

Original article

Anti-CD74 IgA autoantibodies in radiographic axial spondyloarthritis: a longitudinal Swedish study

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Abstract

Objectives. Antibodies against anti-CD74 are related to axial spondyloarthritis (axSpA). The objectives were (i) to study IgA anti-CD74 in radiographic (r)-axSpA patients in the Backbone cohort and to calculate the sensitivity and specificity of anti-CD74, (ii) to study the fluctuation of IgA anti-CD74 levels in prospectively collected samples, and (iii) to explore the relation between IgA anti-CD74 and radiographic spinal changes.

Methods. IgA anti-CD74 was analysed by ELISA in 155 patients with r-axSpA and age- and sex-matched controls. BASDAI, ASDAS, BASFI and BASMI were assessed and spinal radiographs were scored for r-axSpA-related changes with mSASSS. Previously donated samples, before inclusion in the Backbone study, were identified in the Medical Biobank of Northern Sweden.

Results. A total of 155 patients comprising 69% men and 31% women, age [mean (s.d.)] 55.5 (11.4) years and 152 (98.1%) HLA-B27 positive, were included. The plasma level of IgA anti-CD74 was significantly higher in the patients [median (interquartile range), 12.9 (7.9–17.9) U/ml] compared with controls [10.9 (7.2–14.6) U/ml, $P=0.003$]. IgA anti-CD74 was above the cut-off level of 20 U/ml in 36/155 (23.2%) patients and in 15/151 (9.9%) controls ($P=0.002$). Multivariable logistic regression analyses revealed ≥ 1 syndesmophyte associated with IgA anti-CD74 (odds ratio 5.64; 95% CI: 1.02, 35.58; $P=0.048$) adjusted for hsCRP, smoking, BMI, sex and age. No distinct pattern of IgA anti-CD74 over time was revealed.

Conclusion. Plasma levels of IgA anti-CD74 were increased in r-axSpA and independently associated with radiographic spinal changes, which suggests that IgA anti-CD74 could play a role in the pathogenesis of r-axSpA.

Key words: radiographic axial spondyloarthritis ankylosing spondylitis, IgA anti-CD74, outcomes research

Rheumatology key messages

- IgA anti-CD74 plasma level was significantly higher in patients with r-axSpA compared with controls.
- IgA anti-CD74 plasma level was associated with r-axSpA-related changes in the spine.
- There was no difference in IgA anti-CD74 concentrations between HLA-B27 positive and HLA-B27 negative patients or controls.

Introduction

Ankylosing spondylitis (AS) is a chronic rheumatic inflammatory disease that belongs to the spondyloarthritides (SpA) [1]. Axial (ax)SpA with predominant

involvement of the spine and sacroiliac joints is divided into radiographic (r)-axSpA and non-radiographic (nr)-axSpA depending on whether or not there is typical radiographic structural changes in the sacroiliac joints [2]. Recently, it was suggested that AS and r-axSpA can be used as interchangeable terms [3], and therefore we

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will use the name r-axSpA in this report. Due to the lack of specific tests, the diagnosis of axSpA in early phases is challenging and there often remains a considerable delay to diagnosis [4, 5]. HLA-B27 plays a role in the diagnosis and prognosis of axSpA. Only about 1.3–6% of the HLA-B27 positive persons develop axSpA and the prevalence of HLA-B27 varies in different populations, with about 15% of the population in northern Scandinavia [1, 6–8]. Also, the HLA-B27 occurrence differs between nr-axSpA and r-axSpA and between different cohorts around the world [9–11]. CRP or ESR reflect merely systemic inflammation and are thus not disease-specific. MRI of the sacroiliac joints is a crucial tool to identify inflammation but is also not disease-specific since signs of inflammations may also be found in ice-hockey players and runners [12, 13].

