

CD74 auto-antibodies display little clinical value in Chinese Han population with axial spondyloarthritis

Chao-Jun Hu, MD^a, Meng-Tao Li, MD^a, Xi Li, MD^{a,b}, Lin-Yi Peng, MD^a, Shang-Zhu Zhang, MD^a, Xiao-Mei Leng, MD^a, Jin-Mei Su, MD^a, Xiao-Feng Zeng, MD^{a,*} 

Abstract

The European cohort study has indicated about CD74 IgG-autoantibodies as potential marker for axial spondyloarthritis (axSpA) diagnosis. However, multiple studies have questioned the diagnostic value of various disease-specific autoantibodies in different ethnic groups. Here, we have tried to assess the diagnostic value of anti-CD74 IgG and IgA autoantibodies in axSpA patients from Chinese Han population.

The anti-CD74 IgG and IgA autoantibodies were analyzed using ELISA assay in a cohort of 97 axSpA patients, including 47 treatment-naïve axSpA patients never treated with steroids or immunosuppressants and 50 treated axSpA patients. The rheumatic disease control (RDC) group consisted of 40 rheumatoid arthritis, 25 systemic lupus erythematosus, 18 psoriatic arthritis patients, and 60 healthy controls (HC).

Our data demonstrated the presence of anti-CD74 IgA auto-antibodies in 25.8% of the axSpA patients, 30.1% of the RDC group patients and none in HC. Similarly, anti-CD74 IgG autoantibodies were observed in 23.7% of the axSpA patients, 18.1% of the RDC patients and 18.3% of the HC. The sensitivity, specificity, and accuracy of IgA autoantibodies were 21.3%, 82.5%, & 67.4%, respectively, while for IgG, it was 27.7%, 81.8%, and 68.4%, in treatment-naïve axSpA patients. Furthermore, weak positive relationship between anti-CD74 IgA autoantibodies and bath ankylosing spondylitis disease activity index ($r=0.253$, $P=.012$) and functional index (bath ankylosing spondylitis functional index; $r=0.257$, $P=.011$) was observed.

Overall, our study demonstrated little clinical and predictive value of CD74 autoantibodies in the diagnosis of axSpA and its related manifestations, among Chinese Han population.

Abbreviations: AS = ankylosing spondylitis, AUC = area under the curve, axSpA = axial spondyloarthritis, BASDAI = bath ankylosing spondylitis disease activity index, BASFI = bath ankylosing spondylitis functional index, BASMI = bath ankylosing spondylitis metrology index, HC = healthy controls, HLA = human leukocyte antigen, PsA = psoriatic arthritis, RA = rheumatoid arthritis, RDC = rheumatic disease control, SLE = systemic lupus erythematosus, SpA = Spondyloarthritis.

Keywords: anti-CD74, autoantibody, axial spondyloarthritis, biomarker

1. Introduction

Spondyloarthritis (SpA) consists of a group of inflammatory diseases that affect the axial and peripheral skeleton, including ankylosing spondylitis (AS), a prototypic form of SpA. It is commonly diagnosed in the presence of definitive sacroiliitis by

conventional X-rays, and also as non-radiographic axial spondyloarthritis (axSpA), which can cause chronic pain, structural damage, and disability.^[1] Currently, AS is diagnosed based on the modified New York Classification Criteria developed in 1984,^[2] which predominantly relies on radiograph-

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C-JH, M-TL, and XL contributed equally to this work and are the co-first authors.

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^a Department of Rheumatology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences; National Clinical Research Center for Dermatologic and Immunologic Diseases (NCRC-DID); Key Laboratory of Rheumatology & Clinical Immunology, Ministry of Education, Beijing,

^b Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China.

* Correspondence: Xiao-Feng Zeng, Department of Rheumatology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences; National Clinical Research Center for Dermatologic and Immunologic Diseases (NCRC-DID); Key Laboratory of Rheumatology & Clinical Immunology, Ministry of Education, No. 1 Shuafuyuan, Beijing 100730, China (e-mail: zengxfpmc@163.com).

