patients with nr-AxSpA [6]. Therefore, early diagnosis, disease monitoring and therapeutic response prediction are very important.

Several biomarkers have been tested regarding their usefulness in diagnosing disease, monitoring disease activity and predicting therapeutic responsiveness but have thus far not been implemented in clinical practice [7]. HLA-B27 remains the best genetic biomarker for diagnosing AxSpA, and CRP remains the best circulating marker for assessing disease activity and predicting treatment responsiveness and structural progression [7].

MicroRNAs (miRNAs) are small, non-coding RNAs that function as post-transcriptional regulators of gene expression. Altered miRNA expression and target gene dysregulation have been shown to contribute to the pathophysiology of many autoimmune diseases, including rheumatic diseases [8]. Although the (patho) physiological roles of circulating miRNAs remain largely unknown, cell-free circulating miRNAs appear to be promising disease biomarkers [9]. While rheumatoid arthritis (RA) has been extensively investigated, comprehensive studies regarding miRNAs in patients with AxSpA are lacking.

The aim of the present study was to identify circulating miRNAs in patients with AxSpA and to investigate their association with disease characteristics, including spinal disease severity.

Material and methods

Patients

This study included 20 patients with nr-AxSpA, 24 AS patients with isolated sacroiliitis without spinal involvement (AS stage I), 24 patients with AS with spinal involvement (presence of

Table 1. Clinical characteristics of healthy controls and patients with axial spondyloarthritis.

Variable	HC	nr-AxSpA	sacroiliitis	AS II-V
n	29	20	24	24
Gender, female//male, n	9/20	9/11	4/20	4/20
Age, years	34.0 ± 9.2 (20.7, 56.8)	34.9 ± 10.3 (21,3, 68.0)	32.6 ± 8.2 (22.1, 57.9)	40.0 ± 8.3 (25.2, 45.3)
HLA-B27 positivity, n	NA	20	22	21
Disease duration, years	NA	1.6 ± 2.5 (0, 9)	4.3 ± 3.7 (0,14)	6.8 ± 4.3 (0,17)
CRP, mg/l	NA	6.8 ± 7.5 (0.3, 29.0)	18.7 ± 26.3 (1.7, 117.0)	15.0 ± 16.3 (0.5, 77.5)
BASDAI score	NA	3.2 ± 2.3 (0.2, 6.7)	6.0 ± 2.6 (0.1, 9.4)	4.6 ± 2.5 (0.7, 8.2)
Peripheral arthritis, n	NA	4	3	1
Enthesitis, n	NA	9	2	8
Uveitis, n	NA	10	6	9
Treatment, n: NSAID DMARDs Biological therapy	 0 0 0	 15 4 1	 17 5 2	 10 2 12

Abbreviations: AS, ankylosing spondylitis; AS II-V, ankylosing spondylitis with spinal involvement; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; DMARDs, disease modifying antirheumatic drugs; HC, healthy controls; NSAID, non-steroidal anti-inflammatory drug; nr-AxSpA, non-radiographic axial spondyloarthritis; NA, not applicable;. Data are expressed as the mean±SD and minimum and maximum values (min, max).

https://doi.org/10.1371/journal.pone.0185323.t001

syndesmophytes, AS stages II-V), including 7 patients with a bamboo spine, and 29 healthy controls (HC). Radiographic staging was performed as previously described [10]. All patients fulfilled the 2011 ASAS classification criteria for the diagnosis of AxSpA [11]. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [12] and CRP. The clinical characteristics of the patients and HCs are shown in Table 1. Patients were recruited from the outpatient clinic of the Institute of Rheumatology, Prague in 2013–2014. Written informed consent was obtained from all participants prior to enrolment, and the study was approved by the local Ethics committee at the Institute of Rheumatology in Prague.

Samples and RNA isolation

Whole blood samples collected to EDTA tubes were obtained from all participants and plasma was separated by centrifugation within 4 hours of collection ensuring constant pre-analytical condition for all samples. All plasma samples were stored at -80˚C and experienced no freeze-thaw cycles before use. Total RNA was extracted from plasma samples using phenol-chloroform extraction, as previously described [9]. Briefly, 500 μl of plasma was homogenized with 500 μl of Trizol LS reagent (Thermo Fisher Scientific, Waltham, MA, USA) and then centrifuged at 12,000 × g for 10 minutes at 4˚C. Three cycles of acid phenol-chloroform (Thermo Fisher Scientific) extraction were performed. RNA was precipitated by adding 5μg of RNase-free glycogen (Roche Diagnostics, Mannheim, Germany) and 100% isopropanol and then incubated for 10 minutes at room temperature before being centrifuged at 12,000 × g for 10 minutes at 4˚C. The pellet was washed with 75% ethanol, and RNA was dissolved in RNase-free water. Three synthesized *C. elegans* miRNAs, cel-miR-39, cel-miR-54 and cel-miR-238 (Integrated DNA Technologies, Coralville, IA, USA), 25 fmol each, were spiked into plasma samples after denaturation and served as internal calibrators, as previously described [13]. RNA sample quality control was initially performed using Agilent 2100 Bioanalyser with

Agilent Small RNA kit (Agilent, CA, USA) as RNA isolation quality measure and then using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific) in remaining samples.

miRNA analysis

First, twenty non-pooled individual samples (5 samples from each group) were analysed using a TaqMan® Low Density Array (Thermo Fisher Scientific). Complementary DNA was obtained by reverse transcription using a TaqMan® MicroRNA Reverse Transcription Kit with Megaplex RT Primers with equal RNA input. cDNA was preamplified using 2x TaqMan® PreAmp Master Mix and Megaplex™ PreAmp Primers (all Thermo Fisher Scientific) on a PCR thermocycler (Bio-Rad Laboratories, CA, USA). The expression of 760 miRNAs was measured using Human Pool A+B TaqMan® Low Density Array platforms for microRNAs on a 7900RT-PCR thermocycler (Thermo Fisher Scientific). All steps were performed according to the manufacturer's instructions. Data were analysed with RQ Manager Software (Life Technologies). The dCt method was used for relative quantification as follows: dCt = Ct(array average)-Ct(miRNA of interest), followed by x-fold change calculations.

All miRNAs exhibiting a minimum 1.5 mean fold difference in expression between at least 2 groups according to across-group comparisons (HC vs. nr-AxSpA vs. AS) were taken forward for pathway analysis and literature search as explained below. In total, 21 miRNAs were selected for further validation using single assays. Total RNA from the remaining non-pooled samples was reverse-transcribed using TaqMan Real Time miRNA specific primers (including primers for cel-miR-39, cel-miR-54 and cel-miR-238) and then amplified by real-time PCR with TaqMan probes and TaqMan Universal PCR Master Mix on a 7900RT-PCR thermocycler (Thermo Fisher Scientific). Data were analysed with RQ Manager Software (Thermo Fisher Scientific). The dCt method was used for relative quantification as follows: dCt = Ct(spike-in average)-Ct(miRNA of interest); therefore, higher dCt values represent higher expression levels of particular miRNAs.

Statistical analysis

Data are expressed as the mean±SD. One-way ANOVA with post-hoc comparison for multiple comparisons or unpaired T test (with Welch's correction in case of homogeneity assumption violations) for comparisons between 2 groups were used where applicable. Pearson's correlation coefficient was used to correlate any two variables. P values less than 0.05 were considered statistically significant. All analyses and graphs were performed and generated, respectively, using GraphPad Prism 5.02 (GraphPad Software, La Jolla, CA).

Literature search

First, DIANA-mirPath tool was used to analyze clustering of miRNAs and pathways. In the next step, an online search (PubMed, performed in March 2016) of the functions of miRNAs was performed and 21 miRNAs with a hypothesized role in the pathogenesis of AxSpA were selected.

Results

Comprehensive analysis of circulating miRNAs

A comprehensive screening of 760 miRNAs was performed using TaqMan Low Density Arrays, as described above. Only miRNAs expressed in all 5 samples were taken forward for the analysis. Overall, 162 miRNAs were detected in HCs, 154 miRNAs were detected in patients with nr-AxSpA, 110 miRNAs were detected in patients with sacroiliitis, and 110

miRNAs were detected in AS patients with spinal involvement (AS II-V). Of those miRNAs, 92 were detected in all tested samples, 10 were detected in HCs, nr-AxSpA patients and AS patients with sacroiliitis and 25 were shared by HC and nr-AxSpA patients (S1 Fig). We found no miRNA unique to AxSpA.

Using the approach described in the Methods, miRNAs exhibiting a minimum 1.5 mean fold difference in expression between at least 2 groups according to across-group comparisons (HC vs. nr-AxSpA vs. AS with sacroiliitis and with spinal involvement) were considered for further analysis (S1 Table).

DIANA mir-Path cluster analysis and literature review enabled selection of 21 miRNAs for further validation (Fig 1, S2 Table).

Differential expression of circulating miRNAs between HC and patients with AxSpA

As mentioned above, 21 selected miRNAs were analysed using single assays to confirm their differential expression (S3 Table).

The expression was compared between HC and patients with AxSpA as follows:

Significantly lower expression (from 1.6 to 3.9 times) of 14 miRNAs most of which are involved in osteoblast differentiation or the Wnt signalling pathway, were noted in all patients with AxSpA irrespective of patient radiographic findings compared to HC (Table 2, extended results shown in S4 Table).

As the group of AxSpA is heterogeneous, we next compared patients with AxSpA according radiographic damage with HC (Table 2, S4 Table, Fig 2):

In patients with nr-AxSpA, only miR-625-3p appeared significantly different and exhibited 2.3 times lower expression levels than in HC. Eighteen miRNAs exhibited 2.1 to 5.6 times lower expression levels in radiographic disease irrespective of spinal involvement than in HC, and 14 miRNAs were 2.0–3.9 times lower in AS patients than in patients with non-radiographic disease (Table 2, S4 Table).

These results indicate that some differences exist in the levels of circulating miRNAs between HC and patients with non-radiographic disease, while more differences exist at radiographic stage reflecting bony changes in patients with more advanced disease.

Effects of spinal involvement on circulating miRNA expression

Next, we evaluated the differences in circulating miRNA levels between patients with nr-AxSpA and definite radiographic disease in patients with isolated sacroiliitis and with spinal involvement (classified as AS II-V) as follows (Fig 2):

nr-AxSpA vs. AS: The vast majority of miRNAs (miR-19a-3p, miR-24-3p, miR-27a-3p, miR-29a-3p, miR-106a-5p, miR-140-3p, miR-146a-5p, miR-146b-5p, miR-151a-3p, miR-221-3p, miR-223-3p, miR-374a-5p) exhibited 2.0–5.2 times lower expression levels in both AS groups than in the nr-AxSpA patients. As most of them are associated with bone remodelling, these data indicate that an inverse association exists between radiographic bone formation and circulating miRNA levels.

Sacroiliitis vs. AS II-V: However, there were no significant differences in the levels of abovementioned 12 miRNAs between sacroiliitis and AS II-V groups. MiR-99b-5p, miR-625-3p and miR-885-5p exhibited significantly lower expression (2.3 times, 2.2 times, 3.3 times, respectively) in AS patients with spinal involvement (AS II-V) than in those with sacroiliitis. Interestingly, we found no data regarding the roles of miR-625-3p and miR-885-5p in bone formation or inflammation (S2 Table).

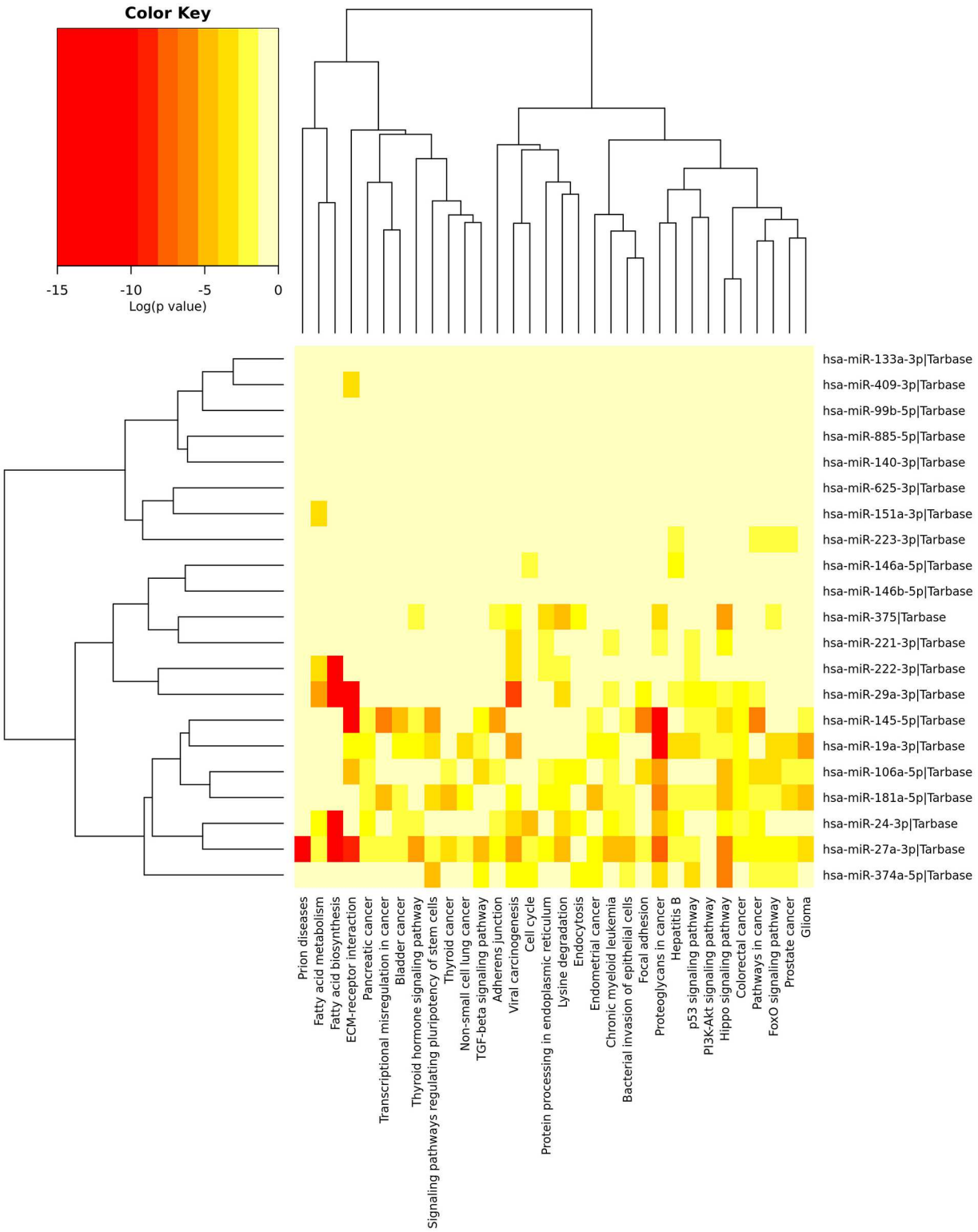

Fig 1. Significance cluster analysis of selected miRNAs using DIANA mirPath tool showing the involvement of miRNAs in different signalling and pathogenic pathways. Although the involvement in certain pathways of several miRNAs overlapped, the function of few miRNAs was unknown and required manual search for their function.