CD74, known as HLA class II invariant chain in humans, is an essential protein in antigen presentation. CD74 is involved in the intracellular assembly of the major histocompatibility complex (MHC) class II and prevents premature binding of peptides to MHC II [14]. When surface-expressed, activation of CD74 may initiate a signal pathway that leads to cell proliferation and the production of pro-inflammatory cytokines such as TNF α [15]. The potential involvement of CD74 in axSpA pathogenesis and diagnosis has been suggested since auto-antibodies against the class II-associated invariant chain peptide (CLIP) domain of CD74 are associated with axSpA. Several studies have analysed IgG, IgG4 or IgA anti-CD74 antibodies in different axSpA cohorts, and mostly found significantly higher serum levels of anti-CD74 antibodies in axSpA patients compared with persons without known axSpA. The isotype of the anti-CD74 antibodies with the best predictive value and the prevalence of the antibodies in relation to disease duration has been inconsistent [16–21]. However, knowledge about the association of anti-CD74 antibodies with disease characteristics and severity of r-axSpA is scarce.

The aims of this investigation were (i) to study IgA anti-CD74 in a cohort of patients with r-axSpA with a very high percentage of HLA-B27 in comparison with matched controls from the same geographical area and to calculate the sensitivity and specificity of anti-CD74, (ii) to study, in prospectively collected samples, the fluctuation of plasma levels of IgA anti-CD74 over time in r-axSpA in comparison with the fluctuation in controls, and (iii) to explore the relation between IgA anti-CD74 and disease-related characteristics including radiographic changes in the spine in the patients with r-axSpA.

Methods

Patients and controls

A total of 155 patients from Västerbotten County, northern Sweden, that fulfilled the modified New York criteria for AS [22] were included in the Backbone study, 2016–2017, at the Department of Rheumatology at Norrlands

University Hospital (NUS). The Backbone study examines severity and comorbidities in r-axSpA. Exclusion criteria were dementia, pregnancy, other inflammatory rheumatic diseases and difficulties in understanding Swedish. The 155 r-axSpA patients were checked if they previously had participated in the Northern Sweden Health and Disease Study (NSHDS) and donated blood samples (after overnight fasting) to the Medical Biobank of Northern Sweden before the recruitment to Backbone. The NSHDS cohort consists of over 135 000 participants, is population-based and started in 1985. All inhabitants in Västerbotten County are asked to take part in NSHDS at 40, 50 and 60 years of age. Controls to the r-axSpA patients were persons from the NSHDS, matched for age, sex, date of blood sampling (pre-Backbone controls ± 1 year and Backbone controls ± 7 years), area of inhabitation (rural or urban), and with no known malignant disease. A total of 151 controls were matched to the 155 patients in Backbone (group C). Altogether, we identified 106/155 (68%) patients who had donated 168 plasma samples after the symptom onset but before 2016–2017 with corresponding controls (group B), and 16/155 (10.3%) individuals who had donated 22 blood samples, with corresponding controls, before the onset of any symptom and referred to as pre-symptomatic individuals (group A). Serum samples of the pre-symptomatic individuals and controls were found in the rubella and virus biobank at NUS. If the r-axSpA patient had donated >1 sample in group B the corresponding control samples was chosen from a control person that had donated >1 sample.

Clinical assessment and questionnaires

At inclusion in Backbone, the patients with r-axSpA underwent clinical examination and answered questionnaires regarding lifestyle habits, medication and disease-related data. Mobility was measured by the BASMI [23]. Disease activity and physical function were assessed using the BASDAI [24], the Ankylosing Spondylitis Disease Activity Score based on CRP (ASDAS-CRP) [25] and the BASFI [26].

Radiography

Spinal radiographic alterations were graded from the lateral projection of spinal radiographs and scored according to the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS). Briefly, the anterior corners of vertebra C2–T1 and T12–S1 are graded in mSASSS with a score between 0 and 3 (0 = normal, 1 = erosion, sclerosis, or squaring, 2 = syndesmophyte, 3 = bridging syndesmophyte). The overall scoring scale ranges from 0 to 72, with 72 representing complete ankylosis [27]. A mSASSS score ≥ 2 at a vertebral corner was considered as having a syndesmophyte. Severe spinal radiographic changes were defined as ≥ 3 consecutive inter-vertebral bridges in the cervical spine and/or the lumbar spine, similar to the definition of severe in the Bath Ankylosing Spondylitis Radiology Index [28].

Laboratory assessment

Blood samples were drawn in the morning after overnight fasting. ESR, high sensitivity CRP (hsCRP) analysed consecutively and HLA-B27 were analysed using standard laboratory techniques. The plasma/sera level of IgA autoantibodies against CD74 was measured, in samples that had been frozen at -80°C , with ELISA (AESKULISA SpA Detect, AESKU Diagnostics, Wendelsheim, Germany) according to the manufacturer's instructions. The limit of detection in the ELISA was 2.7 U/ml and the cut-off level was determined as 20 U/ml.