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ic changes. But radiographic changes develop 6 to 10 years following the disease onset,^[3] thereby results in diagnostic delay and prevents the early treatment in many patients. Henceforth, serum biomarkers have been indicated to have significant value in the management of axSpA patients, as they can be unequivocally identified before sacroiliitis detection with radiographic imaging, and thus are helpful in assessing and monitoring the disease progression.^[4] Currently, the human leukocyte antigen (HLA) B27 is the most common biomarker used to diagnose axial SpA patients with 60% to 95% sensitivity and is a part of the core laboratory testing. However, HLA-B27 specificity is also often insufficient for diagnosing axSpA.^[1,5]

CD74, also known as HLA class II histocompatibility antigen gamma chain or invariant chain expressed on the plasma membrane, is responsible for several biological processes, including the correct folding and transport of major histocompatibility complex class II molecules, the differentiation of B cells, and the inflammatory response as a part of the macrophage migratory inhibitory factor.^[6] Baerlecken et al^[7] first reported on the potential role of CD74 IgG-antibodies (anti-CD74 antibodies) in the diagnosis of SpA. Specifically, they verified the presence of anti-CD74 IgG antibodies in 67% of axSpA patients, along with 45% of psoriatic arthritis (PsA) patients without axial involvement, 11% of rheumatoid arthritis (RA) patients, 15% of systemic lupus erythematosus (SLE) patients, 2.5% of human immunodeficiency virus patients, and 0.8% of blood donors. Several research teams have successively reported the diagnostic value of anti-CD74 antibodies in axSpA, and indicated that it is associated with specific clinical manifestations.^[8–11] Nevertheless, controversial results appeared in the research of de Winter et al^[12] and Liu et al^[13], who showed that anti-CD74 antibodies are inadequate for identifying axSpA patients.

In this context, some studies have also emphasized about differences in the diagnostic value of some disease-specific autoantibodies in different ethnic groups, like the presence of anti-soluble liver antigen/liver pancreas autoantibody in up to 58% of the autoimmune hepatitis patients in North America and Europe,^[14,15] while showing very low frequency in Japanese^[16] and Chinese^[17] adult patients with autoimmune liver disease. Due to the different genetic susceptibility of AS in different ethnic groups, we aimed to confirm the diagnostic value of anti-CD74 IgG and IgA autoantibodies in Chinese Han patients with axSpA.

2. Methods

2.1. Patient samples

Total 97 axSpA patients enrolled in our study were recruited from the Peking Union Medical College Hospital (PUMCH) axSpA registry database, between August 2012 and August 2015. Among these, 62 patients met the modified New York (mNY) criteria for AS diagnosis,^[2] while the remaining 35 met the diagnosis criteria of AS for non-radiographic axial spondylitis.^[18,19] The complete summary of patient characteristics is described in Table 1. In addition, the rheumatic disease control (RDC) group consisted of 40 patients diagnosed with RA (mean age 52.1 ± 11.8, male/female 15/25) based on the criteria outlined by the American College of Rheumatology and European League Against Rheumatism in 2010,^[20] 25 patients with SLE (mean age 33.8 ± 10.6, male/female 8/17) diagnosed based on the 1997 revised classification criteria of the American College of Rheumatology,^[21] 18 patients with PsA (mean age 40.3 ± 11.7, male/female 11/7) who met the CASPAR classification criteria,^[22] and 60 healthy individuals (healthy controls (HCs), mean age 32.2 ± 6.6, male/female 40/12), without any symptoms of autoimmune disease, cardiovascular disease, cancer, metabolic disease, backache, or any other chronic disease. From each subject, 4 mL of blood was collected with the help of the BD vacutainer without anticoagulants. For the next 1 hour, the blood was allowed to clot and was later centrifuged at 4°C for 5 minutes at 3,000 rpm. The serum was collected and stored at -80°C. Before undertaking the final analysis, no sample was subjected to more than 1 freeze-thaw cycle. This study was approved by the Medical Ethics Committee of PUMCH (S-478), and all methods were carried out in accordance with the principles stated in the Declaration of Helsinki. All patients signed the informed consent form before enrollment in our study.

2.2. Measurements of anti-CD74 antibodies in serum

The anti-CD74 IgG and IgA auto-antibodies were measured in patient's serum samples using enzyme-linked immunosorbent assay kit, obtained from AESKU Diagnostics (Wendelsheim, Germany) according to the manufacturer's protocol. After collection and isolation, serum samples were further diluted to 1:100 a ratio with sample buffer. Next, 100 µL of each diluted serum sample was added to the antigen-coated wells of a

Table 1
Summary of axial spondyloarthritis patient's clinical characteristics.

