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Changes in C-reactive protein, erythrocyte sedimentation rate, human leukocyte antigen-B27, and immunoglobulins A, G, M in patients with ankylosing spondylitis

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ABSTRACT

Background: To explore the changes in C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), human leukocyte antigen-B27 (HLA-B27), and immunoglobulin (Ig) (IgA, IgG, and IgM) in patients suffering from ankylosing spondylitis (AS).

Methods: A total of 232 patients with axial spondyloarthritis admitted from January 2020 to December 2021 were selected, among whom 132 were AS patients (AS group) and 100 were non-AS cases (non-AS group). Meanwhile, 100 healthy subjects undergoing physical examination were included as a control group. Peripheral blood was collected to detect the levels of CRP, ESR, HLA-B27, IgA, IgG, and IgM.

Results: The positive rates and levels of CRP, ESR, HLA-B27, IgG, and IgM in non-AS and AS groups were significantly higher, and the IgA level of the AS group was higher than those of the control group ($P<0.05$). The AS group had significantly higher positive rates and levels of CRP, ESR, HLA-B27, and IgA than those of the non-AS group ($P<0.05$). The levels of HLA-B27, ESR and CRP had positive correlations with those of IgA, IgG and IgM ($P<0.05$). The area under the curve (AUC) of HLA-B27 with a cut-off value of 53.18 U/mL for the diagnosis of AS was 0.879. AUC of ESR with a cut-off value of 23.83 mm/h for the diagnosis of AS was 0.811. AUC of CRP with a cut-off value of 14.35 mg/L for the diagnosis of AS was 0.745. With the cut-off values of 2.75 g/L, respectively, AUC of IgA for the diagnosis of AS were 0.668. AUC of the combined diagnosis was 0.929 ($P<0.05$).

Conclusions: AS patients have abnormally elevated levels of HLA-B27, ESR, CRP, and IgA, and the combined detection has a higher diagnostic value. The abnormal increase in IgA, IgG, and IgM is positively correlated with rheumatic inflammation marker HLA-B27, CRP, and ESR.

Keywords: ankylosing spondylitis, C-reactive protein, erythrocyte sedimentation rate, human leukocyte antigen-B27, immunoglobulin

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INTRODUCTION

As chronic arthritis mediated by autoimmune factors, ankylosing spondylitis (AS) has a high mutilation rate. The lesions primarily involve the axial joints such as the spine and the attachment points of the muscles and ligaments, mainly manifested as spinal swelling and pain, and stiffness, showing a strong genetic susceptibility (1,2). Though the pathogenesis of AS remains to be clarified, it is now well-accepted that the type 17 immune response essentially participates in the occurrence and development of AS. Human leukocyte antigen-B27 (HLA-B27) with abnormal expressions in AS and close correlations with the occurrence of AS is a key reference index for the diagnosis of AS (3). Moreover, one of the pathogenic factors of AS is bacterial infection. As a recognized marker of bacterial infection, C-reactive protein (CRP) can activate the complement and enhance the phagocytosis of macrophages to exert an immune function (4). AS patients also suffer from an abnormal increase in CRP (5). Additionally, erythrocyte sedimentation rate (ESR) has been commonly utilized to diagnose rheumatic diseases, with an upward trend in AS patients. As an important index in humoral immunity and an immune inflammatory factor, immunoglobulin (Ig) is found to have correlations with the occurrence of AS (6,7). Nonetheless, the abnormality of the above factors can also be triggered by other non-AS pathological and physiological changes. Thus, the changes in CRP, ESR, HLA-B27, IgA, IgG, and IgM in AS patients were explored and their diagnostic value was evaluated in this study, aiming to provide a reference for AS diagnosis and further treatment.

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MATERIALS AND METHODS

Subjects

This study was approved by the medical ethics committee of our hospital, and all patients signed the informed consent. A total of 232 patients with axial spondyloarthritis (SpA) admitted from January 2020 to December 2021 were selected. Inclusion criteria: Patients who were 1) diagnosed based on the diagnostic criteria for SpA proposed by the American College of Rheumatology/American Spondylitis Association (ASAS) in 2019 (8), and 2) aged ≥18 years old. Exclusion criteria: Patients who 1) were complicated with other autoimmune diseases or endocrine and metabolic diseases, 2) were complicated with other chronic inflammatory diseases like hypertension, cardiovascular and cerebrovascular diseases, 3) had malignant tumors or were pregnant or lactating women, or (4) had a recent history of glucocorticoid use or surgery. There were 135 males and 97 females who were aged 18-44 years old, (28.52±6.56) years on average.