https://doi.org/10.1371/journal.pone.0185323.g001

Table 2. Summary of expression and function of selected miRNAs as markers of disease activity and hypothesized role in AxSpA.

miRNA	Diagnosis				Disease activity				Treatment response		Hypothesized role in AxSpA
	HC vs. AxSpA^T	HC vs. nr-AxSpA	HC vs. AS	nr-AxSpA vs. AS	nr-AxSpA	AS	sacroiliitis	AS II-V	NSAID vs. antiTNF	DMARDs. vs. antiTNF	
miR-19a-3p	*	-	**	*	-	-	-	-	-	**	bone formation
miR-24-3p	*	-	**	**	-	-	-	-	***	***	bone formation
miR-27a-3p	**	-	***	**	-	-	-	-	***	**	bone formation
miR-29a-3p	**	-	***	*	-	-	-	BASDAI, CRP	*	**	bone formation,
miR-99b-5p	**	-	***	*	CRP	BASDAI	-	-	***	**	bone formation
miR-106a-5p	-	-	**	**	-	-	-	-	***	***	bone formation
miR-133a-3p	*	-	*	-	-	BASDAI	-	CRP	-	*	differentiation?
miR-140-3p	-	-	*	**	CRP	-	-	-	**	**	inflammation
miR-145-5p	-	-	*	-	CRP	-	-	-	**	*	bone formation
miR-146a-5p	*	-	**	*	-	CRP	-	-	*	*	inflammation
miR-146b-5p	*	-	**	**	-	-	-	-	**	***	migration, invasion
miR-151a-3p	-	-	*	*	-	CRP	-	CRP	-	-	migration
miR-181a-5p	-	-	-	-	-	CRP	-	-	-	-	?
miR-221-3p	-	-	**	*	-	CRP	-	-	-	*	immunopathogenesis
miR-222-3p	***	-	***	*	-	-	-	BASDAI	-	-	bone formation
miR-223-3p	*	-	**	*	-	-	-	-	**	**	bone formation
miR-374a-5p	**	-	***	*	CRP	-	-	-	*	*	bone formation
miR-375	*	-	-	-	-	-	-	BASDAI	-	-	bone formation
miR-409-3p	**	-	***	-	-	-	-	CRP	-	-	proliferation, invasion
miR-625-3p	***	*	***	-	-	BASDAI	-	BASDAI	-	-	?
miR-885-5p	-	-	-	-	-	BASDAI	-	BASDAI	-	-	?

Abbreviations: HC, healthy controls; nr-AxSpA, non-radiographic axial spondyloarthritis; AS, ankylosing spondylitis; AS II-V, ankylosing spondylitis with spinal involvement; DMARDs, disease modifying antirheumatic drugs; NSAID, non-steroidal anti-inflammatory drugs; T, T test; -, not significant;

*p<0.05,

**p<0.01,

***p<0.001.

Statistical significance was calculated using ANOVA unless stated otherwise.

https://doi.org/10.1371/journal.pone.0185323.t002

Moreover, patients with a bamboo spine exhibited 1.6–8.0 times significantly lower expression levels of some of the miRNAs mentioned above (miR-19a-3p, miR-24-3p, miR-27a-3p, miR-99b-5p, miR-106a-5p, miR-140-3p, miR-145-5p, miR-146a-5p, miR-146b-5p, miR-222-3p, miR-223-3p, miR-374a-5p, miR-375), including miR-625-3p and miR-885-5p than AS patients with less severe spinal damage (Fig 2).

Association between circulating miRNAs and disease activity

We next aimed to analyse the associations between circulating miRNA levels and disease activity parameters (Fig 3).

In patients with nr-AxSpA, no significant associations between miRNA levels and BASDAI were noted. CRP was positively correlated with the levels of miR-99b-5p, miR-140-3p, miR-145-5p and miR-374a-5p expression (Fig 3A).

In all AS patients, BASDAI positively correlated with the levels of miR-99b-5p, miR-133a-3p, miR-625-3p and miR-885-5p, while CRP was correlated with the levels of miR-146a-5p, miR-151a-3p, miR-181a-5p and miR-221-3p (Fig 3B).

When further sub-analysis was performed, we noted no correlations between miRNAs and CRP or BASDAI in patients with sacroiliitis. However, in AS patients with spinal involvement

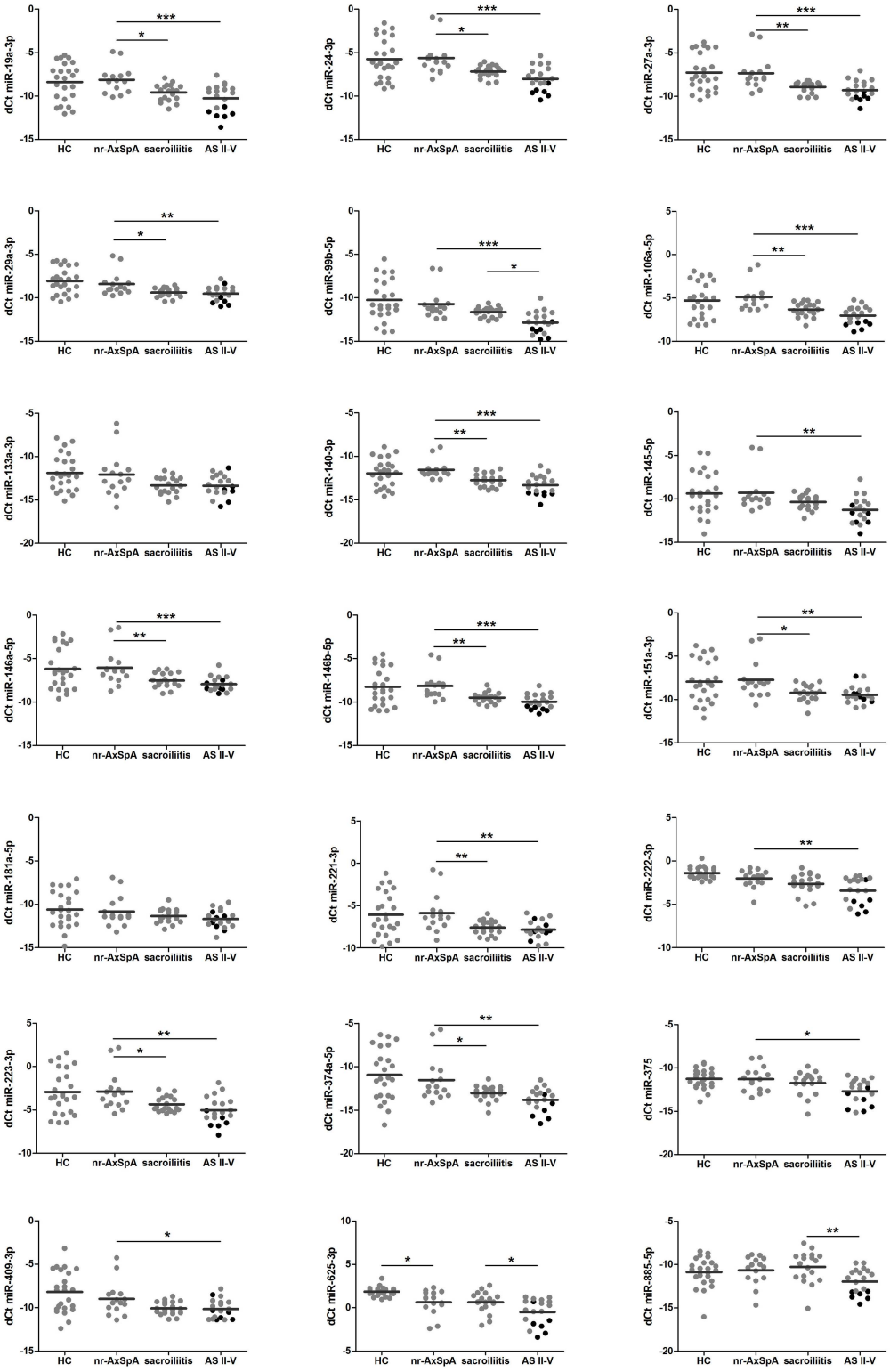

Fig 2. Differences in the levels of circulating miRNAs between healthy controls, patients with non-radiographic AxSpA (nr-AxSpA) and patients with ankylosing spondylitis (AS) with isolated sacroiliitis and spinal involvement (AS II-V). Black symbols indicate patients with bamboo spine. *p<0.05, **p<0.01, ***p<0.001.

https://doi.org/10.1371/journal.pone.0185323.g002

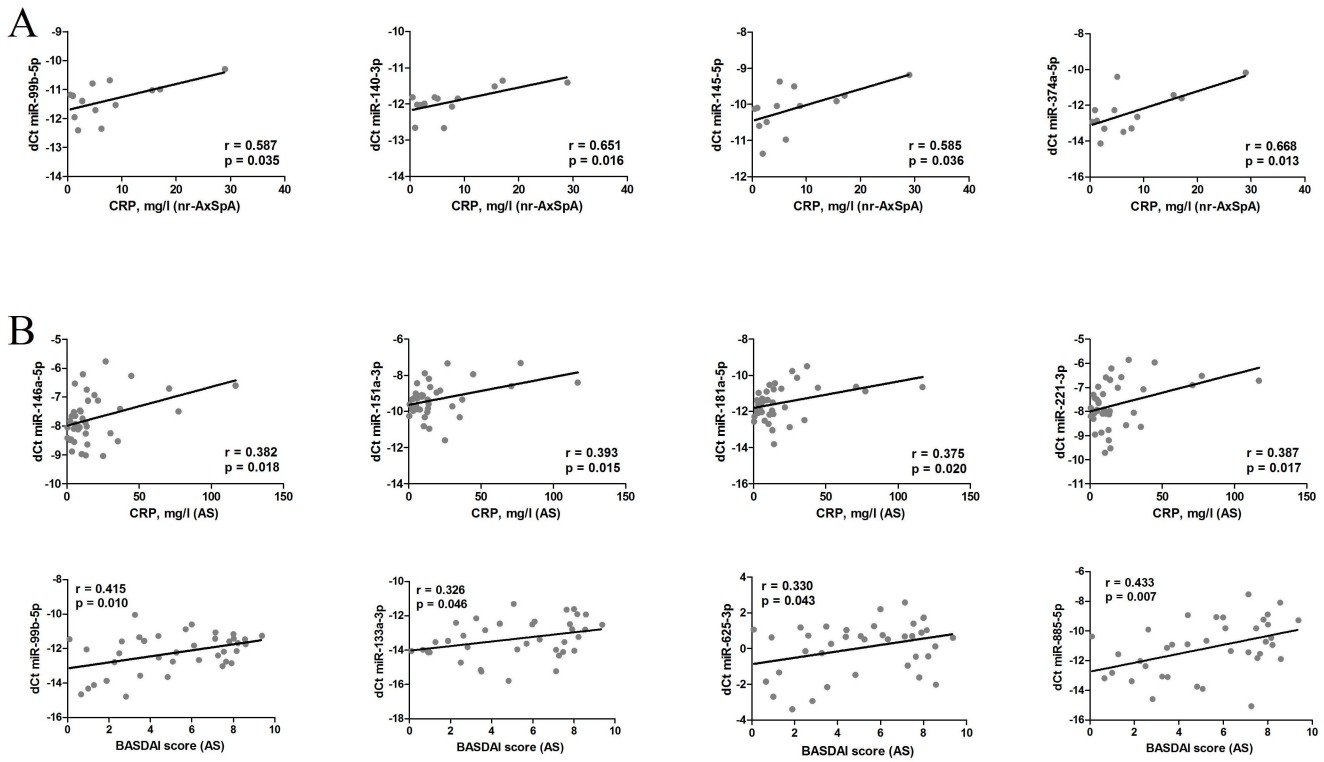

Fig 3. Correlation between circulating miRNAs, CRP and BASDAI in nr-AxSpA (A) and AS patients (B).

https://doi.org/10.1371/journal.pone.0185323.g003

(AS II-V), we noted a positive correlation between BASDAI and mi-29a-3p, miR-222-3p, miR-375, miR-625-3p and miR-885-5p levels and a positive correlation between CRP and miR-29a-3p, miR-133a-3p, miR-151a-3p and miR-409-3p levels.

We then considered different HLA-B27 status, peripheral arthritis or extraarticular manifestations on levels of miRNAs but no effect was found in any of these confounders. While no significant differences were noted in miRNA levels between patients receiving non-steroidal anti-inflammatory drugs (NSAID) or disease modifying antirheumatic drugs (DMARDs), the patients receiving anti-TNF therapy exhibited significantly lower levels of all remaining miRNAs than anti-TNF naïve patients (Table 2, S4 Table).

Discussion

To our knowledge, this is the first study to perform comprehensive analyses of 760 circulating miRNAs in patients with various stages of AxSpA. We observed differential expression of some miRNAs in patients with more advanced spinal disease.

Circulating miRNAs have been shown to be unexpectedly stable, which makes them accessible via body fluid sampling and potentially useful as biomarkers [13]. Some associations between circulating miRNA levels and disease activity e.g., in early RA [9] or systemic lupus erythematosus [14], have previously been shown; however, data on circulating miRNAs in AxSpA are lacking.

Of 760 screened miRNAs, 21 exhibiting differential expression among patients at various disease stages were selected. We confirmed that 14 miRNAs (miR-19a-3p, miR-24-3p, miR-27a-3p, miR-29a-3p, miR-99b-5p, miR-133a-3p, miR-146a-5p, miR-146b-5p, miR-222-3p, miR-223-3p, miR-374a-5p, miR-375, miR-409-3p and miR-625-3p) had lower expression

levels in all AxSpA patients, irrespective of radiographic findings, than in HC. Most of them were shown to be associated with osteoblast differentiation or the Wnt signalling pathway, while others participate in cell differentiation and proliferation or inflammation. These data support our hypothesis that dysregulation of circulating miRNA occurs in patients with AxSpA.