Variables	AS	nr-axSpA	P
N	62	35	
Age (yr; mean ± SD)	29.9 ± 9.5	30.9 ± 10.1	.65
Gender (M/F)	52/10	26/9	.25
Treatment-naïve	32 (51.61%)	15 (42.86%)	.41
Clinical features			
Median disease duration (mo, P25–P75)	33.5 (5.8–62.9)	21.3 (4.1–53.8)	.87
HLA-B27 positive (n, %)	32/10 (76.19%)	24/5 (82.76%)	.51
Positive family history (n, %)	13 (21.0%)	10 (28.6%)	.40
Smoking status (n, %)	25 (40.3%)	9 (25.7%)	.15
BASDAI (median, P25–P75)	2.6 (1.0–4.4)	2.3 (1.4–4.8)	.58
BASFI (median, P25–P75)	1.1 (0.3–3.6)	1.0 (0.3–3.5)	.70
BASMI (median, P25–P75)	2.0 (1.0–5.0)	1.0 (1.0–2.0)	.00

AS = ankylosing spondylitis, BASDAI = Bath ankylosing spondylitis disease activity Index (scale 0–10), BASFI = Bath Ankylosing Spondylitis Functional Index (scale 0–10), BASMI = Bath Ankylosing Spondylitis Metrology Index (scale 0–10), HLA-B27 = human leukocyte antigen B27, nr-axSpA = non-radiographic axial spondyloarthritis, P25–P75 = percentile 25–75.

microplate, along with the reference standards, negative controls, and positive control solutions. After 1 hour incubation at room temperature, the microplate was washed with 300 μ L of washing buffer 3 times, and 100 μ L of the conjugate was added to each well, followed by another incubation of 1 hour at room temperature. Subsequently, the microplate was washed with 300 μ L of washing buffer 3 times before 100 μ L of the substrate was added into each well. Additionally, the microplate was again incubated for another 1 hour at room temperature. Finally, the optical density of each well was assessed using a microplate reader after adding 100 μ L stop solution to each well. Cut-off values were determined strictly in accordance with the kit instructions. The cut-off values were 20 U/mL for anti-CD74 IgG and 25 U/mL for anti-CD74 IgA. All samples having an optical density higher than the cut-off value were categorized as positive. Moreover, the concentration of each antibody type was assessed, according to the respective standard curve.

2.3. Statistical analysis

All of the differences between axSpA and control groups were analyzed using the Student *t* test for continuous variables, and χ^2 or Fisher exact test for proportions. Moreover, the anti-CD74 antibodies concentrations in the same axSpA patient, before and after treatment were analyzed using the paired *t* test. The sensitivity, specificity and area under the curve (AUC) for anti-CD74 antibodies were calculated as measures of diagnostic accuracy. Receiver operating characteristic curves were used to calculate the AUC. The investigation of the associations between anti-CD74 antibodies and different clinical variables in axSpA patients were conducted using the χ^2 or Fisher exact test. The correlation between concentrations of anti-CD74 antibodies and SpA-related indexes (including both ankylosing spondylitis disease activity index (BASDAI), both ankylosing spondylitis functional index (BASFI), and Bath ankylosing spondylitis metrology index (BASMI)) were assessed using the Spearman's analysis. The SPSS statistical software package (version 16.0, IBM, Chicago, IL) was used for conducting all statistical analyses, and 2-tailed *P*-values of $<.05$ represented statistical significance.

3. Results

3.1. Anti-CD74 IgA & IgG auto-antibodies analysis in axSpA patients

Based on our analysis, axSpA patients displayed significantly higher mean concentration of anti-CD74 IgA antibody (25.7 ± 39.1 U/mL) in comparison to HC group (9.0 ± 3.6 U/mL; $P = .000$), but not against RDC group (27.3 ± 39.25 U/mL; $P = .78$). Also, the positive average rate of anti-CD74 IgA antibody in axSpA patients was significantly higher (25.8%) than HC group (0.00%, $P = .000$). However, there was no significant difference in the positive rate between axSpA and RDC groups (30.1%, $P = .36$). The frequency of anti-CD74 IgA antibodies in patients with RA, SLE, PsA was 42.5%, 28.0%, and 5.6% respectively. Surprisingly, there were no significant difference in anti-CD74 IgG antibody concentrations between axSpA (22.3 ± 42.8 U/mL), RDC (14.7 ± 43.3 U/mL; $P = .13$) and HC groups (19.8 ± 33.0 U/mL; $P = .70$). The positive rate of anti-CD74 IgG antibody in 97 axSpA patients was 23.7% with no significant difference from RDC group (18.1%, $P = .36$) or HC group (18.3%, $P = .43$). The frequency of anti-CD74 IgG antibodies in patients with RA, SLE, PsA was 15.0%, 24.0%, and 16.7% respectively. The quantification summary of anti-CD74 IgA and IgG antibodies serum concentration, in axSpA patients, RDC, and HC, has been shown in Figure 1.