Statistical analysis

All data were analysed using IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA) and R 3.6.2 [R Core Team (2019), Vienna, Austria]. Descriptive statistics are presented as mean with (s.d.), median with interquartile range (IQR), or absolute number with percentages. When comparing cases and controls, the Wilcoxon signed-rank test or Student's paired *t*-test was applied and when comparing groups with AS, the Mann-Whitney *U*-test or an independent *t*-test was used for continuous variables depending on the distribution of the variable. A χ^2 test or Fisher's exact test was applied for categorical data. Correlation analysis was performed using Spearman's rank correlation test. Univariable and multivariable linear regression analyses were used to identify the association between IgA antibodies against CD74 and AS related factors and demographics as covariates. Independent variables hypothesized to be of importance for anti-CD74 were considered for the multivariable models in addition to age and sex. Multivariable logistic regression analyses were run with ≥ 1 syndesmophyte or severe radiographic alterations in the spine as dependent variables and IgA anti-CD74 and known risk factors for radiographic changes; age, sex, BMI, smoking and hsCRP were independent variables. Also, BASMI and BASFI were modelled using linear regression adjusting for age, sex, BMI, smoking and hsCRP. Differences in age trend on IgA anti-CD74 levels between patients and controls were longitudinally modelled using a linear mixed-effect model with a random intercept for subjects and a random intercept for each matched case and control pair. The mixed-effect models were adjusted for BMI and smoking. Due to its skewed distribution, IgA anti-CD74 levels, hsCRP and mSASSS were log-transformed when used in the regression models. The variability in measurements of IgA anti-CD74 levels was analysed by calculation of the intraclass correlation using an intercept-only mixed effect model on the longitudinal logarithmic measurements stratified by cases and controls. To have a characteristic was coded 1 and to not have a characteristic was coded 0 in dichotomous variables. Female sex was coded 1 and male sex 0. All tests were two-tailed and $P \leq 0.05$ was considered statistically significant.

Results

Characteristics of the patients with r-axSpA at inclusion in Backbone

A total of 155 patients comprising 69% men and 31% women with a mean age of 55.5 (11.4) years and a mean symptom duration of 31.8 (11.9) years were included. Patient characteristics are presented in Table 1. HLA-B27 was present in 152 (98.1%) patients. Mean mSASSS was 18.0 (20.7) and 86 (56.2%) had ≥ 1 syndesmophyte.

Comparisons between the patients with r-axSpA at inclusion in Backbone and the controls

The patients with r-axSpA were numerically slightly but still significantly older than the matched controls, while there was no difference in sex distribution (Table 2). HLA-B27 was positive in 152/155 (98.1%) of the r-axSpA patients in comparison with 30/117 (25.6%) of the controls ($P < 0.001$). The plasma level of IgA anti-CD74 was significantly higher in the patients [median

TABLE 1 Descriptive characteristics of 155 patients with radiographic axial spondyloarthritis

Characteristic	Value (<i>n</i> = 155)
Sex, <i>n</i> (%)	
Women	48 (31.0)
Men	107 (69.0)
Age, mean (s.d.), years	55.5 (11.4)
BMI, mean (s.d.), kg/m ²	27.9 (5.3)
Ever smoker, <i>n</i> (%)	71 (45.8)
AS related variables	
Symptom duration, mean (s.d.), years	31.8 (11.9)
HLA-B27 positive, <i>n</i> (%)	152 (98.1)
ESR, median (IQR), mm/h	11.0 (5.0–20.0)
hsCRP, median (IQR), mg/l	2.7 (1.0–6.0)
History of anterior uveitis, <i>n</i> (%)	80 (51.6)
History of peripheral arthritis, <i>n</i> (%)	83 (53.5)
ASDAS-CRP score, median (IQR)	1.8 (1.3–2.2)
BASDAI score, median (IQR)	3.5 (2.2–5.3)
BASFI score, median (IQR)	2.6 (1.4–4.2)
BASMI score, median (IQR)	4.0 (3.0–5.4)
NSAID, regular use, <i>n</i> (%)	95 (61.3)
csDMARD, <i>n</i> (%)	19 (12.3)
bDMARD, <i>n</i> (%)	27 (17.4)
csDMARD and/or bDMARD, <i>n</i> (%)	38 (24.5)
Prednisolone, <i>n</i> (%)	13 (8.4)
mSASSS score ^a , median (IQR)	18 (1–30.5)
≥ 1 syndesmophyte ^a , <i>n</i> (%)	86 (56.2)
Severe spinal radiographic changes ^{a,b} , <i>n</i> (%)	32 (20.9)

