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Immunohistological analysis of active sacroiliitis in patients with axial spondyloarthritis

Jianhua Peng, PhD, Yao Gong, MD, Yuping Zhang, PhD, Danmin Wang, PhD, Zhengyu Xiao, BS*

Abstract

The sacroiliac joints (SIJs) are one of the most common sites involved in axial spondyloarthritis (axSpA), and there are few studies on the histopathology of the SIJ in this group of patients.

Mononuclear cell infiltrates in the bone marrow and fibrous tissue resembling a pannus formation were the pathological features of early sacroiliitis in our previous study. We undertook a further immunohistological evaluation of these features in patients with axSpA.

Biopsy specimens from the SIJ of 6 patients with established ankylosing spondylitis (AS) and 13 patients with nonradiographic axial spondyloarthritis (nr-axSpA) were analyzed. An immunohistological method was performed to examine the macrophages (CD163), T cells (CD3), and B cells (CD20).

Mononuclear cell infiltrates in the bone marrow were observed in only 6 patients with nr-axSpA. Fibrous tissue was observed in all patients with established AS and 9 patients with nr-axSpA. Macrophage, T cell, and B cell infiltrates could be detected in both the bone marrow and fibrous tissue. All bone marrow specimens from 6 nr-axSpA patients exhibited CD163+ macrophage infiltrates; of these, 5 exhibited CD20+ B cell infiltrates and 3 exhibited CD3+ T cell infiltrates. Among the fibrous tissue specimens, all exhibited macrophage infiltrates, 9 exhibited B cell infiltrates, and 4 exhibited T cell infiltrates.

In addition to macrophages and T cells, B cells are also involved in active sacroiliitis in patients with axSpA.

Abbreviations: axSpA = axial spondyloarthritis, nr-axSpA = nonradiographic axial spondyloarthritis, SIJ = sacroiliac joints.

Keywords: ankylosing spondylitis, pathology, sacroiliac joints, spondyloarthritis

1. Introduction

The sacroiliac joints (SIJs) are one of the most common sites involved in ankylosing spondylitis (AS), and sacroiliitis is crucial for the diagnosis of AS.[1] The classification criteria for axial spondyloarthritis (axSpA) were developed due to the diagnostic limitations in early disease according to the modified New York criteria.[2,3] axSpA includes the established AS that met the modified New York criteria, and nonradiographic axSpA (nr-axSpA), without definite radiographic sacroiliitis. Early active sacroiliitis can be detected both by magnetic resonance imaging (MRI) and by needle biopsy of SIJ.[4,5]

The location of the SIJ is poorly accessible; therefore, there are only a few studies on the histopathology of SIJ in this group of patients, showing inconsistent findings.[6–9] Shichikawa [8] observed that subchondral granulation tissue but not inflammatory cells is the feature of sacroiliitis in open biopsies of 5 patients with AS. However, inflammatory cell infiltrates were demonstrated in the SIJ of 5 patients with active AS in another study using needle biopsy.[6] Moreover, there is a paucity of data on the immunohistology of SIJ in patients with AS, particularly of early cases.[5,10] T cells and macrophages are the most frequent cells in active sacroiliitis.[5,6,10]

These key features of early sacroiliitis, the presence of mononuclear cell infiltrates in the bone marrow, and the invasion of the subchondral bone plate and cartilage by fibrous tissue, resembling a pannus, also have been described in our previous paper.[9] In the present study, we applied immunohistochemical methods to further characterize these findings.

2. Patients and methods

2.1. Patients

Nineteen patients who met the Assessment of SpondyloArthritis international Society classification criteria for axSpA were recruited between 2003 and 2013 by our department.[2] Only patients with SIJ biopsy sections of $>3\,mm^2$ were recruited. Six patients with established AS, which met the revised New York criteria,[3] and 13 nr-axSpA patients with the absence of definite radiographic sacroiliitis (3 of them met the clinical arm of axSpA). Clinical, laboratory, and imaging data of the SIJ were collected. This study was approved by the ethics committee of the first Affiliated Hospital of Shantou University Medical College. All patients signed an informed consent.

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JP and YG contributed equally to this work.