3.2. Diagnostic performance analysis of anti-CD74 IgG & IgA autoantibodies in axSpA patients

We performed receiver operating characteristic curves curve analysis to determine the diagnostic accuracy of anti-CD74 IgG and IgA in axSpA patients. The AUC of anti-CD74 IgG between axSpA patients and controls was 0.673 (95% CI: 0.606–0.739; $P < .001$), sensitivity was 46.94% (95% CI: 0.328–0.616) and specificity was 59.11% (95% CI: 0.516–0.642). Similarly, the AUC of anti-CD74 IgA between axSpA patients and controls was 0.666 (95% CI: 0.598–0.734; $P < .001$), sensitivity was 50.98% (95% CI: 0.368–0.650) and specificity was 60.33% (95% CI: 0.527–0.674). Further analysis of anti-CD74 autoantibodies between treatment-naïve and treated axSpA patients revealed AUC of 0.520 (95% CI: 0.403–0.637), sensitivity of 56.52% (95% CI: 0.348–0.761), and specificity of 54.05% (95% CI:

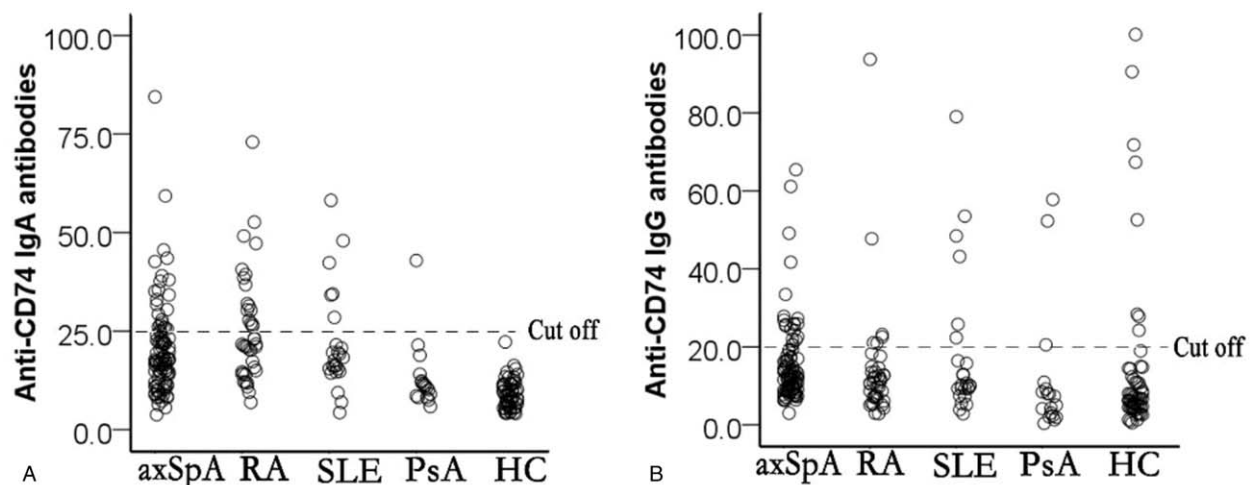


Figure 1. Estimation of antibody concentration in the serum of axial spondyloarthritis patients, rheumatic disease, and healthy controls; (A) anti-CD74 IgA and (B) anti-CD74 IgG.