Interestingly, only miR-625-3p was significantly different in nr-AxSpA patients compared to HC. Eighteen miRNAs were confirmed to have lower expression levels in AS patients than in HCs. Of these, 14 exhibited lower expression levels in AS patients with radiographic disease than in AS patients with non-radiographic disease. The levels of 9 miRNAs (miR-19a-3p, miR-24-3p, miR-27a-3p, miR-106a-5p, miR-140-3p, miR-146a-5p, miR-146b-5p, miR-223-3p, miR-374a-5p) were lower in patients with a bamboo spine than in other patients with AS. Interestingly, these 9 miRNAs that exhibited differential expression in conjunction with progressive spinal damage, 6 miRNAs (miR-19a-3p, miR-24-3p, miR-27a-3p, miR-106a-5p, miR-223-3p, miR-374a-5p) play roles in bone formation by mediating Wnt signalling pathway activity, and 3 (miR-140-3p, miR-146a-5p, miR-146b-5p) play roles in inflammation or cell migration that may be related to the pathogenesis of AxSpA.

At present, it is not technically feasible to show the effect of native biofluid circulating miRNA on target genes in tissues using functional experiments due to lack of data on their specific source, trafficking and targeting mechanisms. Drawing firm conclusions on the role of circulating miRNAs in the pathogenesis is therefore difficult and these are mostly inferred from data published on intracellular miRNAs reviewed by literature and databases search. Similarly, we inferred the potential of differentially expressed circulating miRNAs in the pathogenesis of AxSpA based on published data on other diseases.

Many miRNAs shown here to be differentially expressed are involved in bone turnover. MiR-19a was described as a negative regulator of the Wnt signalling pathway in endothelial cells [15]. MiR-24 overexpression significantly inhibited osteogenic differentiation in osteoblastic cells [16]. Runx2, a transcription factor essential for osteoblastogenesis, was shown to negatively regulate the expression of miR-27a [17]. Another study showed that miR-27 promote osteoblast differentiation by modulating Wnt signalling [18]. MiR-106a inhibits osteogenesis in mesenchymal stem cells [19] and is a negative regulator of IL-8, the levels of which are known to be elevated in AS patients [20, 21]. MiR-223 affects bone metabolism, especially osteoclast and osteoblast differentiation [22] and blocking miR-223 inhibits osteoclastogenesis [23]. Moreover, miR-223 appears to be a biomarker of disease activity and treatment response in RA [9, 24]. MiR-374a has been shown to be an activator of the Wnt signalling pathway [25]. In summary, we believe that lower systemic levels of miRNAs may reflect their low local expression levels. Our data show a trend towards lower levels according the extent of spine involvement with the lowest levels in patients with most advanced disease. We hypothesize that lower systemic levels of miRNAs negatively correlate with new bone formation promoted by induction of osteoblastogenesis and to lesser extend also by local inflammation or osteitis initiated by osteoclast infiltration. However, the data regarding miR-27a and miR-374a remain controversial and do not entirely support our theory.

In addition, several miRNAs modulate inflammatory process. IL-1β suppressed miR-140 expression and induced ADAMTS5, a member of the extracellular protease enzyme family. Conversely, transfection of chondrocytes with miR-140 downregulated IL-1β-induced ADAMTS5 expression [26]. In keeping with these findings, miR-140-3p was shown to ameliorate autoimmune arthritis [27]. Administration of miR-146a prevents joint destruction in arthritic mice presenting miR-146a as a negative regulator of inflammation [28]. These data suggest that miR-140-3p and miR-146a dysregulation may be associated with the

proinflammatory state characteristic of AxSpA. Also, miR-146a polymorphisms have been suggested to be a potential pathogenic factor for AS [29].

Here we propose circulating miRNAs as markers of disease activity. In nr-AxSpA patients, miR-140-3p, miR-223-3p, miR-99b-5p and miR-145-5p levels correlated with CRP. Both miR-99b and miR-145 were shown to be associated with osteoclast/osteoblast differentiation [30, 31]. However, no associations with BASDAI were observed in these patients or those with sacroiliitis.

Furthermore, in AS patients with spinal involvement, we observed correlations between miR-29a-3p and both BASDAI and CRP, as well as associations between miR-222-3p, miR-625-3p and miR-885-5p and BASDAI. The data on miR-29a levels in AS are rather inconsistent. While miR-29a expression in peripheral blood mononuclear cells was lower in active AS patients than in controls and decreased after anti-TNF therapy [32], another showed otherwise [33]. TGF-β, an important stimulator of bone formation [34], inhibits miR-29a expression [35, 36]. In addition, miR-29a has been described as a negative regulator of Wnt signalling and production of extracellular matrix [35, 36]. We hypothesize that advanced-stage AS patients with extensive bone formation have higher levels of TGF-β, which ultimately results in miR-29a suppression and increased bone formation. MiR-222 has been predicted to exert inhibitory effects on genes associated with osteogenic differentiation [37], and low levels of miR-222 may result in increased MMP-13 expression in osteoarthritic cartilage [38]. As mentioned above, miR-625-3p was significantly decreased in nr-AxSpA and may be associated with early disease. There is no pathophysiologic explanation for this finding, nor is there an explanation for its association with BASDAI in advanced AS patients. Similarly, data regarding the potential role of miR-885-5p in AxSpA are lacking.

There are some limitations in our study. The selection of miRNAs for validation analysis may appear biased. The initial mirPath screen of all miRNAs of 1.5 mean fold difference obtained by TLDA provided us with a broad-spectrum unspecific data since such an online tool is based on validated/predicted targets genes coming from different fields (mostly cancer) that may be yet largely unknown in AxSpA. Due to practical reasons, it was not feasible to validate all miRNAs fulfilling the abovementioned cut-off. Therefore, a manual review was implemented to narrow down the list of miRNAs taken forward for further analysis and to enable drawing hypotheses. Next, two different normalization methods were used in our study. Array data were normalized to Ct average of all miRNAs as miRNAs of non-human origin are not included on the array platform, while the normalization to the average of 3 spike-in controls was performed in single-assay analysis. At present, there is no consensus on normalization of cell-free miRNAs. As the normalization to endogenous cell-free miRNAs may be conditioned by their altered expression due to (patho)physiological condition of each individual, the use of spike-in controls of non-human origin appears an acceptable alternative. Moreover, this approach reflects potential errors during the workflow, although these were minimized. We appreciate that the differential expression pattern based on TLDA analysis was not always reflected in single assay analysis. More studies involving larger patient cohorts are needed to confirm these data, as only a small proportion of the circulating mirnome has been analysed, and the functions of many miRNAs remain unknown. Moreover, due to nature of circulating miRNAs direct functional experiments are not feasible and the involvement of miRNAs in the pathogenesis of AS was hypothesized based on published data.

Conclusions

We have shown for the first time the differential expression levels of several circulating miRNAs in radiographic AxSpA patients compared to non-radiographic disease patients and HC.

Moreover, the levels of these miRNAs appear to reflect progressive spinal involvement. Interestingly, while some of these miRNAs play roles in bone formation and associated signalling pathways, others play roles in inflammation. It can be hypothesized that differential systemic miRNA expression levels reflect their dysregulation at sites of spinal inflammation or bone formation where they contribute to the development of pathophysiological features characteristic of AS. However, only miR625-3p, whose role in AS is unknown, exhibited significantly different expression levels in nr-AxSpA patients compared to HC and, interestingly, correlated with disease activity in AS. Our data support the role of circulating miRNAs in the pathogenesis of AxSpA and their potential as biomarkers of disease progression. Additional studies involving larger patient cohorts are needed to confirm these data, as only a small proportion of the circulating mirnome has been analysed, and the functions of many miRNAs remain unknown.

Supporting information

S1 Fig. The expression of miRNAs in healthy controls (HC) and patients with non-radiographic axial spondyloarthritis (nr-AxSpA), sacroiliitis and ankylosing spondylitis with spinal involvement (AS II-V) using TaqMan Low Density Array and their shared expression among groups.
(TIF)

S1 Table. Original Taq Man Low Density Array (TLDA) data obtained in 5 healthy controls (HC), and 5 patients with non-radiographic axial spondyloarthritis (nr-AxSpA), 5 patients with sacroiliitis and 5 patients with ankylosing spondylitis with spinal involvement (AS II-V). The statistical analysis was performed as described in Methods paragraph.
(XLSX)

S2 Table. Functions of the 21 miRNAs selected for further analysis based on a literature search as explained in Methods paragraph.
(DOCX)

S3 Table. Data obtained from single assay analysis of 21 miRNAs. The data are provided as mean±SD calculated from data obtained from 29 healthy controls (HC), 20 patients with non-radiographic axial spondyloarthritis (nr-AxSpA), 24 patients with sacroiliitis and 24 patients with ankylosing spondylitis with spinal involvement (AS II-V). The statistical analysis was performed as described in Methods paragraph. Abbreviations: HC, healthy controls; nr-AxSpA, non-radiographic axial spondyloarthritis; AS, ankylosing spondylitis.
(DOCX)

S4 Table. Extended summary of expression and function of selected miRNAs as markers of disease activity and hypothesized role in AxSpA. Abbreviations: HC, healthy controls; nr-AxSpA, non-radiographic axial spondyloarthritis; AS, ankylosing spondylitis; AS II-V, ankylosing spondylitis with spinal involvement; DMARDs, disease modifying antirheumaticdrugs; NSAID, non-steroidal anti-inflammatory drugs; T, T test; -, not significant. Statistical significance was calculated using ANOVA unless stated otherwise.
(DOCX)

Author Contributions

Conceptualization: Klára Prajzlerová, Astrid Jüngel, Steffen Gay, Jiří Vencovský, Karel Pavelka, Ladislav Šenolt, Mária Filková.

Data curation: Klára Prajzlerová, Kristýna Grobelná, Markéta Hušáková, Šárka Forejtová.

Formal analysis: Klára Prajzlerová, Kristýna Grobelná.

Investigation: Klára Prajzlerová, Kristýna Grobelná, Markéta Hušáková, Šárka Forejtová, Mária Filková.

Methodology: Klára Prajzlerová, Astrid Jüngel, Karel Pavelka, Ladislav Šenolt, Mária Filková.

Supervision: Steffen Gay, Jiří Vencovský, Karel Pavelka, Ladislav Šenolt, Mária Filková.

Writing – original draft: Klára Prajzlerová, Mária Filková.

Writing – review & editing: Klára Prajzlerová, Kristýna Grobelná, Markéta Hušáková, Šárka Forejtová, Astrid Jüngel, Steffen Gay, Jiří Vencovský, Karel Pavelka, Ladislav Šenolt, Mária Filková.

References

1. Braun J, van den Berg R, Baraliakos X, Boehm H, Burgos-Vargas R, Collantes-Estevez E, et al. 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis. Annals of the rheumatic diseases. 2011; 70(6):896–904. https://doi.org/10.1136/ard.2011.151027 PMID: 21540199
2. Dougados M, Baeten D. Spondyloarthritis. Lancet. 2011; 377(9783):2127–37. https://doi.org/10.1016/S0140-6736(11)60071-8 PMID: 21684383.
3. Sieper J, Appel H, Braun J, Rudwaleit M. Critical appraisal of assessment of structural damage in ankylosing spondylitis: implications for treatment outcomes. Arthritis and rheumatism. 2008; 58(3):649–56. https://doi.org/10.1002/art.23260 PMID: 18311819.
4. Lories RJ, Luyten FP, de Vlam K. Progress in spondylarthritis. Mechanisms of new bone formation in spondyloarthritis. Arthritis research & therapy. 2009; 11(2):221. https://doi.org/10.1186/ar2642 PMID: 19439035
5. Rudwaleit M, Landewe R, van der Heijde D, Listing J, Brandt J, Braun J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. Annals of the rheumatic diseases. 2009; 68(6):770–6. https://doi.org/10.1136/ard.2009.108217 PMID: 19297345.
6. Sieper J, van der Heijde D, Dougados M, Mease PJ, Maksymowych WP, Brown MA, et al. Efficacy and safety of adalimumab in patients with non-radiographic axial spondyloarthritis: results of a randomised placebo-controlled trial (ABILITY-1). Annals of the rheumatic diseases. 2013; 72(6):815–22. https://doi.org/10.1136/annrheumdis-2012-201766 PMID: 22772328
7. Prajzlerova K, Grobelna K, Pavelka K, Senolt L, Filkova M. An update on biomarkers in axial spondyloarthritis. Autoimmunity reviews. 2016; 15(6):501–9. https://doi.org/10.1016/j.autrev.2016.02.002 PMID: 26851549.
8. Filkova M, Jungel A, Gay RE, Gay S. MicroRNAs in rheumatoid arthritis: potential role in diagnosis and therapy. BioDrugs: clinical immunotherapeutics, biopharmaceuticals and gene therapy. 2012; 26(3):131–41. https://doi.org/10.2165/11631480-000000000-00000 PMID: 22494429.
9. Filkova M, Aradi B, Senolt L, Ospelt C, Vettori S, Mann H, et al. Association of circulating miR-223 and miR-16 with disease activity in patients with early rheumatoid arthritis. Annals of the rheumatic diseases. 2014; 73(10):1898–904. Epub 2013/07/31. https://doi.org/10.1136/annrheumdis-2012-202815 PMID: 23897768
10. Braun J, van der Heijde D, Dougados M, Emery P, Khan MA, Sieper J, et al. Staging of patients with ankylosing spondylitis: a preliminary proposal. Annals of the rheumatic diseases. 2002; 61 Suppl 3:iii9–23. Epub 2002/10/17. https://doi.org/10.1136/ard.61.suppl_3.iii19 PMID: 12381507
11. Rudwaleit M, van der Heijde D, Landewe R, Akkoc N, Brandt J, Chou CT, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Annals of the rheumatic diseases. 2011; 70(1):25–31. https://doi.org/10.1136/ard.2010.133645 PMID: 21109520.
12. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. The Journal of rheumatology. 1994; 21(12):2286–91. PMID: 7699630.
13. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008; 105(30):10513–8. https://doi.org/10.1073/pnas.0804549105 PMID: 18663219

14. Carlsen AL, Schetter AJ, Nielsen CT, Lood C, Knudsen S, Voss A, et al. Circulating microRNA expression profiles associated with systemic lupus erythematosus. Arthritis and rheumatism. 2013; 65 (5):1324–34. Epub 2013/02/13. https://doi.org/10.1002/art.37890 PMID: 23401079.

15. Landskroner-Eiger S, Qiu C, Perrotta P, Siragusa M, Lee MY, Ulrich V, et al. Endothelial miR-17 approximately 92 cluster negatively regulates arteriogenesis via miRNA-19 repression of WNT signaling. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112 (41):12812–7. https://doi.org/10.1073/pnas.1507094112 PMID: 26417068

16. Zhao W, Wu C, Dong Y, Ma Y, Jin Y, Ji Y. MicroRNA-24 Regulates Osteogenic Differentiation via Targeting T-Cell Factor-1. International journal of molecular sciences. 2015; 16(5):11699–712. https://doi.org/10.3390/ijms160511699 PMID: 26006243

17. Hassan MQ, Gordon JA, Beloti MM, Croce CM, van Wijnen AJ, Stein JL, et al. A network connecting Runx2, SATB2, and the miR-23a~27a~24–2 cluster regulates the osteoblast differentiation program. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107 (46):19879–84. https://doi.org/10.1073/pnas.1007698107 PMID: 20980664

18. Wang T, Xu Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. Biochemical and biophysical research communications. 2010; 402(2):186–9. https://doi.org/10.1016/j.bbrc.2010.08.031 PMID: 20708603.