Authorship: All the authors were involved in drafting the article or critically revising it for important intellectual content, and all the authors approved the publication of the final version. Dr Xiao had full access to all data in the study and took responsibility for the integrity of these data and the accuracy of the data analysis.

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First Affiliated Hospital of Shantou University Medical College, Shantou University Medical College, Shantou, Guangdong Province, China.

** Correspondence: Zhengyu Xiao, First Affiliated Hospital of Shantou University Medical College, No.22 Xin Ling Road, Shantou 515041, Guangdong Province, China (e-mail: deanyu@163.com).*

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2.2. Materials

A computed tomography (CT)-guided needle biopsy of the SIJ was performed as previously described.[11] At the same time of sacroiliac biopsy, an intra-articular corticosteroid injection was performed. SIJ needle biopsy specimens were obtained from both sides from the patients, but only the specimens from one side met the inclusion criteria. Sections of biopsy tissue with a thickness of 4 to 5 μm were cut for a routine hematoxylin and eosin (H&E) staining for the diagnosis of early sacroiliitis.

2.3. Grading of radiographic sacroiliitis

Radiographic sacroiliitis was graded according to the revised New York criteria for AS.[3]

2.4. Scoring of bone marrow edema (BME) on magnetic resonance imaging (MRI)

BME on MRI was defined according to the Assessment of SpondyloArthritis International Society (ASAS)/Outcome Measures in Rheumatology (OMERACT) MRI Group.[4] BME scoring was based on the Spondyloarthritis Research Consortium of Canada (SPARCC) criteria.[12]

2.5. Immunohistochemical procedures

Immunohistochemistry studies were performed to detect CD3+ T cells, CD20+ B cells, and CD163+ macrophages using a 2-step polymer horseradish peroxidase (HRP) detection system. All primary antibodies (monoclonal antibodies) against T cells, B cells, and macrophages, secondary antibodies, and the Polink-2 Plus detection system Kit were purchased from Zhongshan Biotechnology Company Limited (Beijing, China).

Each specimen was cut into 4 μm thick sections, mounted on chrome alum-gelatin-coated slides, and then baked for an hour at 60°C before staining. The slides were deparaffinized in xylene, rehydrated and subjected to heat-induced antigen retrieval in a high-pressure cooker for 3 minutes (CD20 and CD163) or 5 minutes (CD3). For antigen retrieval, the slides were immersed in a Tris-EDTA buffer solution at pH 9.0. After heating, the tissues were cooled to room temperature, incubated with the peroxide block for 10 minutes to block endogenous peroxidase activity, washed in phosphate-buffered saline (pH 7.4), and incubated with the primary antibodies overnight at 4°C. The primary antibodies included monoclonal antibodies against CD3 (clone EP41, dilution 1:50), CD20 (EP7, dilution 1:50), and CD163 (10D6, dilution 1:300). A 2-step polymer HRP detection system was used for detection. Fresh 3, 3′-diaminobenzidine (DAB) (prepared in a ratio of 1:20) was used as the chromogen.

The number of lymphocyte infiltrates per high-power field [HPF (400×)] was counted. Lymphocyte aggregates were defined as ≥50 T cells or B cells per HPF.

2.6. Statistical analysis

All data were analyzed using SPSS software (version 20.0 for Windows; IBM Corp., Armonk, NY) The Mann–Whitney *U* test was used to compare the differences between groups. *P* values less than .05 were considered statistically significant.

3. Results

3.1. Patient characteristics

The demographic and clinical characteristics of 19 axSpA patients are summarized in Table 1. Approximately 68.4% of the patients were HLA-B27-positive. The mean age was 22.5 ± 8.7 years (range: 12.0–49.0 years), and the mean age of onset was 18.2 ± 6.7 years (range: 8.0–33.0 years). The mean disease duration was 4.3 ± 5.8 years (median 2.0 years; range: 0.3–25.0 years). Uveitis, psoriasis, and inflammatory bowel disease were not found in this group of patients, and the other SpA features are summarized in Table 1. The laboratory evaluations showed that the mean erythrocyte sedimentation rate (ESR) was 42.2 ± 28.5 mm/h and the mean C-reactive protein (CRP) was 22.5 ± 23.5 mg/L. According to the radiographic sacroiliitis grading, 5 patients had radiographic sacroiliitis Grade 0, 7 had Grade 1, 4 had Grade 2, and 3 had Grade 3. MRI of the SIJ was performed on 14 of the 19 patients, all of whom had BME on MRI. The mean score was 15.8 ± 10.6 (median 14.0, bilateral). None of the patients received antitumor necrosis factor (anti-TNF) α therapies.