^aTwo missing. ^b ≥ 3 consecutive inter-vertebral bridges, cervical and/or lumbar spine. ASDAS: Ankylosing Spondylitis Disease Activity Score; bDMARD: biologic DMARD; csDMARD: conventional synthetic DMARD; hsCRP: high sensitivity CRP; IQR: interquartile range; mSASSS: Modified Stoke Ankylosing Spondylitis Score.

TABLE 2 Comparisons of IgA anti-CD74, HLA-B27, smoking and demographics between patients with radiographic axial spondyloarthritis and matched controls

Characteristic	r-axSpA (n = 155)	Controls (n = 151)	P-value
Sex, n (%)			
Women	48 (31.0)	48 (31.8)	0.9
Men	107 (69.0)	103 (68.2)	
Age, mean (s.d.), years			
All	55.5 (11.45)	53.0 (9.0)	<0.001
Women	57.7 (10.6)	53.7 (8.9)	<0.001
Men	54.5 (11.7)	52.6 (9.0)	<0.001
Ever smoker, n (%)	71 (45.8)	59 (39.3) ^a	0.25
HLA-B27 positive, n (%)			
All	152 (98.1)	30 (25.6) ^b	<0.001
Women	47 (97.9)	11 (28.9)	<0.001
Men	105 (98.1)	19 (24.0)	<0.001
IgA anti-CD74			
All, median (IQR), U/ml	12.9 (7.9–17.9)	10.9 (7.2–14.6)	0.003
mean (s.d.), U/ml	14.9 (10.1)	12.7 (7.8)	
Women, median (IQR), U/ml	13.2 (8.4–20.4)	9.6 (6.6–13.9)	0.011
Men, median (IQR), U/ml	12.9 (8.7–16.6)	11.5 (8.1–15.9)	0.076
IgA anti-CD74 ⁺ (cut-off 20 U/ml), n (%)			
All	36 (23.2)	15 (9.9)	0.002
Women	13 (27.1)	5 (10.4)	0.036
Men	23 (21.5)	10 (9.7)	0.019

	r-axSpA		Controls	
HLA-B27 status	B27 ⁺	B27 ⁻	B27 ⁺	B27 ⁻
IgA anti-CD74 ⁺	36	0	1	13
IgA anti-CD74 ⁻	116	3	29	74
Total	152	3	30	87
P-value ^c	0.45		0.11	
IgA anti-CD74, median (IQR), U/ml	13.0 (8.7–18.8)	11.8 (6.6–12.2)	11.0 (7.2–14.2)	11.4 (7.6–18.9)
P-value	0.34		0.38	

^aOne missing. ^bHLA-B27 was analysed in 117 controls, 38 women and 79 men. ^cFisher's exact test. IQR: interquartile range; r-axSpA; radiographic axial spondyloarthritis.

(IQR), 12.9 (7.9–17.9) U/ml] compared with the controls [10.9 (7.2–14.6) U/ml; $P=0.003$]. Using a cut-off level of 20 U/ml, according to the ELISA kit instructions, IgA anti-CD74 was above the cut-off level in 36/155 (23.2%) patients with r-axSpA and 15/151 (9.9%) controls ($P=0.002$). Thus, the sensitivity with a cut-off level at 20 U/ml was 0.23 with a specificity of 0.90. Based on the IgA anti-CD74 values of the patients and the controls in this study, the area under the curve was 0.58 (95% CI: 0.51, 0.64). Using the Youden index the cut-off level of 11.75 U/ml resulted in the best balance between sensitivity (0.57) and specificity (0.56) (Supplementary Fig. 1, available at *Rheumatology* online). Using the cut-off level of 11.75 U/ml, IgA anti-CD74 was above this cut-off level in 89/155 (57.4%) patients with r-axSpA and in 66/151 (43.7%) controls ($P=0.016$).