19. Li H, Li T, Wang S, Wei J, Fan J, Li J, et al. miR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem cell research. 2013; 10(3):313–24. Epub 2013/02/13. https://doi.org/10.1016/j.scr.2012.11.007 PMID: 23399447.

20. Hong Z, Hong H, Liu J, Zheng X, Huang M, Li C, et al. miR-106a Is Downregulated in Peripheral Blood Mononuclear Cells of Chronic Hepatitis B and Associated with Enhanced Levels of Interleukin-8. Mediators of inflammation. 2015; 2015:629862. https://doi.org/10.1155/2015/629862 PMID: 26265888

21. Azevedo VF, Faria-Neto JR, Stinghen A, Lorencetti PG, Miller WP, Goncalves BP, et al. IL-8 but not other biomarkers of endothelial damage is associated with disease activity in patients with ankylosing spondylitis without treatment with anti-TNF agents. Rheumatology international. 2013; 33(7):1779–83. Epub 2013/01/09. https://doi.org/10.1007/s00296-012-2631-x PMID: 23297014.

22. Xie Y, Zhang L, Gao Y, Ge W, Tang P. The Multiple Roles of Microrna-223 in Regulating Bone Metabolism. Molecules. 2015; 20(10):19433–48. https://doi.org/10.3390/molecules201019433 PMID: 26512640.

23. M'Baya-Moutoula E, Louvet L, Metzinger-Le Meuth V, Massy ZA, Metzinger L. High inorganic phosphate concentration inhibits osteoclastogenesis by modulating miR-223. Biochimica et biophysica acta. 2015; 1852(10 Pt A):2202–12. https://doi.org/10.1016/j.bbadis.2015.08.003 PMID: 26255635.

24. Castro-Villegas C, Perez-Sanchez C, Escudero A, Filipescu I, Verdu M, Ruiz-Limon P, et al. Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNFalpha. Arthritis research & therapy. 2015; 17:49. Epub 2015/04/11. https://doi.org/10.1186/s13075-015-0555-z PMID: 25860297

25. Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, et al. MicroRNA-374a activates Wnt/beta-catenin signaling to promote breast cancer metastasis. The Journal of clinical investigation. 2013; 123(2):566–79. Epub 2013/01/17. https://doi.org/10.1172/JCI65871 PMID: 23321667

26. Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis and rheumatism. 2009; 60(9):2723–30. Epub 2009/08/29. https://doi.org/10.1002/art.24745 PMID: 19714579

27. Peng JS, Chen SY, Wu CL, Chong HE, Ding YC, Shiau AL, et al. Amelioration of Experimental Autoimmune Arthritis Through Targeting of Synovial Fibroblasts by Intraarticular Delivery of MicroRNAs 140-3p and 140-5p. Arthritis & rheumatology. 2016; 68(2):370–81. https://doi.org/10.1002/art.39446 PMID: 26473405.

28. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. Arthritis and rheumatism. 2011; 63(6):1582–90. Epub 2011/03/23. https://doi.org/10.1002/art.30321 PMID: 21425254.

29. Xu HY, Wang ZY, Chen JF, Wang TY, Wang LL, Tang LL, et al. Association between ankylosing spondylitis and the miR-146a and miR-499 polymorphisms. PloS one. 2015; 10(4):e0122055. Epub 2015/04/04. https://doi.org/10.1371/journal.pone.0122055 PMID: 25836258

30. de la Rica L, Garcia-Gomez A, Comet NR, Rodriguez-Ubreva J, Ciudad L, Vento-Tormo R, et al. NF-kappaB-direct activation of microRNAs with repressive effects on monocyte-specific genes is critical for osteoclast differentiation. Genome biology. 2015; 16:2. https://doi.org/10.1186/s13059-014-0561-5 PMID: 25601191

31. Fukuda T, Ochi H, Sunamura S, Haiden A, Bando W, Inose H, et al. MicroRNA-145 regulates osteoblastic differentiation by targeting the transcription factor Cbfb. FEBS letters. 2015; 589(21):3302–8. https://doi.org/10.1016/j.febslet.2015.09.024 PMID: 26450370.

32. Lv Q, Li Q, Zhang P, Jiang Y, Wang X, Wei Q, et al. Disorders of MicroRNAs in Peripheral Blood Mononuclear Cells: As Novel Biomarkers of Ankylosing Spondylitis and Provocative Therapeutic Targets. BioMed research international. 2015; 2015:504208. https://doi.org/10.1155/2015/504208 PMID: 26273623

33. Huang J, Song G, Yin Z, Luo X, Ye Z. Elevated miR-29a expression is not correlated with disease activity index in PBMCs of patients with ankylosing spondylitis. Modern rheumatology / the Japan Rheumatism Association. 2014; 24(2):331–4. https://doi.org/10.3109/14397595.2013.854077 PMID: 24593209.

34. Noda M, Camilliere JJ. In vivo stimulation of bone formation by transforming growth factor-beta. Endocrinology. 1989; 124(6):2991–4. https://doi.org/10.1210/endo-124-6-2991 PMID: 2721454.

35. Le LT, Swingler TE, Crowe N, Vincent TL, Barter MJ, Donell ST, et al. The microRNA-29 family in cartilage homeostasis and osteoarthritis. Journal of molecular medicine. 2015. https://doi.org/10.1007/s00109-015-1374-z PMID: 26687115.

36. Maurer B, Stanczyk J, Jungel A, Akhmetshina A, Trenkmann M, Brock M, et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. Arthritis and rheumatism. 2010; 62(6):1733–43. https://doi.org/10.1002/art.27443 PMID: 20201077.

37. Yu F, Cui Y, Zhou X, Zhang X, Han J. Osteogenic differentiation of human ligament fibroblasts induced by conditioned medium of osteoclast-like cells. Bioscience trends. 2011; 5(2):46–51. Epub 2011/05/17. PMID: 21572246.

38. Song J, Jin EH, Kim D, Kim KY, Chun CH, Jin EJ. MicroRNA-222 regulates MMP-13 via targeting HDAC-4 during osteoarthritis pathogenesis. BBA clinical. 2015; 3:79–89. https://doi.org/10.1016/j.bbacli.2014.11.009 PMID: 26673737.

Autoimmunity Reviews 15 (2016) 501–509

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

An update on biomarkers in axial spondyloarthritis

Klára Prajzlerová *, Kristýna Grobelná, Karel Pavelka, Ladislav Šenolt, Mária Filková

Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

ARTICLE INFO

Article history:
Received 15 January 2016
Accepted 28 January 2016
Available online 4 February 2016

Keywords:
Axial spondyloarthritis
Pathogenesis
Biomarker
Inflammation

ABSTRACT

Axial spondyloarthritis is a chronic inflammatory disease with the onset at a young age, and, if undiagnosed and untreated, it may result in permanent damage and lifelong disability. Rates of early diagnosis have improved, due in particular to the addition of magnetic resonance imaging into the diagnostic armamentaria; however, it is costly, not widely available, and requires experienced readers to interpret the findings. In addition to clinical measures and imaging techniques, biomarkers that will be described in this review may represent useful tools for diagnosis, monitoring disease activity and outcomes as well as therapeutic responses. Currently, HLA-B27 remains the best genetic biomarker for making a diagnosis, while CRP currently appears to be the best circulating measure for assessing disease activity, predicting structural progression and therapeutic response. Interestingly, key molecules in the pathogenesis of the disease and essential therapeutic targets, such as tumour necrosis factor (TNF)α, interleukin (IL)-17 and IL-23, show only limited association with disease characteristics or disease progression. Some genetic biomarkers and particularly anti-CD74 antibodies, may become a promising tool for the early diagnosis of axSpA. Further biomarkers, such as matrix metalloproteinases (MMP)-3, calprotectin (S100A8/9), vascular endothelial growth factor (VEGF), C-terminal telopeptide of type II collagen (CTX-II) or dickkopf-1 (DKK-1), are not sufficient to reflect disease activity, but may predict spinal structural progression. In addition, recent data have shown that monitoring calprotectin might represent a valuable biomarker of therapeutic response. However, all of these results need to be confirmed in large cohort studies prior to use in daily clinical practice.

Contents

* Corresponding author at: Institute of Rheumatology, Na Slupi 4, 128 50 Prague, Czech Republic. Tel.: +420 234 075 329; fax: +420 224 914 451.
E-mail address: prajzlerova@revma.cz (K. Prajzlerová).

http://dx.doi.org/10.1016/j.autrev.2016.02.002
1568-9972/

1. Introduction

Axial spondyloarthritis (axSpA) is a group of heterogeneous inflammatory rheumatic diseases predominantly affecting the axial skeleton, which is associated with chronic back pain due to sacroiliac joint and spinal inflammation [1]. Diverse clinical manifestations are common in axSpA and include enthesitis, peripheral arthritis (mostly affecting the large joints of the lower extremities) and characteristic extra-articular manifestations, such as uveitis, psoriasis or inflammatory bowel diseases.

The Assessment of SpondyloArthritis international Society (ASAS) has recently developed new set of classification criteria for axSpA that were initially intended for clinical research purposes [2]. These criteria cover the whole spectrum of axial involvement ranging from patients with non-radiographic axSpA (nr-axSpA) to the well-known group of patients who already fulfil the modified New York classification criteria for ankylosing spondylitis (AS) [3]. Because magnetic resonance imaging (MRI) is now a common practice in many countries for evaluating patients suspected of having axSpA, MRI-detected active sacroiliitis has become an important tool for the recognition and early diagnosis of axSpA [4].

However, the diagnosis of the early phases of axSpA still represents a clinical challenge in daily practice because chronic back pain is a common symptom and MRI inflammatory abnormalities can also be detected in healthy individuals. Furthermore, HLA-B27 can be often positive with no associated clinical presentation. Assessment and monitoring of disease activity is mostly limited to patient reported outcomes that do not necessarily correlate with MRI-detected inflammation [5] and to measuring the CRP, which is less sensitive in axSpA [6], and can also be used in assessing progression of structural damage [7], particularly of the spine, and response to therapy [8]. In the context of pathogenic mechanisms (Fig. 1, Table 1), we summarize here the genetic components, biomarkers of inflammation and tissue remodelling, cytokines and other immune mediators, including parameters of angiogenesis and autoantibodies that may improve the diagnosis, prognosis and treatment outcomes in axSpA.

2. Genetic components

HLA-B27 is a class I surface antigen that plays a major role in protective immunity. It is encoded by the B locus of the major histocompatibility complex (MHC) and presents peptide antigens to the T-cells. Whether the major role of HLA-B27 in axSpA is the auto-antigen presentation to CD8 + T-cells suggestive of autoimmune disease, or whether its main function is triggering the innate immune responses secondary to bacterial or mechanical stress in keeping with autoinflammatory disease remains to be elucidated [1]. Irrespective of its pathophysiological function, HLA-B27 is strongly associated with axSpA and represents a core laboratory test for the diagnosis of axSpA. No clear associations between HLA-B27 and sacroiliac or spinal radiographic progression over two years were found in patients with early axSpA [7], however, more severe radiographic progression may be observed in HLA-B27 positive AS patients in long-term [9].

Although HLA-B27 is positive more often in established disease—AS (80–90%), it is less commonly present in early phases of the disease in nr-axSpA patients (73–75%) [7,10]. Although the test has a good sensitivity, it has a low specificity as the prevalence of HLA-B27 in healthy subjects varies across regions ranging between 4 to 25% in western to northern European countries [11] and only 1.3–6% of the HLA-B27 positive population develops AS [1,12]. This is suggestive of contribution of additional genes to susceptibility to axSpA [13].

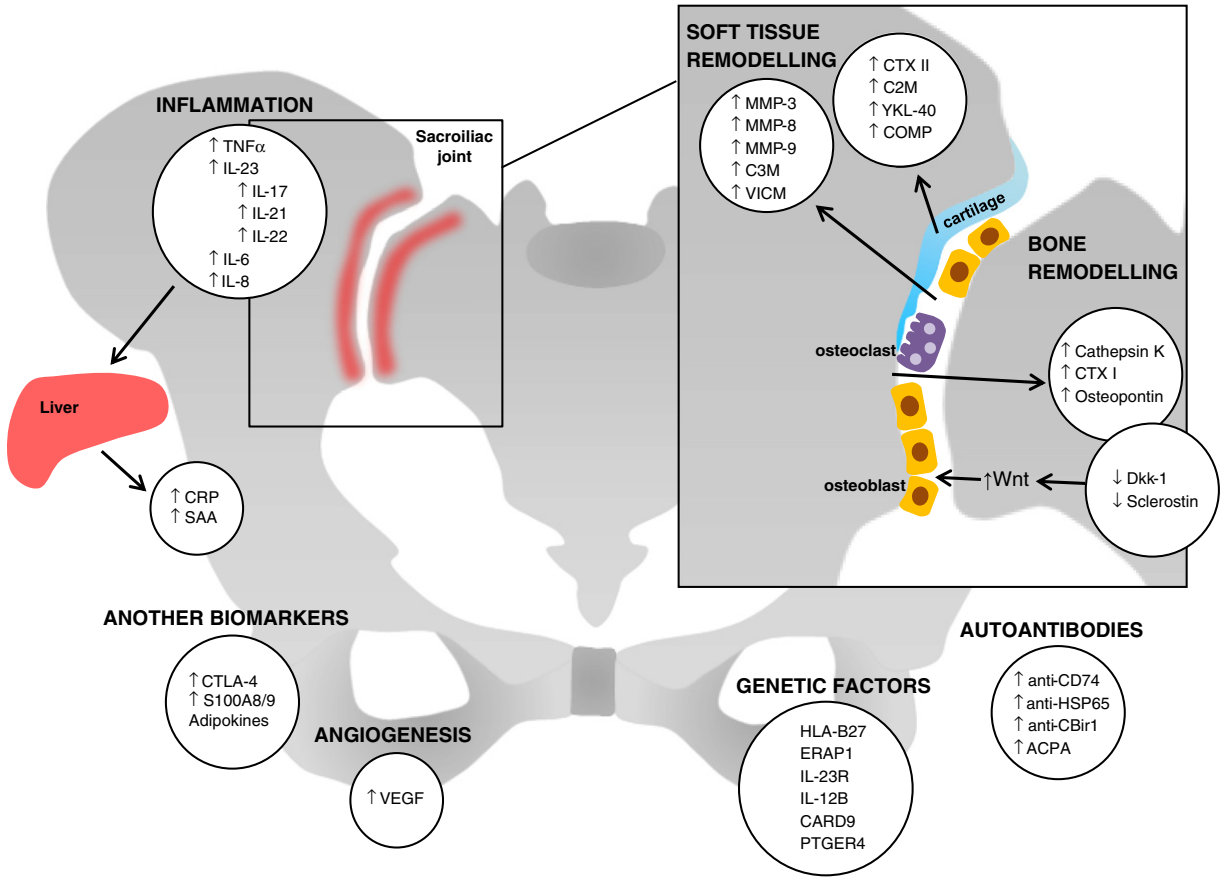

Fig. 1. Biomarkers of axial spondyloarthritis according to pathogenic mechanisms.