Table 1

Demographic and clinical characteristics of 19 patients with axSpA.

Characteristic	AS	nr-axSpA	Total
Age, y	25.5 ± 11.6	21.2 ± 7.1	22.5 ± 8.7
Age of onset, y	18.8 ± 4.4	17.9 ± 7.7	18.2 ± 6.7
Male, n (%)	5 (83.3)	8 (61.5)	13 (68.4)
HLA-B27 (+), n (%)	6 (100)	7 (53.8)	13 (68.4)
Disease duration, y	6.7 ± 9.4	3.2 ± 3.1	4.3 ± 5.8
IBP, n (%)	6 (100)	6 (46.2)	12 (63.2)
Arthritis, n (%)	3 (50.0)	9 (69.2)	12 (63.2)
Dactylitis, n (%)	2 (33.3)	6 (46.2)	8 (42.1)
Enthesitis, n (%)	1 (16.7)	5 (38.5)	6 (31.6)
Family history of SpA, n (%)	0 (0)	4 (30.8)	4 (21.1)
Good response to NSAIDs, n (%)	6 (100)	11 (84.6)	17 (89.5)
ESR, mm/1 h	48.5 ± 37.3	39.7 ± 26.2	42.2 ± 28.5
CRP, mg/L	17.1 ± 11.9	24.7 ± 27.0	22.5 ± 23.5
Grading of radiographic sacroiliitis*			
<2, n (%)	2 (33.3)	10 (76.9)	12 (63.1)†
2, n (%)	1 (16.7)	3 (23.1)	4 (21.1)‡
3, n (%)	3 (50.0)	0 (0)	3 (15.8)
BME ratio on MRI§	3/6	11/13	14/14
BME score on MRI§	26.7 ± 7.6	11.1 ± 8.2	15.8 ± 10.6

IBP = inflammatory back pain, NSAIDs = nonsteroidal anti-inflammatory drugs.
* Only the biopsy side was graded.
† Two AS patients displayed Grade 1 on one side of the SIJ and Grade 3 on the other.
‡ Three nr-axSpA patients displayed Grade 2 on one side of the SIJ and Grade 1 on the other.
§ Bilateral SIJ was scored.

3.2. Histopathological features of SIJ

Mononuclear cell infiltrates in the bone marrow were observed in only 6 of the patients with nr-axSpA, but not in the patients with AS. Fibrous tissue was observed in both nr-axSpA patients (n = 9) and AS patients (n = 6). (Histopathological features of SIJ are shown in Fig. 1). In 4 patients with nr-axSpA, only mononuclear cell infiltrates were observed in the bone marrow sections. The mean BME score on MRI of these patients was 3.0 (median). In 13 patients with AS (n = 6) and nr-axSpA (n = 7), only fibrous tissue that invaded the subchondral bone plate and cartilage was observed in the sections. The mean BME score on MRI was 14.0, which was significantly higher than the group with inflammatory cell infiltrates ($P = .002$). Both pathological features of SIJ were observed in 2 patients with nr-axSpA. The mean BME score on MRI was 4.5 (Table 2).