There was no significant association between HLA-B27 status and IgA anti-CD74 status (cut-off 20 U/ml) in either patients or controls. Additionally, there was no difference in IgA anti-CD74 concentrations between HLA-B27 positive and HLA-B27 negative patients or controls.

No significant correlation between age and IgA anti-CD74 level was found in the r-axSpA patients ($r_s=-0.089$, $P=0.27$) but in the controls ($r_s=0.17$, $P=0.038$).

IgA anti-CD74 in patients with r-axSpA at inclusion in Backbone

Correlation analyses revealed that plasma level of IgA anti-CD74 was significantly correlated with ESR ($r_s=0.25$, $P=0.002$), hsCRP ($r_s=0.21$, $P=0.007$) and platelets ($r_s=0.16$, $P=0.043$), with a tendency with mSASSS ($r_s=0.14$, $P=0.079$). No significant associations were found between IgA anti-CD74 level and symptom duration, BMI, ASDAS-CRP, BASDAI, BASMI or BASFI. Concerning medication, patients on conventional synthetic DMARDs and/or biologic DMARDs had numerically slightly higher IgA anti-CD74 compared with patients without DMARDs [median (IQR) 14.2 (10.4–21.9) vs 12.8 (8.3–16.4) U/ml; $P=0.084$]. The same pattern was found between patients on prednisolone vs not on

TABLE 3 Multivariable linear regression analysis in 155 patients with radiographic axial spondyloarthritis (r-axSpA) with logarithmic plasma level of IgA anti-CD74 concentrations as dependent variable and r-axSpA related and demographics as covariates

Covariate	Logarithmic plasma level of IgA anti-CD74, U/ml			
	B	95% CI	B, standardized	P-value
Age, years	-0.005	-0.008, -0.001	-0.24	0.006
Sex	-0.039	-0.121, 0.043	-0.07	0.35
BMI, kg/m ²	0.005	-0.002, 0.012	0.11	0.13
Ever smoker	0.080	0.009, 0.152	0.17	0.028
NSAID, regular use	-0.081	-0.154, -0.009	-0.16	0.028
csDMARD and/or bDMARD	0.056	-0.028, 0.14	0.10	0.19
Prednisolone	0.192	0.064, 0.320	0.22	0.004
ESR, mm/h	0.005	0.002, 0.008	0.23	0.003
mSASSS, score	0.002	0.002, -0.004	0.17	0.041
		<i>R</i> ² 0.26		

bDMARD: biologic DMARD; csDMARD: conventional synthetic DMARD; mSASSS: Modified Stoke Ankylosing Spondylitis Score.

prednisolone [18.9 (11.2–36.9) vs 12.2 (8.7–16.5) U/ml; $P=0.020$] whereas patients on regular use of NSAID had lower IgA anti-CD74 levels compared with non-regular users [11.8 (8.7–16.3) vs 14.0 (8.9–21.5) U/ml; $P=0.032$]. Ever smokers tended to have higher IgA anti-CD74 levels compared with never smokers [13.2 (10.2–20.4) vs 12.2 (8.3–16.3) U/ml; $P=0.093$]. Furthermore, patients with severe spinal radiographic changes had significantly higher IgA anti-CD74 levels than those without severe changes [14.0 (11.3–22.2) vs 11.9 (8.2–16.6) U/ml; $P=0.046$]. No significant differences in IgA anti-CD74 levels were found between sexes, patients with a history of anterior uveitis, peripheral arthritis, or overweight or not.

Multivariable linear regression analyses demonstrating factors associated with IgA anti-CD74 levels in patients with r-axSpA

In multivariable linear regression analyses, the logarithmic plasma levels of IgA anti-CD74 showed significant associations with ever smoker, ESR, mSASSS and use of prednisolone, and inversely with age and regular treatment with NSAIDs ($R^2=0.26$) (Table 3). The results of univariable regression analyses are shown in Supplementary Table 1, available at *Rheumatology* online.