Table 1
Summary of biomarkers with established roles and exploratory value in the diagnosis and monitoring of disease activity and treatment response in patients with axial spondyloarthritis, and their potential for becoming therapeutic targets.

Diagnosis	Disease activity	Structural progression	Therapeutic response	Therapeutic targets
HLA B27 [12]	YKL-40 [41] TNFα [75]	CTX-II [36] CTX-I [48]	CRP [23]	TNFα [77]
anti-CD74 [137]	MMP-3 [42] MMP-8 [70] MMP-9 [70]	C2M/C3M [40]	ESR [23]	IL-17 [87]
	IL-8 [101]	COMP [8]	Sclerostin [63] SAA [23]	IL-23 [90]
	IL-17 [83]	Cathepsin K [50]	S100A8/9 [25]	
	IL-23 [83]	Osteopontin [46]		
	IL-21 [94]	Dkk-1 [58]		
	SAA [103]	Sclerostin [61] MMP-3 [43]		
	CTLA-4 [107]	VICM [72]		
	Leptin [123]	IL-6 [8]		
		S100A8/9 [130]		
		VEGF [114]		
		Visfatin [116]		

Other genes are involved in the pathogenesis of axSpA, for instance, there is a close link between HLA-B27 and endoplasmic reticulum aminopeptidase 1 (ERAP1), which trims peptides so they can be loaded onto the MHC. Specific haplotypes are strongly associated with increased risk of AS whereas some variants are AS protective [14–16]. Moreover, ERAP1 inhibition seems to be a potential treatment for AS [15]. Other gene polymorphisms revealed to be involved with AS and that play some role in the IL23-IL17 pathway include IL23R, IL-12B, CARD9 and PTGER4 [16–19].

3. Routine inflammatory biomarkers

3.1. C-reactive protein

C-reactive protein (CRP) is a commonly used acute-phase protein that reflects systemic inflammation. However, the degree of inflammation observed fluctuates during the course of axSpA. In general, the level of CRP is higher in patients with the full-blown radiographic form of the disease compared with nr-axSpA [6,7,20]. Although CRP is not elevated in a large proportion of patients with active axSpA, it is widely used as a reliable parameter of disease activity. CRP correlates with the Ankylosing Spondylitis Disease Activity Score (ASDAS) and, to a lesser extent, to the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), while its correlation with the Bath Ankylosing Spondylitis Functional Index (BASFI) is inconclusive. CRP levels are moderately correlated with MRI inflammation, which is presently the best tool for assessing disease activity [6,8,21,22].

In addition, CRP has been demonstrated to be a reliable biomarker for the monitoring of treatment response and the prediction of further structural progression of the disease. Several studies and clinical practice have demonstrated that elevated CRP levels decrease significantly during anti-TNF therapy [8,21,23–25]. Importantly, the extent of change in CRP levels correlate with changes in BASDAI and MRI scores [21,23], and elevated baseline CRP levels are associated with good treatment response [23].

However, Pedersen et al. recently demonstrated that the development of new syndesmophytes is associated with a more significant decrease in CRP, while persistent systemic inflammation is associated with a lack of radiographic progression over a short period of time [8]. This finding is in line with the hypothesis that, as a result of resolved inflammation, the increased rate of ossification of repaired tissue may occur, while chronic inflammation prevents syndesmophyte formation [26]. In addition, because several studies failed to show inhibition of structural progression in patients with AS over two years of anti-TNF therapy [27,28], it can be hypothesized that ossification appears independent of systemic inflammation, at least in terms of spinal involvement. However, longer duration (e.g. eight years) of treatment with anti-TNF may be necessary to inhibit new syndesmophyte formation in patients with AS [29].

Although the progression of radiographic sacroiliitis over two years of anti-TNF therapy was considered low in patients with nr-axSpA as well as AS, it is evident that elevated CRP levels at the baseline represent a strong positive predictor of radiographic sacroiliitis progression, especially for the progression from nr-axSpA to full-blown AS [7]. In addition, several prospective studies have demonstrated that elevated CRP levels are independently associated with radiographic spinal progression in patients with axSpA [7,30,31]. This adds more complexity to how the inflammation contributes to osteoproliferation in the context of axSpA and how available treatment prevents further structural damage. At present, CRP appears to be the most versatile and reliable biomarker for assessing disease activity and predicting structural progression as well as response to therapy.

3.2. Erythrocyte sedimentation rate

Erythrocyte sedimentation rate (ESR) is a nonspecific measure of inflammation that may be influenced by a variety of other conditions. It appears a worse marker of disease activity than CRP, although these data are not consistent [23,32,33]. Furthermore, in patients with early axSpA, elevated ESR is independently associated with radiographic spinal progression [31].

4. Markers of tissue remodelling

During the course of axSpA, both tissue degradation and the production of new tissue occur. Biomarkers of tissue remodelling are therefore expected to be differentially expressed during the disease.

4.1. Markers of cartilage remodelling

Type II collagen is a structural protein of cartilage and type III collagen is a component of most connective tissues. The C-terminal telopeptide of type II collagen (CTX-II) specifically indicates cartilage turnover [34]. It has been shown that urinary levels of CTX-II are higher in AS patients compared with healthy controls [8,35] and that they correlate with markers of systemic inflammation [36,37]. In addition, axSpA patients with MRI proven inflammation had higher CTX-II levels [8], and treatment with anti-TNF agents contributed to significant decreases in urinary CTX-II levels that correlated with improvement in disease activity [37]. There is data showing that urine levels of CTX-II are found to correlate with joint destruction in patients with rheumatoid arthritis [38]. Similarly, CTX-II appears to be a biomarker of radiographic progression in axSpA; CTX-II levels were associated with radiographic spinal damage and structural progression in patients with AS [35,36].

Other biomarkers of cartilage and synovium turnover include the degradation products C2M and C3M, respectively, that are both significantly higher in patients with AS compared with healthy controls [39,40]. C3M, but not C2M, correlated with acute phase reactants and with radiographic modified Stoke Ankylosing Spondylitis Spine Score

(mSASSS). Furthermore, the combination of both biomarkers had a high predictive value for radiographic progression of the disease [39,40].

Serum human cartilage glycoprotein-39 (YKL-40) is a protein secreted by human chondrocytes and synoviocytes and can be used as a marker for cartilage remodelling and synovial hyperplasia. YKL-40 levels are significantly higher in SpA patients compared with healthy controls [41]. While the change in YKL-40 after therapy varies [8,41,42], there is some evidence supporting a correlation between the change in YKL-40 with a change in disease activity using BASDAI, ESR and CRP, but not with radiographic progression [42,43].

Cartilage Oligomeric Matrix Protein (COMP) is an extracellular matrix protein that catalyzes the assembly of type I and type II collagen and reflects increased cartilage turnover [44]. A weak inverse relationship between COMP levels and MRI inflammation in patients with axSpA is suggestive of increased collagen synthesis while the inflammatory process is suppressed [8].

4.2. Markers of bone remodelling

New bone formation (syndesmophytes) and bone resorption (erosions) are typical features of axSpA, suggesting that parameters of bone metabolism may be impaired during the course of the disease.

C-terminal telopeptide of type I collagen (CTX-I) is a marker of osteoclast activity reflecting bone degradation [45]. Both urinary and serum CTX-I levels are higher in AS patients compared with healthy controls [8,35,46]. Urinary CTX-I reflects disease activity and loss of femoral bone mass density (BMD) [35], but not radiographic spine damage [36]. In patients receiving anti-TNF therapy, baseline serum levels of CTX-I correlated with increases in spinal BMD over periods of 24 and 102 weeks [47]. Furthermore, in a cross-sectional study, patients with AS with more than one bridging syndesmophyte had significantly higher serum levels of CTX-I than patients without bridging syndesmophytes [48].

Cathepsin K, an enzyme expressed predominantly in osteoclasts triggered by inflammatory cytokines, is involved in bone remodelling and resorption. Although systemic levels of cathepsin K in patients with AS were similar to those in healthy controls [49], there was a strong expression of cathepsin K in mononuclear cells, fibroblast-like cells and cells attached to bone in patients with AS in contrast to those with degenerative disc disease [50].

Osteopontin is a secreted protein expressed by various cells including inflammatory cells, chondrocytes or cells involved in bone metabolism, such as osteoclasts and osteoblasts. Circulating osteopontin levels as well as its intracellular expression in inflammatory cells were higher in patients with AS than in controls. Although the data are not robust, osteopontin appears a marker of tissue remodelling rather than a marker of inflammation in AS based on its strong correlation with other markers of bone metabolism (osteocalcin, CTX-I) and the lack of association with inflammatory parameters [46].

Dickkopf-1 (DKK-1) is an inhibitor of Wnt signalling, a crucial pathway affecting bone formation [51]. The deregulation of DKK-1 leads to different bone pathologies [52], including AS [53]. In a mouse model, inhibition of DKK-1 did not affect inflammatory signs of sacroiliitis, but significantly reduced bone erosions and number of osteoclasts and triggered ankylosis of the sacroiliac joints [54]. However, conflicting data on systemic DKK-1 levels have been reported. Few studies showed significantly higher DKK-1 levels in patients with rheumatoid arthritis compared with healthy controls, while DKK-1 levels in AS patients were lower than in healthy subjects [55,56]. In contrast, another study showed high levels of serum DKK-1 in patients with AS compared with healthy controls or other types of arthritis [57]. There was a trend toward higher functional DKK-1 levels in AS patients without syndesmophytes compared with those with syndesmophytes, while the total DKK-1 levels were not different. In addition, the DKK-1 levels were significantly higher in patients with no syndesmophyte growth compared with those with syndesmophyte formation over two years, irrespective of the baseline presence of syndesmophytes [58]. These data suggest that high serum levels of functional DKK-1 are protective and are associated with lower syndesmophyte formation in AS patients [58]. While levels of DKK-1 remained unchanged [56] or even decreased [59] after therapy with TNF inhibitors [56], higher serum DKK-1 levels in AS patients treated with anti-TNF agents versus untreated AS patients were noted [57]. This was not a disease activity–related effect because patients in clinical remission had similar DKK-1 levels to AS patients with active disease, and only anti-TNF administration was a significant predictor of serum DKK-1 levels irrespective of other disease characteristics [57].

Sclerostin is another Wnt signalling inhibitor that negatively regulates osteoblast function and suppresses bone formation [60]. Sclerostin expression is impaired in AS as shown in a mouse model of spondylitis [53]. Similarly, low local expression of sclerostin as well as low circulating levels were demonstrated in patients with AS compared with healthy controls or patients with inflammatory arthritis [61,62]. In addition, low circulating sclerostin in patients with AS was significantly associated with the formation of new syndesmophytes [61]. Sclerostin levels progressively increased over 12 months of anti-TNF therapy, although they remained significantly lower in AS patients compared with controls [63]. In addition, AS patients with lower sclerostin serum levels had higher baseline CRP levels and an increased risk of having high CRP levels after 12 months of treatment than patients with higher baseline sclerostin levels [62,63]. These data indicate that baseline levels of sclerostin seem to be predictive of anti-TNF therapy response.

4.3. Other biomarkers of tissue degradation

Matrix metalloproteinases (MMPs), enzymes regulating the breakdown of the extracellular matrix, are involved in multiple pathologies [64]. In general, serum levels of MMP-3 are increased in patients with AS compared with healthy controls [25,49,65–67]. In addition, MMP-3 correlated with CRP level, disease activity and functional status assessed by BASDAI and BASFI, respectively, and was suggested to be a more accurate parameter than acute phase reactants in identifying AS patients with high disease activity [22,65–68]. Although serum MMP-3 represents a reliable parameter of disease activity, it is often not reflected in MRI studies of inflammation of the sacroiliac joints [68]. On the other hand, baseline MMP-3 was demonstrated as an independent predictor of the two year radiographic progression [43], but this was not observed in another study, nevertheless of a shorter duration [8]. Change in MMP-3 levels following therapy in patients with AS showed inconsistent results with either unchanged or decreased levels [8,25,42,69]. Although investigated to a lesser extent, MMP-8 and MMP-9 were shown to be associated with disease activity assessed by BASDAI score [70].

Vimentin is a part of the cytoskeleton expressed by various cells. Citrullinated fragments of vimentin (VICM) are MMP-degraded fragments modified by citrullination, an unspecific modification associated with the inflammatory process [71]. Levels of VICM are significantly higher in patients with AS than in healthy controls and, moreover, patients with higher levels of VICM have more structural damage and higher disease activity as measured by mSASSS and BASDAI. Importantly, patients with higher levels of VICM and high baseline mSASSS are at higher risk of radiographic progression over two years [72].

5. Cytokines as promising therapeutic targets

TNF-alpha (TNFα) is a crucial cytokine that plays an essential role during the inflammatory response via the upregulation of adhesive molecules, lymphocyte activation, fibroblast proliferation and the production of the main pro-inflammatory cytokines [73]. TNFα levels are significantly higher in patients with AS compared with subjects with non-inflammatory back pain or healthy controls [74,75]. TNFα expression is strongly up-regulated in sacroiliac joint biopsies of AS patients

[76]. Although it is not a reliable biomarker of disease activity, targeting TNFα has become a major step forward in the management of axSpA [77].

In addition to TNFα, the IL-23/IL-17 immune axis appears to be a very important pathway in the pathogenesis of SpA [78]. Despite the lack of association between these two cytokines and disease activity, both cytokines have become promising therapeutic targets [78]. IL-17 is mainly produced by Th17+ T-helper cells that are initially activated by IL-23. This axis is crucially involved in inflammation and autoimmune diseases [78]. The number of IL-17+ T cells in peripheral blood is higher in patients with AS compared with healthy individuals [79–81]. Interestingly, the number of IL-17+ peripheral T cells is equally increased in patients with early active axSpA both with and without MRI abnormalities [80]. IL-17 is up-regulated in facet joints [82] as well as in the blood circulation [83–86] of AS patients compared with subjects with degenerative changes or healthy controls, respectively. However, in most studies, systemic levels of IL-17 correlated neither with inflammatory markers nor with clinical disease activity [83,84,86].

To stress the role of the IL-17 axis in AS, treatment with TNFα blocking agents significantly reduced the number of Th17 cells as well as serum IL-17 levels in responders, but it increased both in non-responders [83]. Moreover, in a recent phase 2 trial, treatment with the anti-IL-17 A monoclonal antibody secukinumab was effective in patients with AS [87].