3.3. Immunohistological features

We applied an immunohistochemical method to further characterize these pathological features of the SIJ. Lymphocyte

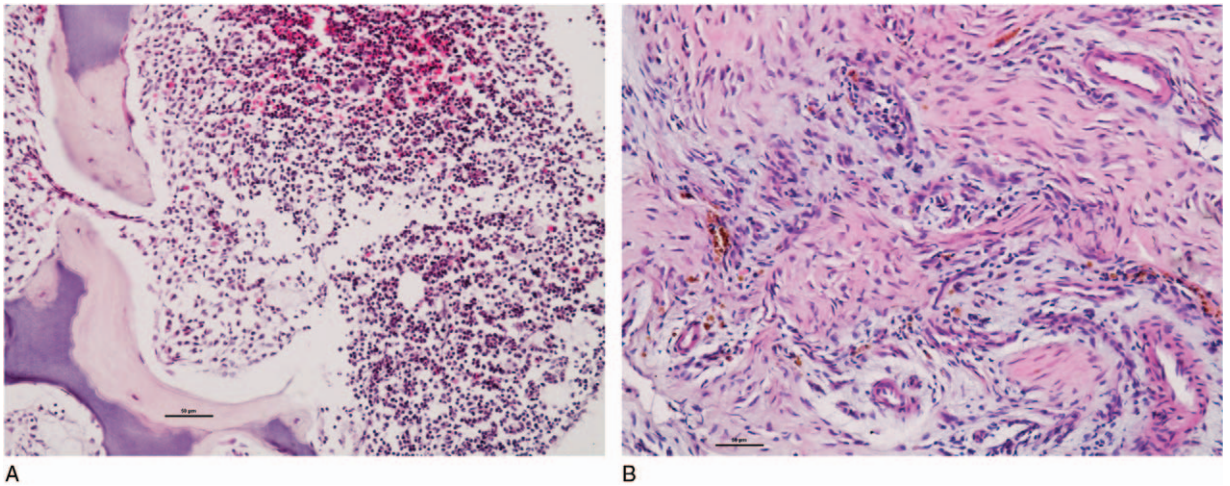

Figure 1. (A) Mononuclear cell infiltrates, SIJ biopsy sections of nr-axSpA patients, a 15-year-old man, with HLA-B27+, with a disease duration of 5 months and severe unilateral hip pain. (B) Fibrous tissue resembling a pannus formation. SIJ biopsy sections of a nr-axSpA patient, a 24-year-old woman, who was HLA-B27+, with a disease duration of 8 months and alternating buttock pain. Bars = 50 μm.

aggregates were defined as clusters of ≥50 T cells or B cells per HPF (400×).

3.3.1. Mononuclear cell infiltrates in bone marrow. In all 6 specimens from the nr-axSpA patients, CD163+ macrophages were observed as a dense cellular infiltrate in the bone marrow. Both CD3+ T cell and CD20+ B cell aggregates were observed in 2 specimens. Interstitial CD20+ B cell infiltrates were observed in 3 of the 6 specimens, and interstitial CD3+ T cell infiltrates were seen in 1 of the 6 specimens. The numbers of B cell and T cell infiltrates were 30.3 ± 6.3 per HPF (400×) and 40.0 per HPF (400×), respectively (Representative immunohistochemical staining of bone marrow is showed in Fig. 2).

3.3.2. Fibrous tissue. CD163+ macrophages were also observed as a dense cellular infiltrate in the fibrous tissue in all 15 specimens. Due to the limited biopsy material, only 13 of the 15 specimens that contained fibrous tissue could be stained for CD3 and CD20. CD20+ B cells were detected in 9 of the 13 specimens; these B cells were detected as lymphocyte aggregates in three specimens, and the number of the cells in the other 6 specimens was 30.3 ± 6.3 per HPF (400×). CD3+ T cells were detected in 4 specimens; these cells were detected as lymphocyte aggregates in 2 specimens, and the number of cells in the other specimens was 25.0 per HPF (400×) (Table 3, and representative immunohistochemical staining of fibrous tissue is showed in Fig. 3).

Table 2

Distribution of the histopathological features, BME scores on the MRI, and grade of radiographic sacroiliitis of 19 axSpA patients.

	Mononuclear cell infiltrates	Fibrous tissue	Both
Number of patients	4	13	2
BME ratio on MRI*	4/4	7/8	2/2
BME score on MRI, median*	3.0	14.0	4.5
Grading of radiographic sacroiliitis†			
Grade <2, n (%)	4 (100.0)	6 (46.2)	2 (100.0)
Grade ≥2, n (%)	0 (0)	7 (53.8)	0 (0)

BME = bone marrow edema, MRI = magnetic resonance imaging.
* Only the biopsy side was scored
† The biopsy side was graded.