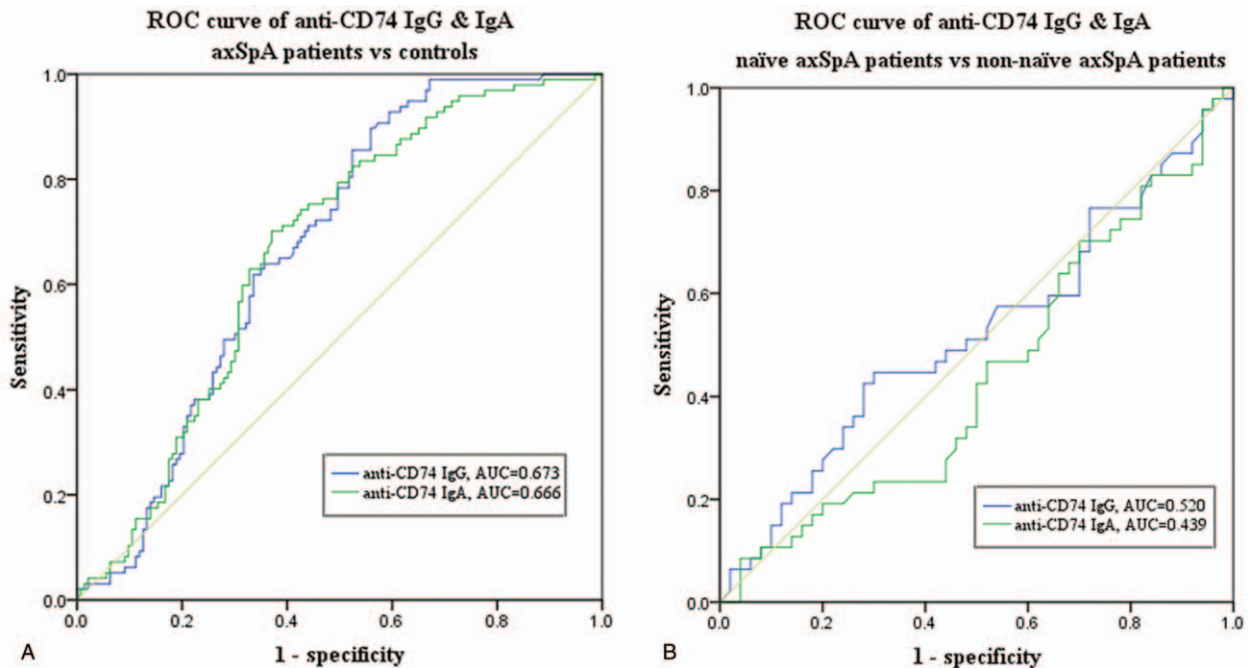


Figure 2. Receiver operating characteristic curve analysis of anti-CD74 IgG & IgA. (A) Estimation of anti-CD74 IgG and IgA area under the curves between axial spondyloarthritis patients and controls. (B) Estimation of anti-CD74 IgG and IgA area under the curves between treatment-naïve and treated axial spondyloarthritis patients. AUC=area under the curve.

0.421–0.656) for IgG, while AUC of 0.439 (95% CI: 0.324–0.554), sensitivity of 42.32% (95% CI: 0.240–0.628) and specificity of 49.30% (95% CI: 0.373–0.613). Overall our data, as shown in Figure 2, indicated no significant differences in the diagnostic ability of anti-CD74 IgG and IgA autoantibodies in axSpA Chinese patients.

3.3. Dual-Positivity analyses of anti-CD74 IgA & IgG antibodies in axSpA patients

Among the 47 treatment-naïve axSpA and 50 treated axSpA patients, only 3 patients from each group tested positive

for 2 autoantibodies. The positivity of the anti-CD74 IgA antibody alone was only 17.0% in the treatment-naïve axSpA group, and was lower than 24.0% in treated axSpA group. However, this difference was not significant ($\chi^2 = 1.28, P = .26$). In contrast, the positivity of anti-CD74 IgG alone was 21.3% in the treatment-naïve axSpA group, and was higher than 14.0% observed in treated axSpA group. However, again the difference was not significant ($\chi^2 = 0.89, P = .35$). Overall, dual-positivity for both anti-CD74 IgA and IgG was nearly identical in both treatment-naïve and treated axSpA patients at 6.4% and 6.0%, respectively (Fig. 3).