As IL-23 induces the production of IL-17, both cytokines positively correlated with each other in AS patients [84]. Although IL-23 serum levels [84,85,88–90] and IL-23 expression in the subchondral bone marrow from the facet joints [91] are up-regulated in AS patients, they do not correlate with the CRP level, MRI inflammation or clinical disease activity [83,89]. Similarly to IL-17, the levels of IL-23 significantly decreased in responders to anti-TNF therapy while they increased in non-responders [83]. Furthermore, in a proof-of-concept clinical trial, ustekinumab, a monoclonal antibody against the p40 subunit shared by IL-12 and IL-23, demonstrated clinical improvement in patients with AS [90].

In addition, IL-21 and IL-22, both interconnected with IL-17 and Th17 cells [92], are elevated in patients with AS [89,93–95]. Although no significant correlation between IL-21 and disease activity has been demonstrated, IL-21 is decreased after the initiation of conventional and anti-TNF therapy [94,96].

Interleukin 6 (IL-6), an important cytokine regulating the immune response, acute phase reaction and haematopoiesis, is involved in the pathogenesis of multiple diseases, including immune-mediated disorders [97]. Most studies have demonstrated elevated serum levels of IL-6 in patients with AS compared with healthy controls [25,41,75,83,98]. Interestingly, high local expression of IL-6 has been found in sacroiliac biopsies of AS patients, more pronounced in patients with early disease compared with advanced stages [99]. Serum levels of IL-6 were shown to be associated with disease activity, including acute phase reactants, BASDAI and ASDAS [74,83]. Decrease in serum IL-6 levels were shown in AS patients following anti-TNF treatment [8,21,41], in good responders in particular [83]. Thus, IL-6 may be used as a predictor of therapeutic response. Moreover, early drop in levels of IL-6 (unlike CRP) two weeks after treatment initiation was associated with significant improvement in disease activity measured by BASDAI and the spinal inflammation detected by MRI after six months of treatment [21]. These results were supported in another study where normalization of IL-6 levels correlated with a decrease in MRI inflammation scores [8]. Baseline IL-6 levels correlated with increased spinal BMD after anti-TNF therapy and decrease in IL-6 levels was associated with new syndesmophytes formation [8,47]. However, it was recently demonstrated that IL-6 receptor blockade with sarilumab was not an effective treatment for AS [100].

IL-8, a member of the CXC family (known as CXCL 8), is an inflammatory chemokine which induces chemotaxis in target cells. Serum levels of IL-8 are significantly higher in patients with AS compared with controls and were shown to correlate with laboratory and clinical disease activity [98,101]. However, further studies are needed to confirm the role of IL-8 as a biomarker of disease activity or treatment response in patients with axSpA, particularly in early phases of the disease.

6. Other inflammatory mediators

Serum amyloids A (SAA) are proteins associated with high-density lipoprotein that are produced as a response to inflammatory stimuli [102]. Patients with AS have significantly higher levels of serum SAA than healthy controls, and the levels of SAA were shown to correlate with laboratory as well as clinical parameters of disease activity [103,104]. Given that SAA is a precursor of amyloid A protein leading to complications in severe form of AS, it is hypothesizedd that high circulating SAA is a predisposing indicator of disease activity [104]. Levels of SAA were significantly decreased after anti-TNF therapy, were associated with BASDAI and, importantly, elevated SAA levels prior therapy in combination with CRP predicted treatment response [23,105].

Cytotoxic T lymphocyte associated molecule (CTLA-4) is involved in inhibition of immune response. While the membrane-bound CTLA-4 is a negative regulator of T cell-mediated immune response, the role of soluble CTLA-4 is still not completely understood [106]. Serum levels of CTLA-4 were significantly higher in the patients with SpA than in healthy controls and correlated with CRP and BASDAI [107]. While treatment of RA patients with abatacept, a fusion CTLA-Ig protein, has demonstrated good efficacy, a pilot study in patients with AS failed to prove satisfactory treatment response [108,109].

7. Biomarkers of angiogenesis

Vascular endothelial growth factor (VEGF), a signalling protein that plays a crucial role in angiogenesis, is involved in the pathogenesis of arthritis [110]. Because angioneogenesis is important for new bone formation as well as sacroiliitis and enthesitis, VEGF has been suggested to be a promising biomarker in SpA. Indeed, patients with AS have higher systemic levels of VEGF [41,111,112] that were shown to correlate with acute phase reactants and functional status [83,111,113]. There is a body of evidence demonstrating that VEGF is significantly decreased following anti-TNF therapy [8,21,25,41,113]. Although this decrease does not seem to correlate with the change in MRI inflammation or BASDAI improvement, elevated baseline VEGF levels were associated with increases in spinal BMD over time [21,47]. While Drouart et al. found no association between VEGF and the presence of extra-articular manifestations, syndesmophytes or with the severity of sacroiliitis [111], other studies have shown that elevated serum VEGF is a reliable predictor of radiographic progression in patients with axSpA, especially in patients with the presence of syndesmophytes at baseline [8,114].

8. Controversial adipokines

Adipokines, although originally thought to be produced specifically by adipose tissue, are released by various cells and have been associated with a variety of inflammatory conditions [115]. While resistin and visfatin levels were demonstrated to be higher, adiponectin levels were either higher or comparable in patients with AS compared with healthy controls [116–119]. Several studies have demonstrated either lower or comparable levels of leptin in patients with AS compared with healthy controls [118–120]. While adipokines have been extensively studied in RA with controversial results, most data in AS show a lack of association with disease activity [121,122]. However, high baseline visfatin levels were predictive of radiographic progression over two years [116]. AS patients with hip involvement, synovitis and/or enthesitis had higher adiponectin levels than patients without complications [121]. In addition, systemic levels of leptin were found to be elevated in patients with AS with syndesmophytes and were associated with BASDAI and IL-6 [123,124].

9. Calprotectin (S100A8/9)—biomarker of clinical response?

Calprotectin is a heterodimer composed of the two calcium-binding proteins S100A8 and S100A9 with pro-inflammatory properties and an indisputable role in RA [125,126]. Faecal calprotectin is used to distinguish inflammatory bowel disease from non-inflammatory conditions [127]. The levels of faecal calprotectin in AS patients were associated with age, disease duration, acute phase reactants, but, interestingly, not with gastrointestinal symptoms. However, faecal calprotectin was higher in AS patients using NSAIDs, while it was lower in patients on disease modifying antirheumatic drugs, which may suggest subclinical intestinal inflammation [128]. Serum calprotectin was shown to be increased in SpA patients compared with healthy controls [25,129]. Although serum calprotectin was positively correlated with acute-phase reactants, it did not correlate with disease activity indices [128–130]. More interestingly, baseline calprotectin was higher in patients with more rapid radiographic spinal progression, was decreased upon treatment with TNFα-inhibitors and may represent a biomarker for clinical response [25,130].

10. Autoantibodies as biomarkers?

Several (auto)antibodies have already been studied in axSpA with some showing promising results. Antibodies to different microbial antigens have been studied in axSpA. Mycobacteria are arthritogenic microorganisms involved in the pathogenesis of SpA and inflammatory bowel disease (IBD) [131,132]. As demonstrated, autoantibodies against mycobacterial HSP 65 kDa (HSP65) were detected in the sera of patients with AS [133,134]. While the first report did not show any differences, more recent data showed higher levels of anti-HSP65 in patients with AS than in healthy controls [134]. However, these antibodies did not differentiate between axial and peripheral disease, did not distinguish between the presence or absence of extraarticular features, and did not correlate with disease activity [134].

Flagellin, a molecular component of bacterial surfaces, triggers the production of pro-inflammatory cytokines. While there were either higher or no differences in positivity rates of antibodies against flagellin (anti-CBir-1) between patients with IBD and patients with AS [131,132], these antibodies were able to distinguish AS from healthy controls [131].

While anti-citrullinated peptide antibodies (ACPA) are a useful diagnostic tool in RA, their relevance to axSpA remains largely unknown. Recently, antibodies against (modified) citrullinated vimentin (anti-MCV) were identified in 14% of patients with SpA, compared with 62% with RA or 15% of PsA patients [135] or in 37% of AS patients compared with 0% of healthy controls [134]. Similar to anti-HSP65 antibodies, no association with disease activity was shown.

Recently, IgG antibodies against CD74 were found to be elevated in patients with axSpA compared with non-SpA patients (67–85 vs. <15%) or to healthy individuals (<1%) [136,137]. CD74 is a gamma chain of HLA class II involved in antigen presentation, B-cell differentiation and inflammatory signalling [138]. Its extracellular portion has two domains: the thyroglobulin type-1 and the class II-associated invariant chain peptide (CLIP). Binding of CLIP antibodies to CD74 may lead to the activation of cells and the production of pro-inflammatory cytokines such as TNFα [139]. Anti-CLIP antibodies were strongly associated with a short disease duration of axSpA [136] and with a higher likelihood of confirming the diagnosis than using HLA-B27 (sensitivity of anti-CLIP vs. HLA-B27 for the diagnosis of axSpA: 85.1 vs. 77.8%) [137]. Therefore, anti-CLIP antibodies may become a promising tool for the early diagnosis of axSpA.

11. Synovial tissue analysis—an underestimated tool?

In addition to axial presentation, the involvement of peripheral joints with chronic synovitis may also be observed in axSpA patients. The unique information obtained from synovial tissue may be superior to markers obtained from peripheral blood. Synovial tissue analyses revealed distinct histology between patients with RA and axSpA, although the pattern was not significantly different between SpA subtypes (e.g., AS, psoriatic, reactive or enteropathic arthritis) [140]. Considering the relation between synovial histopathology and disease activity in SpA, the following relationships were revealed: swollen joint count correlated with lining-layer thickness and sublining macrophage infiltrations, while CRP levels correlated significantly with synovial infiltration with polymorphonuclear cells and macrophages. It appears that global disease activity in SpA is essentially associated with inflammatory infiltration as well as with lining hyperplasia [140]. Moreover, synovial tissue analysis of biopsies in AS patients following anti-TNF therapy showed that changes in CD163 + synovial tissue macrophages, polymorphonuclear cells and MMP-3 levels were associated with therapeutic response and significantly distinguished responders and nonresponders [141]. These pilot data support the idea that analysis of synovial tissue in SpA may be of additional value in monitoring disease activity and therapeutic response, particularly in early-phase clinical trials.

12. Conclusion

Axial spondyloarthritis is a chronic disease with typical onset at a young age, and, if undiagnosed and untreated, it may result in permanent damage and lifelong disability. Early diagnosis has been improved particularly due to the addition of MRI to the diagnostic armamentaria; however, it is costly, not widely available and requires experienced readers to interpret the findings.

Basic research has shed some light on the complex pathogenesis of axSpA and has resulted in the discovery of new biomarkers from inflammation, angiogenesis and tissue turnover. Some genetic biomarkers and particularly anti-CD74 antibodies, may become promising tools for the early diagnosis of axSpA. Interestingly, key molecules in the pathogenesis of the disease, such as TNFα, IL-17 and IL-23, have shown only limited associations with disease characteristics. Further biomarkers, such as MMP-3, S100A8/9, VEGF, CTX-II or DKK-1, are not sufficient to reflect disease activity, but may predict structural spinal progression. In addition, recent data showed that monitoring of S100A9/9 might represent a valuable biomarker of therapeutic response. However, all of these results need to be confirmed in large cohort studies and are not yet available in daily clinical practice.

In conclusion, HLA-B27 is the best genetic biomarker for making a diagnosis, while CRP remains the best circulating measure for assessing disease activity, predicting structural progression and response to therapy.

Take-home messages

- In addition to clinical measures and imaging techniques such as MRI, new biomarkers have a potential in diagnosis and disease monitoring in the early phases of axSpA.
- New genetic markers and anti-CD74 antibodies are a promising tool for the early diagnosis of axSpA.
- Some biomarkers of inflammation, tissue remodelling or angiogenesis are not sufficient to reflect disease activity, but may predict spinal structural progression.
- Calprotectin represents a valuable biomarker of therapeutic response.
- At present, HLA-B27 remains the best genetic diagnostic marker and CRP the best tool for assessing disease activity, predicting structural progression and treatment response.

Acknowledgment

This work was supported by a project of the MHCR for conceptual research development No. 023728.