In addition, 100 healthy subjects [58 males, 42 females, aged 18-80 years old, averaging (41.74±11.99) years] were enrolled as a control group. SpA and control groups had comparable gender ratio and age (P>0.05).

Sample collection

Fasting peripheral venous blood was collected from every subject (6 mL). Specifically, 3 mL was placed in a test tube containing K3EDTA anticoagulant for flowcytometry and 3 mL were placed in an anticoagulant-free test tube, and the upper serum was harvested and stored in a -20°C refrigerator for later detection after 30-min agglutination and 10-min centrifugation at 3000 rpm.

Sample detection

A flow cytometer (Attune NxT, Thermo Fisher, USA) was used to perform flow cytometry for the expression of HLA-B27 in peripheral blood lymphocytes. First of all, peripheral blood lymphocytes were collected through gradient centrifugation of blood samples and then they were incubated with PE-labeled mouse anti-human HLA-B27 monoclonal antibody at 4°C for 30 min. Afterwards, the direct fluorescence assay was used to detect the expression rate of membrane-type HLA-B27. Moreover, after the cryopreserved serum samples were taken out and thawed at room temperature, the CRP and HLA-B27 serum levels were measured with the enzyme-linked immunosorbent assay following the manufacturer's instructions (MyBioSource, USA), while their concentrations were also detected in standard samples. The ESR detection was performed by NF-9910 (Olabor, Shandong, China). The IgA, IgG, and IgM levels in the serum were measured with rate turbidimetry and determined with an automatic biochemical analyzer (AU600, Olympus). Relevant test kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. The normal reference range was as follows: CRP<10 mg/L, ESR<20 mm/h, 0.71-3.85 g/L (IgA), 7-16.6 g/L (IgG), and 0.4-3.45 g/L (IgM). Values outside the normal reference range were identified to be positive.

Diagnosis of AS

In accordance with the classification criteria of ASAS for axial SpA, patients younger than 45 years old, who had had the symptoms of low back pain for 3 months or longer and met any of the following conditions, were diagnosed as AS: 1) Imaging examinations suggested sacroiliitis with 1 or more SpA characteristics; 2) with positive HLA-B27 expression and two or more SpA characteristics.

SpA characteristics: 1) Arthritis; 2) inflammatory back pain; 3) enthesitis (Achilles tendon); 4) ophthalmitis; 5) finger (toe) inflammation; 6) ulcerative colitis or Crohn's disease; 7) psoriasis; 8) family SpA history; 9) highly responsive to non-steroidal anti-inflammatory drugs; 10) positive HLA-B27 expression; 11) elevated CRP level. The SpA cases which met the diagnostic criteria for AS were assigned to the AS group (n=132), and the remaining cases were assigned to the non-AS group (n=100).

Statistical analysis

SPSS 20.0 software was used to carry out statistical analysis. The measurement data and count data were expressed as (± s) and [*n* (%)], and detected by the ***t*** test and χ^2 test, respectively. Pearson correlation analysis was conducted. Analysis of variance was performed to compare the count data of multiple groups, and pairwise comparison was carried out using the LSD-t test. The efficiencies of these indices for diagnosing AS were determined by plotting receiver operating characteristic (ROC) curves, and a higher efficiency was reflected by a larger area under the curve (AUC). GraphPad Prims software was used for plotting. A statistically significant difference was defined by P<0.05.

RESULTS

Positive rates of CRP, ESR, HLA-B27, IgA, IgG, and IgM

The positive rates of CRP, ESR, HLA-B27, IgG, and IgM in non-AS and AS groups were significantly higher than those of the control group (P<0.05). Besides, the positive rates of CRP, ESR, HLA-B27, and IgA in the AS group

were significantly higher than those of the non-AS group (P<0.05) (**Table 1**).

Levels of CRP, ESR, HLA-B27, IgA, IgG, and IgM

The levels of CRP, ESR, HLA-B27, IgA, IgG, and IgM in non-AS and AS groups were significantly higher than those of the control group (P<0.05). The AS group had significantly higher levels of CRP, ESR, HLA-B27, and IgA than those of the non-AS group (P<0.05) (**Table 2**).

Correlation analysis of HLA-B27, ESR and CRP levels with IgA, IgG and IgM levels

Pearson correlation analysis revealed the positive correlations of HLA-B27, ESR, and CRP levels with IgA, IgG, and IgM levels (P<0.05) (**Figure 1** and **Table 3**).