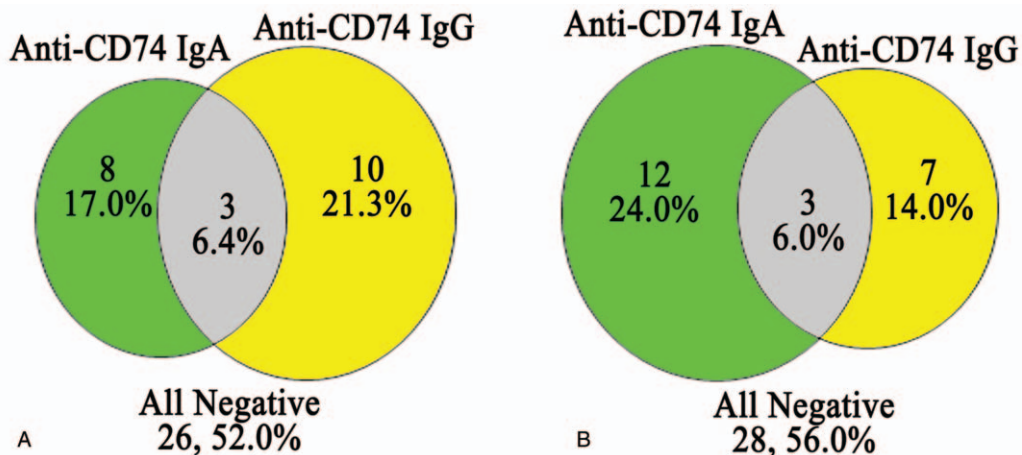


Figure 3. Dual-positivity analysis of anti-CD74 IgA and IgG antibodies in axial spondyloarthritis (axSpA) patients. Panel A, show data in 47 treatment-naïve axSpA patients, while panel B display data from 50 treated axSpA patients.

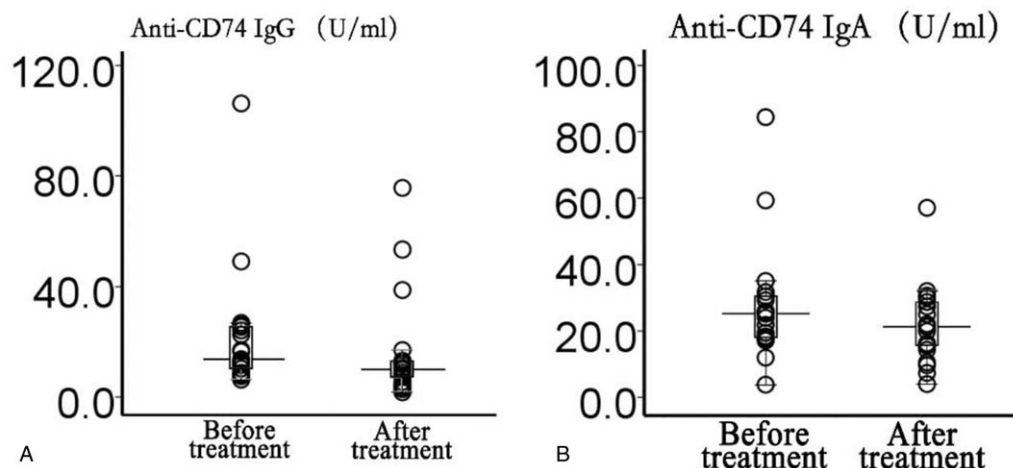


Figure 4. Antibody concentration assessment in 21 treatment-naïve axial spondyloarthritis patients, before and after treatment; (A) anti-CD74 IgG, and (B) anti-CD74 IgA.

3.4. Assessment of anti-CD74 IgA and IgG antibody concentrations in axSpA patients, before and after treatment

From the 97 total axSpA patients, we only analyzed anti-CD74 IgA and IgG antibody concentrations in the serum of 21 patients, before and after treatment (Fig. 4). Following logarithmic transformation of anti-CD74 IgA and IgG antibody concentrations, the values were analyzed using Student paired *t* tests. It was observed that concentrations of anti-CD74 IgG antibodies in the serum of treated axSpA patients were significantly lower than before treatment ($t=3.94, P=.001$). However, no significant differences were observed for the concentrations of anti-CD74 IgA antibodies in the serum of axSpA patients before and after treatment ($t=1.88, P=.07$).

3.5. Association analysis between axSpA-related clinical features and anti-CD74 antibodies

Among the various clinical manifestations presented by axSpA patients (Table 2), anti-CD74 IgA antibodies showed significant association with HLA-B27 positivity ($\chi^2=4.57, P=.03$). Other clinical features, including family history and smoking status, were not associated with the presence of anti-CD74 IgG or IgA

antibodies. However, our study confirmed a positive relationship of anti-CD74 IgA antibodies concentration with BASDAI ($r=0.253, P=.012$) and BASFI ($r=0.257, P=.011$).

4. Discussion

The recently introduced term, axial SpA (axSpA),^[18,19] is one of the most common autoimmune inflammatory disease with diverse clinical presentation. It is typically characterized by inflammatory chronic back pain, stiffness, and ankylosis of the spinal joints. Its incidence rate is usually higher in males. The early and correct axSpA diagnosis, along with aggressive intervention is crucial to reduce the potentially destructive effects of this disease. Recently, public awareness about axSpA, advanced training of rheumatologists, the evolution of diagnosis criteria, and development of radiographic techniques have all contributed to the improved patient outcomes. However, the incidences of delayed or incorrect diagnoses remain too frequent due to the considerable delay of 7 to 10 years between the onset of inflammatory back pain and axial SpA diagnosis.^[23,24]

In this study, anti-CD74 IgA and IgG autoantibodies were analysed using ELISA assay in axSpA cohort and other autoimmune diseases patients, along with healthy volunteers. We further divided the axSpA patients into treatment-naïve and

Table 2
Association between axial spondyloarthritis-related clinical features and anti-CD74 antibody levels.