Multivariable logistic regression analyses with radiographic outcomes in patients with r-axSpA

Multivariable logistic regression analyses with ≥ 1 syndesmophyte as the dependent variable and log IgA anti-CD74 as an independent variable adjusted for other known risk factors for radiographic changes in the spine in r-axSpA showed an odds ratio (OR) of 5.64 (95% CI: 1.02, 35.58; $P=0.048$) for log IgA anti-CD74. Multivariable logistic regression analyses with the presence of severe radiographic changes as the dependent

variable and log IgA anti-CD74 as an independent variable adjusted for other known risk factors for radiographic alterations displayed an OR of 8.11 (95% CI: 1.13, 67.31; $P=0.037$) for log IgA anti-CD74. Thus, our results indicate that IgA anti-CD74 is associated with r-axSpA-related changes in the spine (Table 4).

The fluctuation of plasma levels of IgA anti-CD74 over time in r-axSpA in comparison with the fluctuation in controls

Mixed-effects analysis of log IgA anti-CD74 levels, adjusting for BMI and ever smoker (at inclusion in Backbone), revealed an increasing trend in relation to age in the controls ($\beta=0.11$; 95% CI: 0.01, 0.2; $P=0.031$). In contrast, the patients with r-axSpA displayed a more variable pattern (age-group interaction: $\beta=-0.15$; 95% CI: -0.27, -0.02; $P=0.025$). The r-axSpA patients had slightly but not significantly higher baseline IgA anti-CD74 levels with an estimated difference of $\beta=0.02$ (95% CI: -0.04, 0.08; $P=0.502$). Log IgA anti-CD74 levels in group B in patients with r-axSpA and controls concerning age are shown in Fig. 1. The intraclass correlation for the longitudinal measurements was 0.63 for cases and 0.70 for controls where the difference between cases and controls is explained by a higher residual variance among cases compared with controls (0.026 vs 0.017). On the logarithmic scale the variability was comparable between the two groups (s.d. cases: 0.30; s.d. controls: 0.29).

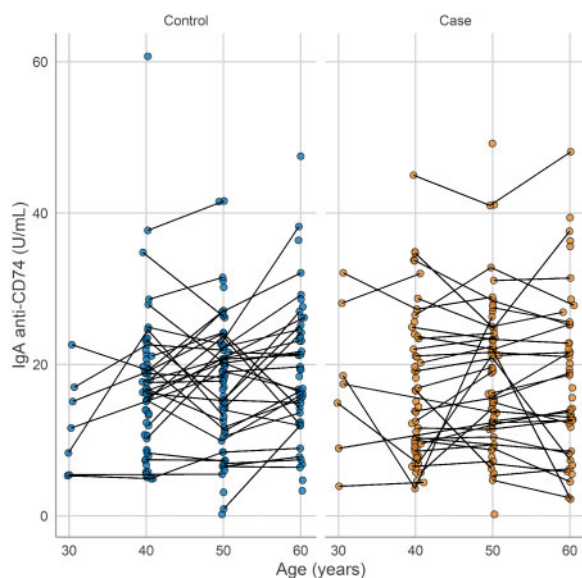
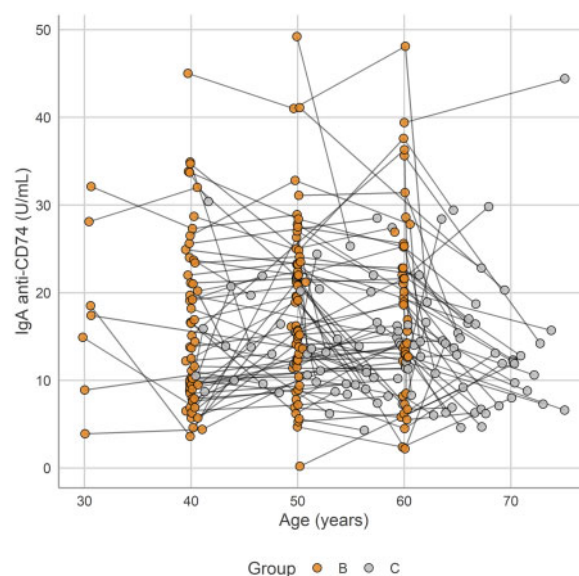
Association between plasma levels of IgA anti-CD74 over time and the severity of r-axSpA

Multivariable regression, adjusted for age, sex, BMI, ever smoking and log CRP, showed that the overall levels and trend in log IgA anti-CD74 levels in group B and C (group C corresponds with the Backbone) in the patients with r-axSpA had no significant influence on