References

[1] Dougados M, Baeten D. Spondyloarthritis. Lancet 2011;377:2127–37.
[2] Rudwaleit M, van der Heijde D, Landewe R, Listing J, Akkoc N, Brandt J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis 2009;68:777–83.
[3] van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
[4] Rudwaleit M, Jurik AG, Hermann KG, Landewe R, van der Heijde D, Baraliakos X, et al. Defining active sacroiliitis on magnetic resonance imaging (MRI) for classification of axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI group. Ann Rheum Dis 2009;68:1520–7.
[5] Navarro-Compan V, Ramiro S. Disease activity is longitudinally related to sacroiliac inflammation on MRI in male patients with axial spondyloarthritis: 2-years of the DESIR cohort; 2015.
[6] Kiltz U, Baraliakos X, Karakostas P, Igelmann M, Kalthoff L, Klink C, et al. Do patients with non-radiographic axial spondylarthritis differ from patients with ankylosing spondylitis? Arthritis Care Res 2012;64:1415–22.
[7] Poddubnyy D, Rudwaleit M, Haibel H, Listing J, Marker-Hermann E, Zeidler H, et al. Rates and predictors of radiographic sacroiliitis progression over 2 years in patients with axial spondyloarthritis. Ann Rheum Dis 2011;70:1369–74.
[8] Pedersen SJ, Sorensen IJ, Lambert RG, Hermann KG, Garnero P, Johansen JS, et al. Radiographic progression is associated with resolution of systemic inflammation in patients with axial spondylarthritis treated with tumor necrosis factor alpha inhibitors: a study of radiographic progression, inflammation on magnetic resonance imaging, and circulating biomarkers of inflammation, angiogenesis, and cartilage and bone turnover. Arthritis Rheum 2011;63:3789–800.
[9] Ramiro S, Stolwijk C, van Tubergen A, van der Heijde D, Dougados M, van den Bosch F, et al. Evolution of radiographic damage in ankylosing spondylitis: a 12 year prospective follow-up of the OASIS study. Ann Rheum Dis 2015;74:52–9.
[10] Landewe R, Braun J, Deodhar A, Dougados M, Maksymowych WP, Mease PJ, et al. Efficacy of certolizumab pegol on signs and symptoms of axial spondyloarthritis including ankylosing spondylitis: 24-week results of a double-blind randomised placebo-controlled phase 3 study. Ann Rheum Dis 2014;73:39–47.
[11] van Tubergen A. The changing clinical picture and epidemiology of spondyloarthritis. Nat Rev Rheumatol 2015;11:110–8.
[12] van der Linden SM, Valkenburg HA, de Jongh BM, Cats A. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. A comparison of relatives of spondylitis patients with the general population. Arthritis Rheum 1984;27:241–9.
[13] Brown MA. Progress in spondylarthritis. Progress in studies of the genetics of ankylosing spondylitis, 11. Arthritis research & therapy; 2009. p. 254.
[14] Maksymowych WP, Inman RD, Gladman DD, Reeve JP, Pope A, Rahman P. Association of a specific ERAP1/ARTS1 haplotype with disease susceptibility in ankylosing spondylitis. Arthritis Rheum 2009;60:1317–23.
[15] Chen L, Ridley A, Hammitzsch A, Al-Mossawi MH, Bunting H, Georgiadis D, et al. Silencing or inhibition of endoplasmic reticulum aminopeptidase 1 (ERAP1) suppresses free heavy chain expression and Th17 responses in ankylosing spondylitis. Ann Rheum Dis 2015.
[16] Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 2007;39:1329–37.
[17] Evans DM, Spencer CC, Pointon JJ, Su Z, Harvey D, Kochan G, et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet 2011;43:761–7.
[18] Pointon JJ, Harvey D, Karaderi T, Appleton LH, Farrar C, Stone MA, et al. Elucidating the chromosome 9 association with AS; CARD9 is a candidate gene. Genes Immun 2010;11:490–6.
[19] Reveille JD. Genetics of spondyloarthritis–beyond the MHC. Nat Rev Rheumatol 2012;8:296–304.
[20] Poddubnyy D, Brandt H, Vahldiek J, Spiller I, Song IH, Rudwaleit M, et al. The frequency of non-radiographic axial spondyloarthritis in relation to symptom duration in patients referred because of chronic back pain: results from the Berlin early spondyloarthritis clinic. Ann Rheum Dis 2012;71:1998–2001.
[21] Visvanathan S, Wagner C, Marini JC, Baker D, Gathany T, Han J, et al. Inflammatory biomarkers, disease activity and spinal disease measures in patients with ankylosing spondylitis after treatment with infliximab. Ann Rheum Dis 2008; 67:511–7.
[22] Pedersen SJ, Sorensen IJ, Garnero P, Johansen JS, Madsen OR, Tvede N, et al. ASDAS, BASDAI and different treatment responses and their relation to biomarkers of inflammation, cartilage and bone turnover in patients with axial spondyloarthritis treated with TNFalpha inhibitors. Ann Rheum Dis 2011;70:1375–81.
[23] de Vries MK, van Eijk IC, van der Horst-Bruinsma IE, Peters MJ, Nurmohamed MT, Dijkmans BA, et al. Erythrocyte sedimentation rate, C-reactive protein level, and serum amyloid a protein for patient selection and monitoring of anti-tumor necrosis factor treatment in ankylosing spondylitis. Arthritis Rheum 2009;61: 1484–90.
[24] Sieper J, Lenaerts J, Wollenhaupt J, Rudwaleit M, Mazurov VI, Myasoutova L, et al. Efficacy and safety of infliximab plus naproxen versus naproxen alone in patients with early, active axial spondyloarthritis: results from the double-blind, placebo-controlled INFAST study, part 1. Ann Rheum Dis 2014;73:101–7.
[25] Turina MC, Yeremenko N, Paramarta JE, De Rycke L, Baeten D. Calprotectin (S100A8/9) as serum biomarker for clinical response in proof-of-concept trials in axial and peripheral spondyloarthritis. Arthritis Res Ther 2014;16:413.
[26] Sieper J, Appel H, Braun J, Rudwaleit M. Critical appraisal of assessment of structural damage in ankylosing spondylitis: implications for treatment outcomes. Arthritis Rheum 2008;58:649–56.
[27] van der Heijde D, Landewe R, Einstein S, Ory P, Vosse D, Ni L, et al. Radiographic progression of ankylosing spondylitis after up to two years of treatment with etanercept. Arthritis Rheum 2008;58:1324–31.
[28] van der Heijde D, Landewe R, Baraliakos X, Houben H, van Tubergen A, Williamson P, et al. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. Arthritis Rheum 2008;58:3063–70.
[29] Baraliakos X, Haibel H, Listing J, Sieper J, Braun J. Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. Ann Rheum Dis 2014;73:710–5.
[30] Poddubnyy D, Sieper J. Radiographic progression in ankylosing spondylitis/axial spondyloarthritis: how fast and how clinically meaningful? Curr Opin Rheumatol 2012;24:363–9.
[31] Poddubnyy D, Haibel H, Listing J, Marker-Hermann E, Zeidler H, Braun J, et al. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. Arthritis Rheum 2012;64:1388–98.
[32] Spoorenberg A, van der Heijde D, de Klerk E, Dougados M, de Vlam K, Mielants H, et al. Relative value of erythrocyte sedimentation rate and C-reactive protein in assessment of disease activity in ankylosing spondylitis. J Rheumatol 1999;26:980–4.
[33] Yildirim K, Erdal A, Karatay S, Melikoglu MA, Ugur M, Senel K. Relationship between some acute phase reactants and the Bath Ankylosing Spondylitis Disease Activity Index in patients with ankylosing spondylitis. South Med J 2004;97:350–3.
[34] Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, Gineyts E, et al. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone 2001;29: 209–15.
[35] Park MC, Chung SJ, Park YB, Lee SK. Bone and cartilage turnover markers, bone mineral density, and radiographic damage in men with ankylosing spondylitis. Yonsei Med J 2008;49:288–94.
[36] Vosse D, Landewe R, Garnero P, van der Heijde D, van der Linden S, Geusens P. Association of markers of bone- and cartilage-degradation with radiological changes at baseline and after 2 years follow-up in patients with ankylosing spondylitis. Rheumatology 2008;47:1219–22.
[37] Maksymowych WP, Rahman P, Shojania K, Olszynski WP, Thomson GT, Ballal S, et al. Beneficial effects of adalimumab on biomarkers reflecting structural damage in patients with ankylosing spondylitis. J Rheumatol 2008;35:2030–7.
[38] Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 2002;46:21–30.
[39] Bay-Jensen AC, Leeming DJ, Kleyer A, Veidal SS, Schett G, Karsdal MA. Ankylosing spondylitis is characterized by an increased turnover of several different metalloproteinase-derived collagen species: a cross-sectional study. Rheumatol Int 2012;32:3565–72.
[40] Bay-Jensen AC, Wichuk S, Byrjalsen I, Leeming DJ, Morency N, Christiansen C, 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, e54504.
[41] Pedersen SJ, Hetland ML, Sorensen IJ, Ostergaard M, Nielsen HJ, Johansen JS. Circulating levels of interleukin-6, vascular endothelial growth factor, YKL-40, matrix metalloproteinase-3, and total aggrecan in spondyloarthritis patients during 3 years of treatment with TNFalpha inhibitors. Clin Rheumatol 2010;29: 1301–9.
[42] Maksymowych WP, Jhangri GS, Lambert RG, Mallon C, Buenviaje H, Pedrycz E, et al. Infliximab in ankylosing spondylitis: a prospective observational inception cohort analysis of efficacy and safety. J Rheumatol 2002;29:959–65.
[43] Maksymowych WP, Landewe R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H, 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.
[44] Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, et al. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 1992;267:6132–6.
[45] Huvelle S, Bothy A, Lepoutre T, Gruson D. Measurement of C-terminal cross-linking telopeptide of type I collagen: evaluation of a new automated assay. Clin Biochem 2013;46:1778–9.
[46] Choi ST, Kim JH, Kang EJ, Lee SW, Park MC, Park YB, et al. Osteopontin might be involved in bone remodelling rather than in inflammation in ankylosing spondylitis. Rheumatology 2008;47:1775–9.
[47] Visvanathan S, van der Heijde D, Deodhar A, Wagner C, Baker DG, Han J, et al. Effects of infliximab on markers of inflammation and bone turnover and associations with bone mineral density in patients with ankylosing spondylitis. Ann Rheum Dis 2009;68:175–82.
[48] Arends S, Spoorenberg A, Efde M, Bos R, Leijsma MK, Bootsma H, et al. Higher bone turnover is related to spinal radiographic damage and low bone mineral density in ankylosing spondylitis patients with active disease: a cross-sectional analysis. PLoS One 2014;9, e99685.
[49] Wendling D, Cedoz JP, Racadot E. Serum levels of MMP-3 and cathepsin K in patients with ankylosing spondylitis: effect of TNFalpha antagonist therapy. Joint Bone Spine 2008;75:559–62.
[50] Neidhart M, Baraliakos X, Seemayer C, Zelder C, Gay RE, Michel BA, et al. Expression of cathepsin K and matrix metalloproteinase 1 indicate persistent osteodestructive activity in long-standing ankylosing spondylitis. Ann Rheum Dis 2009;68:1334–9.