Clinical features		Anti-CD74 IgG			Anti-CD74 IgA		
		Positivity	χ^2 or <i>r</i>	<i>P</i>	Positivity	χ^2 or <i>r</i>	<i>P</i>
HLA-B27 positive	Yes	21.4%	0.92	.34	19.6%	4.57	.03
	No	33.3%			46.7%		
Family history	Yes	26.1%	0.09	.76	17.4%	0.61	.44
	No	23.0%			28.4%		
Smoking status	Yes	26.5%	0.22	.64	35.3%	2.48	.12
	No	22.2%			20.6%		
BASDAI			0.083	.417		0.253	.012
BASFI			0.095	.357		0.257	.011
BASMI			-0.051	.620		0.075	.465

BASDAI = bath ankylosing spondylitis disease activity index, BASFI = bath ankylosing spondylitis functional index, BASMI = Bath ankylosing spondylitis metrology index, HLA-B27 = human leukocyte antigen B27.

treated axSpA groups. Low positivity of anti-CD74 IgA was observed in the axSpA patients, with 23.4% in the treatment-naïve and 30.0% in treated axSpA groups, which was consistent with results from an earlier published study.^[12] In addition, we noted that the positive rate of anti-CD74 IgA in the treatment-naïve, treated, and unselected total axSpA patients were 23.4%, 30.0%, and 25.8% respectively, which indicated that the positive rate of anti-CD74 in axSpA patients varied among different patients. However, compared to HC group, the concentration and positivity rate of anti-CD74 IgA autoantibodies were significantly higher in axSpA group, yet no significant difference was observed between axSpA and RDC groups, thereby indicating little diagnostic value of anti-CD74 IgA autoantibodies in axSpA. Similarly, the anti-CD74 IgG auto-antibodies showed no significant difference in the concentrations and positive rates among axSpA, RDC and HC groups, suggesting that anti-CD74 autoantibody is not useful as a diagnostic marker for axSpA Chinese patients. Our results demonstrate that the positive rate of the PsA anti-CD74 antibody has the lowest level in RDCs. PsA is most closely related to SpA, with similarities in their pathogenesis, clinical manifestations, and treatments.^[25,26] Hence, CD74 autoantibodies cannot distinguish axSpA or PsA from rheumatic diseases. Further research needs to clarify the clinical utility of anti-CD74 antibodies to SpA, especially axSpA.

Similar conclusions have been obtained by Liu et al^[13] and de Winter et al,^[12] who found that anti-CD74 autoantibodies are not good biomarkers for the diagnosis of SpA in Asian and European people, especially in patients under 45 years old presenting with early back pain.^[12] As mentioned earlier, several studies have assessed the diagnostic value of anti-CD74 antibodies for axSpA in European and Middle Eastern patients, finding that antibodies can be used as a new serological marker of axSpA.^[7-11,27] Moreover, the latest research reveals that CD74 is a T cell antigen in SpA, eliciting Th1 and Th17 responses, which may be relevant in SpA disease pathogenesis.^[27] axSpA is a disease of genetic susceptibility.^[28-31] As these contradictory results show, the prevalence of anti-CD74 antibodies may be linked to differences in racial or geographical predilection. Moreover, given the MHC association of this disease,^[32] there is no good reason to expect an autoantibody presence; however, this represents an area that needs further investigation.

In parallel, the dual-positivity of anti-CD74 IgA and IgG autoantibodies was also investigated in axSpA patients. We found that 52.0% and 56.0% of treatment-naïve and treated axSpA patients had no anti-CD74 IgA or IgG autoantibodies. Only 6.4% of the 47 treatment-naïve axSpA and 6.0% of the 50 treated axSpA patients were positive for anti-CD74 IgA and IgG autoantibodies. In addition, no significant difference in the positivity for anti-CD74 IgG alone or anti-CD74 IgA alone in the treatment-naïve and treated axSpA patients was observed. Paired samples from 21 patients analyzed before and after the treatment, showed that concentrations of anti-CD74 IgG antibodies in the serum of treated axSpA patients were significantly decreased after treatment. However, this trend was not observed for anti-CD74 IgA autoantibodies. As a matter of utility, there is substantial overlap between the pre- and post-treatment measures, and further study is needed to reveal the association between anti-CD74 antibodies and treatment.