TABLE 4 Multivariable logistic regression analyses with presence of ≥ 1 syndesmophyte or severe radiographic alterations^a as dependent variables

Covariates	≥ 1 syndesmophyte			Severe radiographic changes		
	OR	95% CI	P-value	OR	95% CI	P-value
Age, years	1.10	1.06, 1.15	<0.001	1.09	1.05, 1.15	<0.001
Sex	0.16	0.06, 0.38	<0.001	0.18	0.05, 0.57	0.002
BMI, kg/m ²	0.98	0.92, 1.06	0.672	1.03	0.95, 1.12	0.495
Ever smoker	1.03	0.47, 2.27	0.932	0.82	0.33, 2.01	0.668
Log hsCRP, mg/l	1.64	0.87, 1.80	0.236	1.19	0.88, 2.00	0.182
Log IgA anti-CD74, U/ml	5.64	1.02, 35.58	0.048	8.11	1.13, 67.31	0.037

^aDefined as ≥ 3 consecutive inter-vertebral bridges in the cervical spine and/or the lumbar spine. Female sex was coded 1 and male sex 0. hsCRP: high sensitivity CRP.

Fig. 1 Plasma levels of IgA anti-CD74 in patients with radiographic axial spondyloarthritis and matched controls over age in group B**Fig. 2** Plasma levels of IgA anti-CD74 in patients with radiographic axial spondyloarthritis over age in group B and C

mSASSS, presence of ≥ 1 syndesmophyte or severe radiographic spinal changes, BASMI or BASFI at inclusion in Backbone. The full results are presented in [Supplementary Table 2](#), available at *Rheumatology* online. In [Fig. 2](#), IgA anti-CD74 levels with age for the r-axSpA patients with ≥ 1 measurement of IgA anti-CD74 [106/155 (68%) in group B and C] are shown. IgA anti-CD74 was analysed in the longitudinally collected samples from each of these 106 r-axSpA patients (2–4 samples/patient, altogether in 274 samples).

IgA anti-CD74 in pre-symptomatic individuals

Concerning the pre-symptomatic individuals, 16/155 (10.3%), 5 men and 11 women, had donated 22 blood

samples, with corresponding samples from controls (group A). No difference in the levels of IgA anti-CD74 was found [median (IQR), in pre-symptomatic individuals 20.8 (16.7–28.8) U/ml vs 25.2 (17.5–37.2) U/ml in controls] or in age [in pre-symptomatic individuals 27.7 (25.0–33.9) years vs 27.8 (25.1–33.1) years in controls].

Discussion

In this study on patients with r-axSpA participating in the Backbone study, which investigates severity and comorbidities in r-axSpA, we found that the plasma level of IgA anti-CD74 and the frequency of IgA anti-CD74 positives were significantly increased compared with age- and sex-

matched controls from the same geographical area. We did not find any difference in sex concerning the plasma level or frequency of IgA anti-CD74 positives (threshold 20 U/ml) in patients or controls. The plasma IgA anti-CD74 levels fluctuated over time in both r-axSpA patients and controls with no distinct pattern. Furthermore, the levels over time did not predict radiographic spinal changes later at the inclusion in Backbone whereas IgA anti-CD74 was significantly associated with radiographic spinal changes at the inclusion in Backbone.

The HLA-B27 positive rate was high in the Backbone cohort (98%), which mirrors the high HLA-B27 frequency in the general population in this part of Sweden. Also, about one-fourth of the controls were HLA-B27 positive. We did not find a relation between HLA-B27 status and IgA anti-CD74 status in patients or controls. Previous studies on axSpA patients are inconsistent. Witte *et al.* reported recently that in one of their two cohorts studied, significantly more HLA-B27 negative patients compared with HLA-B27 positive patients had IgA anti-CD74s [29], whereas no such association was found in the InterSpA [21].

Using the cut-off of 20 U/ml according to the ELISA kit instructions, we found IgA anti-CD74 in 23% of the patients with r-axSpA compared with 10% of the controls. In comparison with the current study, Riechers *et al.* reported that IgA anti-CD74 was present in 36% of patients with r-axSpA, out of which 90% were HLA-B27 positive, and in 47% of patients with nr-axSpA, out of which 81% carried HLA-B27 [21]. De Winter *et al.* measured IgA anti-CD74 in a cohort with r-axSpA and the Spondyloarthritis Caught Early (SPACE) cohort and found the frequency to be 28% and 55%, respectively. The frequency was low in healthy controls (5%) but higher in the control group with chronic back pain (37%) [18]. Thus, the pattern is somewhat different in the diverse studies, which might be explained by different symptom duration, demographics, and cut-off levels of IgA anti-CD74, among others.