[51] Kim JH, Liu X, Wang J, Chen X, Zhang H, Kim SH, et al. Wnt signaling in bone formation and its therapeutic potential for bone diseases. Ther Adv Musculoskelet Dis 2013;5:13–31.
[52] Pinzone JJ, Hall BM, Thudi NK, Vonau M, Qiang YW, Rosol TJ, et al. The role of Dickkopf-1 in bone development, homeostasis, and disease. Blood 2009;113:517–25.
[53] Haynes KR, Pettit AR, Duan R, Tseng HW, Glant TT, Brown MA, et al. Excessive bone formation in a mouse model of ankylosing spondylitis is associated with decreases in Wnt pathway inhibitors. Arthritis Res Ther 2012;14:R253.
[54] Uderhardt S, Diarra D, Katzenbeisser J, David JP, Zwerina J, Richards W, et al. Blockade of Dickkopf (DKK)-1 induces fusion of sacroiliac joints. Ann Rheum Dis 2010;69:592–7.
[55] Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med 2007;13:156–63.
[56] Kwon SR, Lim MJ, Suh CH, Park SG, Hong YS, Yoon BY, et al. Dickkopf-1 level is lower in patients with ankylosing spondylitis than in healthy people and is not influenced by anti-tumor necrosis factor therapy. Rheumatol Int 2012;32:2523–7.
[57] Daoussis D, Liossis SN, Solomou EE, Tsanaktsi A, Bounia K, Karampetsou M, et al. Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis. Arthritis Rheum 2010;62:150–8.
[58] Heiland GR, Appel H, Poddubnyy D, Zwerina J, Hueber A, Haibel H, et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. Ann Rheum Dis 2012;71:572–4.
[59] Korkosz M, Gasowski J, Leszczynski P, Pawlak-Bus K, Jeka S, Siedlar M, et al. Effect of tumour necrosis factor-alpha inhibitor on serum level of dickkopf-1 protein and bone morphogenetic protein-7 in ankylosing spondylitis patients with high disease activity. Scand J Rheumatol 2014;43:43–8.
[60] Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Lowik CW, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J 2005;19:1842–4.
[61] Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. Arthritis Rheum 2009;60:3257–62.
[62] Klingberg E, Nurkkala M, Carlsten H, Forsblad-d'Elia H. Biomarkers of bone metabolism in ankylosing spondylitis in relation to osteoproliferation and osteoporosis. J Rheumatol 2014;41:1349–56.
[63] Saad CG, Ribeiro AC, Moraes JC, Takayama L, Goncalves CR, Rodrigues MB, et al. Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? Arthritis Res Ther 2012;14:R216.
[64] Verma RP, Hansch C. Matrix metalloproteinases (MMPs): chemical–biological functions and (Q)SARs. Bioorg Med Chem 2007;15:2223–68.
[65] Chen CH, Lin KC, Yu DT, Yang C, Huang F, Chen HA, et al. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. Rheumatology 2006;45:414–20.
[66] Vandooren B, Kruithof E, Yu DT, Rihl M, Gu J, De Rycke L, et al. Involvement of matrix metalloproteinases and their inhibitors in peripheral synovitis and down-regulation by tumor necrosis factor alpha blockade in spondylarthropathy. Arthritis Rheum 2004;50:2942–53.
[67] Yang C, Gu J, Rihl M, Baeten D, Huang F, Zhao M, et al. Serum levels of matrix metalloproteinase 3 and macrophage colony-stimulating factor 1 correlate with disease activity in ankylosing spondylitis. Arthritis Rheum 2004;51:691–9.
[68] Soliman E, Labib W, el-Tantawi G, Hamimy A, Alhadidy A, Aldawoudy A. Role of matrix metalloproteinase-3 (MMP-3) and magnetic resonance imaging of sacroiliiitis in assessing disease activity in ankylosing spondylitis. Rheumatol Int 2012;32:1711–20.
[69] Woo JH, Lee HJ, Sung IH, Kim TH. Changes of clinical response and bone biochemical markers in patients with ankylosing spondylitis taking etanercept. J Rheumatol 2007;34:1753–9.
[70] Mattey DL, Packham JC, Nixon NB, Coates L, Creamer P, Hailwood S, et al. Association of cytokine and matrix metalloproteinase profiles with disease activity and function in ankylosing spondylitis. Arthritis Res Ther 2012;14:R127.
[71] Vossenaar ER, Nijenhuis S, Helsen MM, van der Heijden A, Senshu T, van den Berg WB, et al. Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis Rheum 2003;48:2489–500.
[72] Bay-Jensen AC, Karsdal MA, Vassiliadis E, Wichuk S, Marcher-Mikkelsen K, Lories R, et al. Circulating citrullinated vimentin fragments reflect disease burden in ankylosing spondylitis and have prognostic capacity for radiographic progression. Arthritis Rheum 2013;65:972–80.
[73] Davis Jr JC. Understanding the role of tumor necrosis factor inhibition in ankylosing spondylitis. Semin Arthritis Rheum 2005;34:668–77.
[74] Gratacos J, Collado A, Filella X, Sanmarti R, Canete J, Llena J, et al. Serum cytokines (IL-6, TNF-alpha, IL-1 beta and IFN-gamma) in ankylosing spondylitis: a close correlation between serum IL-6 and disease activity and severity. Br J Rheumatol 1994;33:927–31.
[75] Bal A, Unlu E, Bahar G, Aydog E, Eksioglu E, Yorgancioglu R. Comparison of serum IL-1 beta, sIL-2R, IL-6, and TNF-alpha levels with disease activity parameters in ankylosing spondylitis. Clin Rheumatol 2007;26:211–5.
[76] Braun J, Bollow M, Neure L, Seipelt E, Seyrekbasan F, Herbst H, et al. Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. Arthritis Rheum 1995;38:499–505.
[77] Braun J, Baraliakos X, Heldmann F, Kiltz U. Tumor necrosis factor alpha antagonists in the treatment of axial spondyloarthritis. Expert Opin Investig Drugs 2014;23:647–59.
[78] Sherlock JP, Taylor PC, Buckley CD. The biology of IL-23 and IL-17 and their therapeutic targeting in rheumatic diseases. Curr Opin Rheumatol 2015;27:71–5.
[79] Shen H, Goodall JC, Hill Gaston JS. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. Arthritis Rheum 2009;60:1647–56.
[80] Jansen DT, Hameetman M, van Bergen J, Huizinga TW, van der Heijde D, Toes RE, et al. IL-17-producing CD4+ T cells are increased in early, active axial spondyloarthritis including patients without imaging abnormalities. Rheumatology 2015;54:728–35.
[81] Jandus C, Bioley G, Rivals JP, Dudler J, Speiser D, Romero P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. Arthritis Rheum 2008;58:2307–17.
[82] Appel H, Maier R, Wu P, Scheer R, Hempfing A, Kayser R, et al. Analysis of IL-17(+) cells in facet joints of patients with spondyloarthritis suggests that the innate immune pathway might be of greater relevance than the Th17-mediated adaptive immune response. Arthritis Res Ther 2011;13:R95.
[83] Xueyi L, Lina C, Zhenbiao W, Qing H, Qiang L, Zhu P. Levels of circulating Th17 cells and regulatory T cells in ankylosing spondylitis patients with an inadequate response to anti-TNF-alpha therapy. J Clin Immunol 2013;33:151–61.
[84] Mei Y, Pan F, Gao J, Ge R, Duan Z, Zeng Z, et al. Increased serum IL-17 and IL-23 in the patient with ankylosing spondylitis. Clin Rheumatol 2011;30:269–73.
[85] Wang X, Lin Z, Wei Q, Jiang Y, Gu J. Expression of IL-23 and IL-17 and effect of IL-23 on IL-17 production in ankylosing spondylitis. Rheumatol Int 2009;29:1343–7.
[86] Wendling D, Cedoz JP, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. Joint Bone Spine 2007;74:304–5.
[87] Baeten D, Baraliakos X, Braun J, Sieper J, Emery P, van der Heijde D, et al. Anti-interleukin-17 A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. Lancet 2013;382:1705–13.
[88] Chen WS, Chang YS, Lin KC, Lai CC, Wang SH, Hsiao KH, et al. Association of serum interleukin-17 and interleukin-23 levels with disease activity in Chinese patients with ankylosing spondylitis. J Chin Med Assoc 2012;75:303–8.
[89] Andersen T, Rasmussen TK, Hvid M, Holm CK, Madsen KJ, Jurik AG, et al. Increased plasma levels of IL-21 and IL-23 in spondyloarthritis are not associated with clinical and MRI findings. Rheumatol Int 2012;32:387–93.
[90] Poddubnyy D, Hermann KG, Callhoff J, Listing J, Sieper J. Ustekinumab for the treatment of patients with active ankylosing spondylitis: results of a 28-week, prospective, open-label, proof-of-concept study (TOPAS). Ann Rheum Dis 2014;73:817–23.
[91] Appel H, Maier R, Bleil J, Hempfing A, Loddenkemper C, Schlichting U, et al. In situ analysis of interleukin-23- and interleukin-12-positive cells in the spine of patients with ankylosing spondylitis. Arthritis Rheum 2013;65:1522–9.
[92] Qu N, Xu M, Mizoguchi I, Furusawa J, Kaneko K, Watanabe K, et al. Pivotal roles of T-helper 17-related cytokines, IL-17, IL-22, and IL-23, in inflammatory diseases. Clin Dev Immunol 2013;2013:968549.
[93] Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 2007;448:484–7.
[94] Xiao F, Zhang HY, Liu YJ, Zhao D, Shan YX, Jiang YF. Higher frequency of peripheral blood interleukin 21 positive follicular helper T cells in patients with ankylosing spondylitis. J Rheumatol 2013;40:2029–37.
[95] Zhang L, Li YG, Li YH, Qi L, Liu XG, Yuan CZ, et al. Increased frequencies of Th22 cells as well as Th17 cells in the peripheral blood of patients with ankylosing spondylitis and rheumatoid arthritis. PLoS One 2012;7, e31000.
[96] Shan Y, Qi C, Zhao J, Liu Y, Gao H, Zhao D, et al. Higher frequency of peripheral blood follicular regulatory T cells in patients with new onset ankylosing spondylitis. Clin Exp Pharmacol Physiol 2015;42:154–61.
[97] Ataie-Kachoie P, Pourgholami MH, Richardson DR, Morris DL. Gene of the month: interleukin 6 (IL-6). J Clin Pathol 2014;67:932–7.
[98] Limon-Camacho L, Vargas-Rojas MI, Vazquez-Mellado J, Casasola-Vargas J, Moctezuma JF, Burgos-Vargas R, et al. In vivo peripheral blood proinflammatory T cells in patients with ankylosing spondylitis. J Rheumatol 2012;39:830–5.
[99] Francois RJ, Neure L, Sieper J, Braun J. Immunohistological examination of open sacroiliac biopsies of patients with ankylosing spondylitis: detection of tumour necrosis factor alpha in two patients with early disease and transforming growth factor beta in three more advanced cases. Ann Rheum Dis 2006;65:713–20.
[100] Sieper J, Braun J, Kay J, Badalamenti S, Radin AR, Jiao L, et al. Sarilumab for the treatment of ankylosing spondylitis: results of a Phase II, randomised, double-blind, placebo-controlled study (ALIGN). Ann Rheum Dis 2015;74:1051–7.
[101] Azevedo VF, Faria-Neto JR, Stinghen A, Lorencetti PG, Miller WP, Goncalves BP, et al. IL-8 but not other biomarkers of endothelial damage is associated with disease activity in patients with ankylosing spondylitis without treatment with anti-TNF agents. Rheumatol Int 2013;33:1779–83.
[102] Eklund KK, Niemi K, Kovanen PT. Immune functions of serum amyloid A. Crit Rev Immunol 2012;32:335–48.
[103] Jung SY, Park MC, Park YB, Lee SK. Serum amyloid a as a useful indicator of disease activity in patients with ankylosing spondylitis. Yonsei Med J 2007;48:218–24.
[104] Lange U, Boss B, Teichmann J, Klor HU, Neeck G. Serum amyloid A–an indicator of inflammation in ankylosing spondylitis. Rheumatol Int 2000;19:119–22.
[105] van Eijk IC, de Vries MK, Levels JH, Peters MJ, Huizer EE, Dijkmans BA, et al. Improvement of lipid profile is accompanied by atheroprotective alterations in high-density lipoprotein composition upon tumor necrosis factor blockade: a prospective cohort study in ankylosing spondylitis. Arthritis Rheum 2009;60:1324–30.
[106] Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol Rev 2009;229:12–26.

[107] Toussirot E, Saas P, Deschamps M, Pouthier F, Perrot L, Perruche S, et al. Increased production of soluble CTLA-4 in patients with spondylarthropathies correlates with disease activity. Arthritis Res Ther 2009;11:R101.
[108] Genovese MC, Becker JC, Schiff M, Luggen M, Sherrer Y, Kremer J, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med 2005;353:1114–23.
[109] Song IH, Heldmann F, Rudwaleit M, Haibel H, Weiss A, Braun J, et al. Treatment of active ankylosing spondylitis with abatacept: an open-label, 24-week pilot study. Ann Rheum Dis 2011;70:1108–10.
[110] Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cell Signal 2007;19:2003–12.
[111] Drouart M, Saas P, Billot M, Cedoz JP, Tiberghien P, Wendling D, et al. High serum vascular endothelial growth factor correlates with disease activity of spondylarthropathies. Clin Exp Immunol 2003;132:158–62.
[112] Goldberger C, Dulak J, Duftner C, Weidinger F, Falkenbach A, Schirmer M. Vascular endothelial growth factor (VEGF) in ankylosing spondylitis—a pilot study. Wien Med Wochenschr 1946;152(2002):223–5.
[113] Appel H, Janssen L, Listing J, Heydrich R, Rudwaleit M, Sieper J. Serum levels of biomarkers of bone and cartilage destruction and new bone formation in different cohorts of patients with axial spondyloarthritis with and without tumor necrosis factor-alpha blocker treatment. Arthritis Res Ther 2008;10:R125.
[114] Poddubnyy D, Conrad K, Haibel H, Syrbe U, Appel H, Braun J, et al. Elevated serum level of the vascular endothelial growth factor predicts radiographic spinal progression in patients with axial spondyloarthritis. Ann Rheum Dis 2014;73:2137–43.
[115] Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911–9 (quiz 920).
[116] Syrbe U, Callhoff J, Conrad K, Poddubnyy D, Haibel H, Junker S, et al. Serum adipokine levels in patients with ankylosing spondylitis and their relationship to clinical parameters and radiographic spinal progression. Arthritis Rheum 2015; 67:678–85.
[117] Kocabas H, Kocabas V, Buyukbas S, Melikoglu MA, Sezer I, Butun B. The serum levels of resistin in ankylosing spondylitis patients: a pilot study. Rheumatol Int 2012;32:699–702.
[118] Toussirot E, Streit G, Nguyen NU, Dumoulin G, Le Huede G, Saas P, et al. Adipose tissue, serum adipokines, and ghrelin in patients with ankylosing spondylitis. Metab Clin Exp 2007;56:1383–9.
[119] Derdemezis CS, Filippatos TD, Voulgari PV, Tselepis AD, Drosos AA, Kiortsis DN. Leptin and adiponectin levels in patients with ankylosing spondylitis. The effect of infliximab treatment. Clin Exp Rheumatol 2010;28:880–3.
[120] Sari I, Demir T, Kozaci LD, Akar S, Kavak T, Birlik M, et al. Body composition, insulin, and leptin levels in patients with ankylosing spondylitis. Clin Rheumatol 2007;26: 1427–32.
[121] Miranda-Filloy JA, Lopez-Mejias R, Genre F, Carnero-Lopez B, Ochoa R, Diaz de Teran T, et al. Adiponectin and resistin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-alpha antagonist therapy. Clin Exp Rheumatol 2013;31:365–71.
[122] Hulejova H, Levitova A, Kuklova M, Stochl J, Haluzik M, Pavelka K, et al. No effect of physiotherapy on the serum levels of adipocytokines in patients with ankylosing spondylitis. Clin Rheumatol 2012;31:67–71.
[123] Park MC, Lee SW, Choi ST, Park YB, Lee SK. Serum leptin levels correlate with interleukin-6 levels and disease activity in patients with ankylosing spondylitis. Scand J Rheumatol 2007;36:101–6.
[124] Kim KJ, Kim JY, Park SJ, Yoon H, Yoon CH, Kim WU, et al. Serum leptin levels are associated with the presence of syndesmophytes in male patients with ankylosing spondylitis. Clin Rheumatol 2012;31:1231–8.
[125] Abildtrup M, Kingsley GH, Scott DL. Calprotectin as a biomarker for rheumatoid arthritis: a systematic review. J Rheumatol 2015.
[126] Gebhardt C, Nemeth J, Angel P, Hess J. S100A8 and S100A9 in inflammation and cancer. Biochem Pharmacol 2006;72:1622–31.
[127] Meucci G, D'Inca R, Maieron R, Orzes N, Vecchi M, Visentini D, et al. Diagnostic value of faecal calprotectin in unselected outpatients referred for colonoscopy: a multicenter prospective study. Dig Liver Dis 2010;42:191–5.
[128] Klingberg E, Carlsten H, Hilme E, Hedberg M, Forsblad-d'Elia H. Calprotectin in ankylosing spondylitis–frequently elevated in feces, but normal in serum. Scand J Gastroenterol 2012;47:435–44.
[129] Oktayoglu P, Bozkurt M, Mete N, Caglayan M, Em S, Nas K. Elevated serum levels of calprotectin (myeloid-related protein 8/14) in patients with ankylosing spondylitis and its association with disease activity and quality of life. J Investig Med 2014;62: 880–4.
[130] Turina MC, Sieper J, Yeremenko N, Conrad K, Haibel H, Rudwaleit M, et al. Calprotectin serum level is an independent marker for radiographic spinal progression in axial spondyloarthritis. Ann Rheum Dis 2014;73:1746–8.
[131] Wallis D, Asaduzzaman A, Weisman M, Haroon N, Anton A, McGovern D, et al. Elevated serum anti-flagellin antibodies implicate subclinical bowel inflammation in ankylosing spondylitis: an observational study. Arthritis Res Ther 2013;15:R166.
[132] Mundwiler ML, Mei L, Landers CJ, Reveille JD, Targan S, Weisman MH. Inflammatory bowel disease serologies in ankylosing spondylitis patients: a pilot study. Arthritis Res Ther 2009;11:R177.
[133] McLean IL, Archer JR, Cawley MI, Pegley FS, Kidd BL, Thompson PW. Specific antibody response to the mycobacterial 65 kDa stress protein in ankylosing spondylitis and rheumatoid arthritis. Br J Rheumatol 1990;29:426–9.
[134] Bodnar N, Szekanecz Z, Prohaszka Z, Kemeny-Beke A, Nemethne-Gyurcsik Z, Gulyas K, et al. Anti-mutated citrullinated vimentin (anti-MCV) and anti-65 kDa heat shock protein (anti-hsp65): new biomarkers in ankylosing spondylitis. Joint Bone Spine 2012;79:63–6.
[135] Damjanovska L, Thabet MM, Levarth EW, Stoeken-Rijsbergen G, van der Voort EI, Toes RE, et al. Diagnostic value of anti-MCV antibodies in differentiating early inflammatory arthritis. Ann Rheum Dis 2010;69:730–2.
[136] Baerlecken NT, Nothdorft S, Stummvoll GH, Sieper J, Rudwaleit M, Reuter S, et al. Autoantibodies against CD74 in spondyloarthritis. Ann Rheum Dis 2014;73: 1211–4.
[137] Baraliakos X, Baerlecken N, Witte T, Heldmann F, Braun J. High prevalence of anti-CD74 antibodies specific for the HLA class II-associated invariant chain peptide (CLIP) in patients with axial spondyloarthritis. Ann Rheum Dis 2014;73: 1079–82.
[138] Borghese F, Clanchy FI. CD74: an emerging opportunity as a therapeutic target in cancer and autoimmune disease. Expert Opin Ther Targets 2011;15:237–51.
[139] Starlets D, Gore Y, Binsky I, Haran M, Harpaz N, Shvidel L, et al. Cell-surface CD74 initiates a signaling cascade leading to cell proliferation and survival. Blood 2006; 107:4807–16.
[140] Baeten D, Kruithof E, De Rycke L, Boots AM, Mielants H, Veys EM, et al. Infiltration of the synovial membrane with macrophage subsets and polymorphonuclear cells reflects global disease activity in spondyloarthropathy. Arthritis Res Ther 2005;7: R359–69.
[141] Kruithof E, De Rycke L, Vandooren B, De Keyser F, FitzGerald O, McInnes I, et al. Identification of synovial biomarkers of response to experimental treatment in early-phase clinical trials in spondylarthritis. Arthritis Rheum 2006;54:1795–804.