4. Discussion

This study shows that in addition to macrophages and T cells, B cells are also involved in SIJ inflammation in patients with AS or nr-axSpA. A large amount of CD20+ B cell infiltrates were detected in the bone marrow, and CD20+ B cell infiltrates were also observed in fibrous tissue resembling a pannus.

There are limited reports of immunohistological staining for B cells in axSpA patients. Appel et al[13] first identified a significantly increased number of CD20+ B cells in advanced AS patients with persistent inflammation in the zygapophyseal joints. This result supported our finding that CD20+ B cell is an important cell subset in active sacroiliitis in AS or axSpA. In another previous study,[5] researchers performed immunohistological staining for B cells in SIJ biopsy specimens from 18 AS and 12 undifferentiated spondyloarthritis (SpA) patients; however, it seems that the number of B cells was not impressive. The difference in this finding from our study may be due to the different detection systems used in the 2 reports. In our study, the Polink-2 Plus detection system was used that was more sensitive than the alkaline phosphatase-anti-alkaline phosphatase technique (APAAP) used in the previous study. Another reason for the difference might be the difficulty in accessing the SIJ to obtain representative biopsy material. In fact, some of the specimens in our study also showed negative staining for CD3 or CD20 in the fibrous tissue.

The role of B cells in axSpA patients remains unclear. Although rituximab was effective in patients with SpA who were TNF blocker-naive, there was no clear response for whom TNF blockers had failed.[14,15] Unlike rheumatoid arthritis or other connective tissue diseases, no specific autoantibodies were detected in patients with axSpA. B cells could be antigen-presenting cells, as well as precursors of antibody-secreting plasma cells, which can enhance the activation of T cells.[16] This possibility will require more exploration in the future to determine the role of B cells in the pathogenesis of AS.

In a study on the synovium in active SpA patients (half of which were AS), 12 weeks of treatment with anti-TNFα therapies

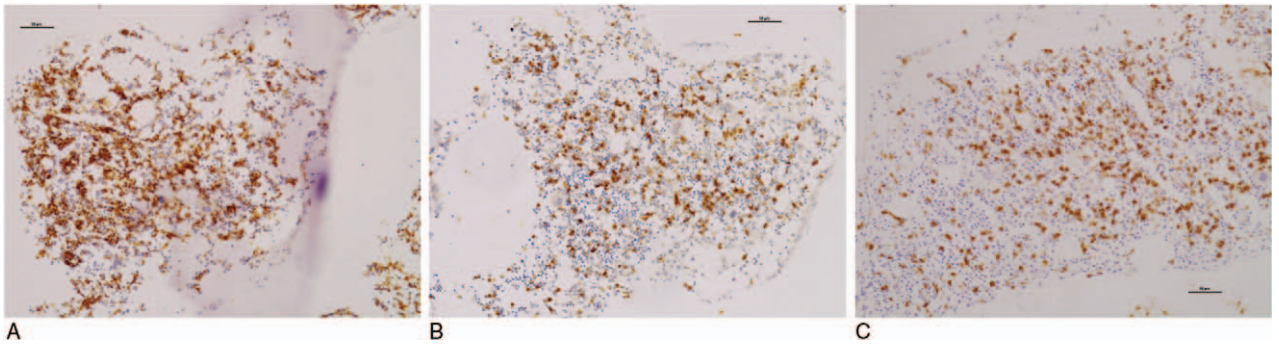

Figure 2. The same patients as shown in Fig. 1A (A–C). Immunohistochemical staining of bone marrow. (A) Dense CD163+ macrophage infiltrates. (B) CD3+ T cell aggregates. (C) CD20+ B cell aggregates. Bars = 50 μm.