Concerning the sensitivity and specificity of IgA anti-CD74, we achieved a sensitivity of 0.23 with a specificity of 0.90 (cut-off plasma level of 20 U/ml), which indicates a good specificity but a low sensitivity. By use of our calculated cut-off level (11.75 U/ml), the sensitivity was 0.57 with a specificity of 0.56, indicating modest sensitivity and specificity of the test in our patients with mostly longstanding r-axSpA. Thus, the overall sensitivity/specificity found by these two different cut-offs was only moderate.

We also analysed, to the best of our knowledge, for the first time plasma levels of IgA anti-CD74 in prospectively collected samples. About 70% of the patients had participated earlier in life in the NSHDS and donated blood samples (frozen at -80°C) where the earliest sample was donated in 1989. The levels fluctuated over time in both patients and controls with no distinct development pattern over time in either group. In addition, we analysed serum/plasma levels of IgA anti-CD74 in 16 pre-symptomatic individuals who later developed

r-axSpA and matched controls. The earliest donated and frozen sample was from 1976. No significant difference in the IgA anti-CD74 level was found between the pre-symptomatic persons and their controls.

Furthermore, we studied the relation between IgA anti-CD74 levels and clinical characteristics, laboratory measures of inflammation, medication, disease activity, physical function and spinal radiographic changes at inclusion in the Backbone study. In multivariable logistic regression analyses, we found that log IgA anti-CD74 was significantly associated with spinal radiographic changes assessed both as the presence of syndesmophytes and as the presence of severe radiographic changes adjusted for other known risk factors for radiographic changes in the spine. In accordance, in multivariable linear regression analysis mSASSS, ever smoker, ESR, use of prednisolone and inversely with age and regular treatment with NSAIDs were significantly associated with log IgA anti-CD74 as the dependent variable. Thus, we demonstrated clear relationships between IgA anti-CD74 levels and the osteoproliferative process in the spine in patients with r-axSpA. In line with our findings, Witte *et al.* recently showed that the axSpA patients with IgA anti-CD74 had significantly higher sacroiliitis scores compared with patients without IgA anti-CD74 and patients with IgA anti-CD74 had higher mSASSS compared with patients without IgA anti-CD74 [29]. Unfortunately, we did not find a predictive value of the IgA anti-CD74 level analysed in the prospectively collected samples and radiographic spinal changes.

The limitations of this study include that some of the samples have been frozen for many years, which may have influenced the results, though the same influence should have occurred in the control samples since they were handled in the same way. The outcomes of severity such as radiographic changes were only evaluated once, which limits the possibility to evaluate changes over time in relation to the IgA anti-CD74 levels. Also, we lack MRI data on the spine that could support the association between IgA anti-CD74 and the radiographic changes. The CI intervals in the logistic regression analyses with radiographic severity outcomes were broad, meaning that the variation in IgA anti-CD74 levels was large. To improve the power a higher number of patients and controls would have been preferable.

In summary, we found that the plasma levels of IgA anti-CD74 were elevated and the frequency of IgA anti-CD74 positivity increased in this cohort of well-characterized patients with r-axSpA with a high rate of HLA-B27. IgA anti-CD74 levels were associated with the radiographic changes independent of hsCRP, smoking, BMI, age and sex, which implies that IgA anti-CD74 could play a part in the pathogenesis of r-axSpA.

Ethics

All participants gave their written informed consent. The study was approved by the Ethics Review Board at Umeå

University, Umeå, Sweden (Dnr 2016/208–31), and carried out in accordance with the Declaration of Helsinki.

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Disclosure statement: T.W. holds a patent on the commercial exploitation of the measurement of antibodies against CD74.

Data availability statement

The data sets generated and/or analysed during the current study are not publicly available due to the General Data Protection Regulation (GDPR). Researchers with a specific question regarding the study are encouraged to contact the corresponding author (H.F.dE.).

Supplementary data

Supplementary data are available at *Rheumatology* online.

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