Values of CRP, ESR, HLA-B27, IgA, IgG, and IgM for diagnosis of AS

HLA-B27, ESR, CRP, and IgA had the optimal cut-off values of 53.18 U/mL, 23.83 mm/h, 14.35 mg/L, and 2.75 g/L, respectively, corresponding to the AUC values for the diagnosis of AS of 0.879, 0.811, 0.745, and 0.668, respectively. The AUC value of combined diagnosis was 0.929, significantly higher than those of each single index (P<0.05) (**Table 4** and **Figure 2**).

DISCUSSION

Severe chronic pain in the sacroiliac and spinal joints can be caused by AS. In the advanced stage, AS may lead to spinal rigidity or even deformity and result in disability, seriously affecting the patient's mobility. In addition, it can also increase the risks of iritis, osteoporosis, spinal compression fractures, and cardiovascular diseases, putting a serious burden on the health and life quality of patients (9,10). Due to the insidious onset, unclear pathogenesis and complex clinical manifestations of AS, early diagnosis and treatment are difficult. Hence, the exploration of serological markers that are easy to access, simple to operate, as well as highly reproducible and sensitive is of great significance to improving the diagnosis accuracy of AS.

The results of this study revealed that the abnormal increase in CRP, ESR, HLA-B27, and IgA was associated with the occurrence of AS. The immune regulation disorders in AS patients are found to be associated with the imbalance of multiple co-stimulatory molecules (11). HLA-B27 antigen, an immune-genetic marker with certain familial inheritance, has a strong correlation with SpA-related diseases. The positive HLA-B27 rate of AS patients is as high as above 90% which markedly exceeds that in non-AS patients, demonstrating a high diagnostic value for AS (12,13). ESR is a manifestation of the sedimentation rate of erythrocytes under certain conditions. Normally, erythrocytes have relatively high specific gravity. Their sedimentation is naturally at a relatively low rate under the action of gravity, but accelerates in such cases as inflammation, rheumatic diseases, anemia, and surgical trauma (14,15). CRP, an acute-phase protein responding to inflammation, plays an immunomodulatory role and can participate in the classical pathway of complement activation by binding to C1q, eliminate immune complexes, and regulate immune responses. For this reason, AS patients are subjected to an increasing CRP level (16,17). It is now well-established that the severity of axial SpA can be assessed by ESR, CRP plus imaging examinations (18), so this method is more specific to AS cases than to non-AS ones. Of IgA, IgM, and IgG as important immune effector molecules, IgG has the highest content in serum and a long half-life, and can produce corresponding antibodies for immune regulation upon pathogenic microbial stimulation (19). As a short-phase acute immune inflammatory index, IgM is characterized

Table 1. Positive rates of indices in different groups [*n* (%)]

Group	HLA-B27	ESR	CRP	IgA	IgG	IgM
AS (*n*=132)	128 (96.97)$^{**\#\#}$	98 (74.24) $^{**\#\#}$	85 (64.39) $^{**\#\#}$	39 (29.55) $^{**\#}$	1 (0.76)	47 (35.61)**
Non-AS (*n*=100)	70 (70.00)**	50 (50.00)**	39 (39.00)**	15 (15.00)	4 (4.00)*	36 (36.00)**
Control (*n*=100)	6 (6.00)	7 (7.00)	2 (2.00)	10 (10.00)	0 (0.00)	3(3.00)
χ^2	203.174	103.983	94.128	15.651	6.221	39.116
P	<0.0001	<0.0001	<0.0001	<0.001	0.045	<0.0001

Compared to the control group, *P<0.05, **P<0.001; compared to the non-AS group, $^{\#}$P<0.05, $^{\#\#}$P<0.001.

Table 2. Levels of indices in different groups (±s)

Group	HLA-B27 (U/mL)	ESR (mm/h)	CRP (mg/L)	IgA (g/L)	IgM (g/L)	IgG (g/L)
AS (*n*=132)	65.06±18.97$^{**\#\#}$	25.14±8.22$^{**\#\#}$	14.37±7.91$^{**\#\#}$	3.22±1.15$^{**\#\#}$	1.84±0.59**	14.47±4.46**
Non-AS (*n*=100)	48.22±12.33**	19.20±5.01**	10.44±3.56**	2.69±0.90*	1.70±0.50*	13.40±4.01**
Control (*n*=100)	17.56±6.23	13.73±4.60	6.23±2.27	2.39±1.07	1.56±0.41	10.33±2.11
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Compared to the control group, *P<0.05, **P<0.001; compared to the non-AS group, $^{\#}$P<0.05, $^{\#\#}$P<0.001.