Additionally, we assessed the association between anti-CD74 autoantibodies and axSpA-related clinical features, including HLA-B27 positivity, family history, smoking status, and disease activity index, as no previous studies have analyzed these

relationships. However, our data showed that HLA-B27, family history, smoking status, BASDAI, BASFI, and BASMI scores did not show any association with anti-CD74 IgG antibodies. In regards to anti-CD74 IgA autoantibodies, the positivity was only associated with HLA-B27 positivity ($\chi^2=4.57$, $P=.03$). A weak positive relationship between their concentrations and BASDAI ($r=0.253$, $P=.012$) and BASFI ($r=0.257$, $P=.011$) scores were found. Different from the study of Riechers et al,^[11] the positive rate of anti-CD74 IgA antibodies in their European cohort was as high as 47%, and the combination of anti-CD74 IgA and HLA-B27 provided post-test probabilities of 80.2% for distinguishing axSpA in chronic back pain (CBP) patients. Thus, our results are not sufficient to support anti-CD74 IgA autoantibodies as a useful tool for diagnosing axSpA.

Importantly, despite the outcome of our study, it has several strengths. First, this is the study analyzing anti-CD74 autoantibodies in Chinese axSpA patients. Second, to avoid the effect of treatment on serum levels of anti-CD74 autoantibodies, we included treatment-naïve axSpA patients. Third, we performed a comparative dynamic analysis of anti-CD74 antibodies in same axSpA patients before and after treatment, along with analyzing the relationship between anti-CD74 antibodies concentrations and disease activity indexes, including BASDAI, BASFI, and BASMI. Nonetheless, the current study has some limitations, such as its relatively small sample size, and data being collected from a single center.

5. Conclusions

In summary, we have comprehensively analyzed the significance of anti-CD74 antibodies in the serum of Chinese Han patients with axSpA, especially in treatment-naïve patients. Our data indicated little clinical value of anti-CD74 autoantibodies, as the positivity of IgA and IgG autoantibodies was only 23.4% and 27.7% in treatment-naïve, while 30.0% and 20.0% in treated axSpA Chinese patients, respectively. Furthermore, our study also showed limited association between anti-CD74 antibodies and specific axSpA clinical features or disease activity indexes. Thus, the utility of anti-CD74 autoantibodies as diagnostic and predictive markers for axSpA diagnosis could not be validated in Chinese axSpA patients. However, due to the small sample size in our study, it would be interesting to conduct additional longitudinal, multicenter studies to further validate if anti-CD74 autoantibodies have any diagnostic and prognostic role in axSpA patients.

Author contributions

All authors were involved in the design of the study. CJH, MTL, and XL were involved in the collection of blood samples, experimental procedures, statistical analysis, and writing of the manuscript. MTL, LYP, SZZ, XML, JMS, and XFZ were involved in the recruitment of patients and evaluation of clinical data. All authors gave intellectual input to the study and approved the final version of the manuscript.

Conceptualization: Chao-Jun Hu.

Data curation: Chao-Jun Hu, Meng-Tao Li, Xi Li, Lin-Yi Peng, Shang-Zhu Zhang, Xiao-Mei Leng, Jin-Mei Su.

Formal analysis: Chao-Jun Hu, Xi Li, Shang-Zhu Zhang.

Funding acquisition: Chao-Jun Hu.

Investigation: Chao-Jun Hu, Xi Li, Shang-Zhu Zhang, Xiao-Mei Leng, Jin-Mei Su.

Methodology: Chao-Jun Hu, Xi Li, Jin-Mei Su.

Project administration: Chao-Jun Hu, Meng-Tao Li, Xiao-Feng Zeng.

Resources: Chao-Jun Hu, Lin-Yi Peng, Xiao-Mei Leng, Jin-Mei Su.

Software: Chao-Jun Hu.

Supervision: Chao-Jun Hu, Meng-Tao Li, Xiao-Feng Zeng.

Validation: Chao-Jun Hu, Meng-Tao Li, Xi Li, Xiao-Feng Zeng.

Visualization: Chao-Jun Hu, Meng-Tao Li, Xiao-Feng Zeng.

Writing – original draft: Chao-Jun Hu, Xi Li.

Writing – review & editing: Chao-Jun Hu, Xi Li, Xiao-Feng Zeng.

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