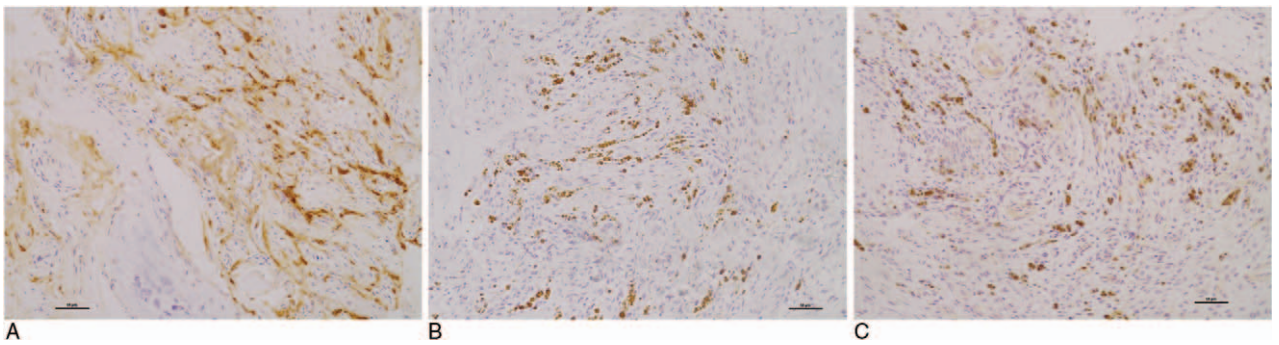

Figure 3. The same patients as shown in Fig. 1B (A ~ C). Immunohistochemical staining of fibrous tissue. (A) Dense CD163+ macrophage infiltrates. (B) CD3+ T cell infiltrates. (C) CD20+ B cell infiltrates. Bars = 50 μm.

(infliximab) induced a reduction in inflammatory cell infiltration by macrophages and T cells, but not B cells.[17] In a study on peripheral blood B cell subsets in patients with AS, the number of CD19+ B cells in the active AS patients was increased and positively correlated with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). After 12 weeks of treatment with anti-TNFα therapies (etanercept), the high percentage of CD19+ B cells in the active AS patients could not be downregulated.[18] It seems that anti-TNFα therapies are ineffective in reversing the inflammation induced by B cells. The development of anti-TNF-α (anti-TNFα) therapies is a milestone in the treatment of AS. It is unusual that the 3 major anti-TNFα inhibitors (etanercept, infliximab, and adalimumab) did not show an effect on radiographic progression in patients with AS after 2 years of treatment.[19–21] The resistance of B cells to anti-TNFα therapies might explain why radiographic progression continued in AS after 2 years of treatment. Future studies need to explore whether a combination of rituximab and TNF blockers will achieve further improvement for AS or axSpA.

In patients with AS, only fibrous tissue was observed in the specimens. Inflammatory cell infiltrates were only observed in patients with nr-axSpA. This finding is consistent with a previous report.[8] Shichikawa[8] described the pathology of the SIJ in open biopsies of 5 patients with AS. All the cases showed subchondral granulation tissue, but did not show inflammatory cells. In this study, we found that fibrous tissue contained not only abundant blood vessels but also a large amount of infiltrated inflammatory cells. Interestingly, the mean BME score on MRI was significantly higher in patients with fibrous tissue than in patients who only showed inflammatory cell infiltrates. It seems that fibrous tissue might be correlated with the BME on MRI.

There are some limitations to this study. First, the sample size of this study is small, because most of the remaining needle biopsy material is not sufficient for the immunohistological analysis. The location of the SIJ is poorly accessible; thus, it is very difficult to obtain a large piece of tissue. Second, the nr-axSpA patients in this study were lost to follow-up. Only 2 of them had progressed to AS after 5 years.

In conclusion, the present study confirms that in addition to macrophages and T cells, B cells are also involved in active sacroiliitis in patients with axSpA. Fibrous tissue with abundant inflammatory cells infiltration might be correlated with the BME on MRI.

Table 3

Cellular infiltrates of sacroiliitis.

Cellular infiltrates	Fibrous tissue (n = 15)	Mononuclear cells infiltrates (n = 6)
Macrophages, n (%)	15 (100.0)	6 (100.0)
B cells, n (%)	9 (69.2)*	5 (83.3)
T cells, n (%)	4 (30.8)*	3 (50.0)

* Due to the limited biopsy material, only 13 of the 15 specimens that contained fibrous tissue could be stained.

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