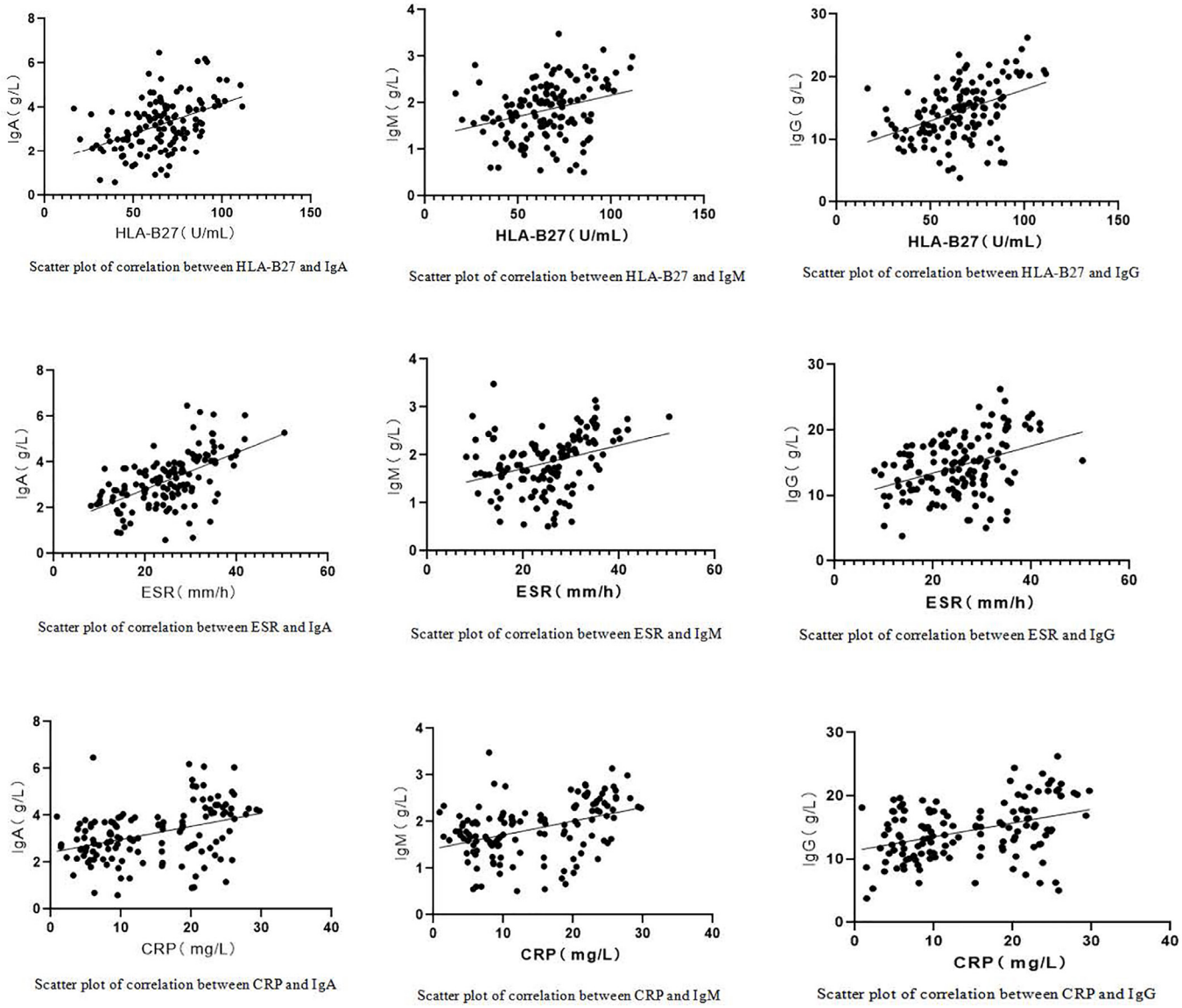

Fig. 1. Correlation analysis results of HLA-B27, ESR, and CRP levels with IgA, IgG, and IgM levels in AS patients.

Table 3. Correlation analysis results in AS patients

Index	IgA		IgM		IgG	
	r	P	r	P	r	P
HLA-B27	0.448	0.001	0291	0.085	0.426	<0.001
ESR	0.571	<0.001	0.334	<0.001	0.381	<0.001
CRP	0.383	<0.001	0.395	<0.001	0.386	<0.001

Table 4. Diagnostic efficiencies of indices for AS

Variable	Cut-off value	AUC	Sensitivity	Specificity	Standard error	P	95% CI
HLA-B27	53.18 U/mL	0.879	74.24%	84.50%	0.015	0.000	0.839~0.912
ESR	23.83 mm/h	0.811	62.88%	90.00%	0.026	0.000	0.765~0.852
CRP	14.35 mg/L	0.745	54.55%	95.00%	0.030	0.000	0.695~0.791
IgA	2.75 g/L	0.668	65.91%	59.50%	0.030	0.000	0.614~0.718
Combination		0.929	81.82%	89.50%	0.013	0.000	0.896~0.955

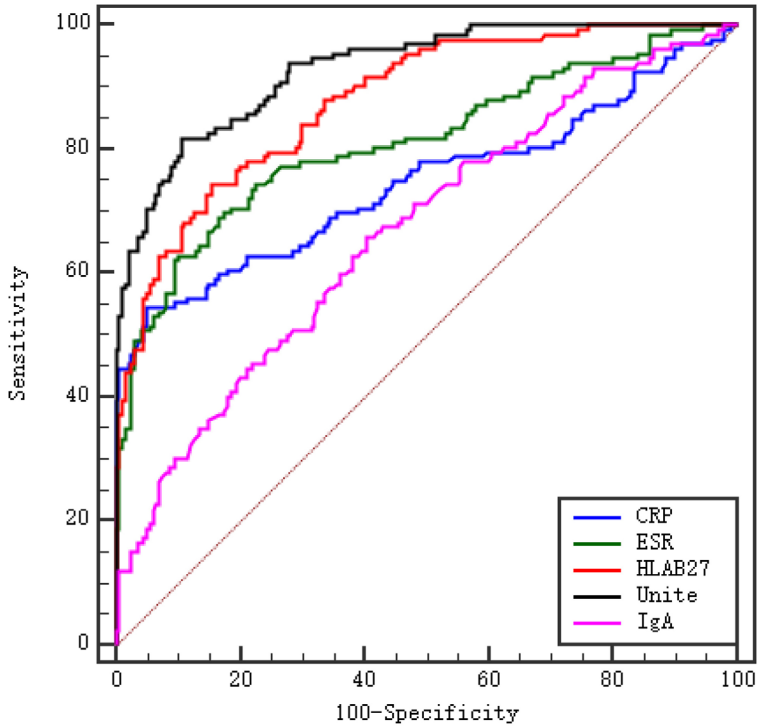

Fig. 2. ROC curves of HLA-B27, ESR, CRP, and IgA for diagnosis of AS.

by early emergence and short residence time (20). IgA, the content of which is second only to that of IgG in the blood, generally indicates autoimmune or mucosal diseases and serves as a crucial component of the mucosal defense (21). Due to the influence of inflammatory antigens, SpA patients have abnormal levels of IgA, IgM, and IgG, but these levels may not be highly specific to AS. The results of the correlation analysis conducted in this study revealed the positive correlations of HLA-B27, ESR, and CRP levels with those of IgA, IgG, and IgM. In other words, IgA, IgG, and IgM levels are strongly correlated with HLA-B27, ESR, and CRP that can be employed to diagnose AS and to reflect the disease severity, so they can serve as reference indices for the diagnosis of AS.

In this study, the combined diagnosis of AS had the largest AUC, indicating that the combined use of indices has the highest diagnostic efficiency. HLA-B27 has correlations with inflammatory changes and infectious diseases in various organs and systems, and has gene polymorphisms and regional differences (22). The diagnosis of AS by HLA-B27 alone may cause misdiagnosis, since the rising ESR level can also originate from other inflammatory and infectious diseases. Complex histocompatibility antigen correlations of AS have been reported, to which non-HLA-B27 HLA made a contribution (22). Moreover, ESR, and CRP, as well as IgA, IgG, and IgM, are not specific indices of AS, and they also present abnormal changes in major surgery, pulmonary tuberculosis, and other inflammatory diseases. The diagnosis of AS by a single factor may lead to incorrect or missed diagnosis. The combined detection of indices can more comprehensively reflect the pathophysiological changes, displaying a higher efficiency for the diagnosis of AS.

In conclusion, AS patients have abnormally elevated levels of HLA-B27, ESR, CRP, and IgA, and the combined detection has a higher diagnostic value. The abnormal increase in IgA, IgG, and IgM is positively correlated with rheumatic inflammation marker HLA-B27, CRP, and ESR. The findings provide valuable evidence for the timely diagnosis and treatment of AS.

AUTHORS' CONTRIBUTIONS

LZ and RC significantly revised the manuscript and designed this study; SZ, YL, ZY, and YT drafted the manuscript and performed this study. All authors have approved the submission and publication of this manuscript.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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