

## Original Article

# Changes of neutrophil-to-lymphocyte ratio and red blood cell distribution width in ankylosing spondylitis

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**Abstract:** Objectives: Neutrophil-to-lymphocyte ratio (NLR) and red blood cell distribution width (RDW) are increased in inflammation-associated diseases. However, a clinical evaluation of these markers in patients with ankylosing spondylitis (AS) has never been reported. In this study we analyzed the NLR and RDW in patients with AS, exploring their value in evaluating inflammation in AS. Methods: According to the New York AS standard revised in 1984, 74 newly-diagnosed AS patients who had received no previous medical treatment were recruited, along with 195 healthy controls who visited the hospital for regular physical examination. Parallel detection of laboratory indicators including NLR, RDW, high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), and other clinical markers was carried out for both groups. SPSS17.0 software was used for statistical analysis. Results: The NLR and RDW in AS patients was significantly higher than in healthy controls, and both were significantly positively correlated with hs-CRP and ESR. Conclusions: NLR and RDW can be used as metrics to evaluate the inflammatory activity in AS.

**Keywords:** NLR, RDW, Ankylosing spondylitis

## Introduction

Ankylosing spondylitis (AS), which is a subtype of spinal joint disease, is a chronic progressive inflammatory disease with the spine as the major lesion site. The most commonly involved joints are the sacral joint, the spinal joint, the soft tissue of the vertebral body and limb joints. AS affects both youths and adults, and the onset of the disease is usually occult and patients present with unstable symptoms. There is no effective treatment for this disease, therefore early diagnosis and treatment are particularly important [1]. The etiology and pathogenesis of AS have are not yet fully understood, and genetic, environmental and immunological factors all play a role in the occurrence of AS.

Neutrophil-to-lymphocyte ratio (NLR) is an easily assessable, repeatable and low cost immunological index, which reflects the oxidative stress and inflammatory state of the body. In recent years, NLR has become a widely used

inflammatory indicator, notably as an independent prognostic factor for coronary artery disease [2], with high levels of NLR increasing the risk of atrial fibrillation [3]. Studies have also shown that NLR can also be used as an index for tumor prognosis and evaluating the therapeutic effect of anti-cancer treatments [4-6]. In addition, NLR also has value for predicting disease recurrence [7]. Red blood cell distribution width (RDW) is a parameter for measuring the heterogeneity of red blood cell volume, which is commonly used in the differential diagnosis and classification of anemia along with mean corpuscular volume. Felker et al [8] firstly reported that RDW can be used as a prognostic indicator of heart failure in 2007, demonstrating that RDW is an independent prognostic factor for morbidity and mortality in patients with heart failure. Subsequent to this report, more studies have found that RDW is closely associated with the occurrence and development of cardiovascular diseases. There are also reports suggesting that RDW is associated with autoim-

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**Table 1.** General clinical characteristics

	AS group	Control group	P value
Cases	74	195	-
Age (year)	40.16±15.22	42.14±12.92	0.323
Gender (M/F)	54/20	104/81	0.012
Neutrophil (10 <sup>9</sup> /L)	4.48±1.45	3.41±0.81	< 0.001
Lymphocyte (10 <sup>9</sup> /L)	2.37±0.96	2.30±0.57	0.554
NLR	2.04±0.74	1.56±0.51	< 0.001
RDW (%)	12.87±1.14	12.26±0.53	< 0.001
hs-CRP (mg/L)	26.34±25.87	-	-
ESR (mm/h)	45.30±27.89	-	-

Note: data are presented as mean ± standard deviation. -, no data available, NLR, Neutrophil-to-lymphocyte ratio; RDW, Red blood cell distribution width; hs-CRP, high-sensitivity C-reactive protein; ESR, Erythrocyte sedimentation rate.

mune disease, liver disease, cancer, and other malignancies [9-11]. Although the molecular mechanisms of the involvement of RDW in these diseases remain unclear, we hypothesize that inflammation may be the key factor linking the two.

Besides HLA-B27, there is no other specific evaluation index in the experimental diagnosis of AS patients. As novel inflammation indexes, NLR and RDW are risk factors of many diseases, and may have value in the assessment of disease activity and severity for AS. The current study analyzed NLR and RDW in AS patients, exploring their value and application to the evaluation of inflammation in AS patients.

### Materials and methods

#### Study subjects

All 74 subjects were patients admitted to the Department of Rheumatology of Qilu Hospital affiliated with Shandong University (Qingdao, China) from Jan. to Dec. 2015. All patients gave written informed consent for participation in the study. The study cohort included 54 male patients and 20 female patients, with ages ranging from 14 to 71 years old who were newly diagnosed with AS and had not received any drug treatment. Additional inclusion criteria included: (1) meeting the New York criteria of AS modified in 1984; (2) exclusion of other autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren syndrome, and inflammatory enteritis; (3) exclusion of liver disease, such as hepatitis or

liver cirrhosis; (4) exclusion of malignant tumors, end-stage renal disease, diabetes, hypertension and other cardiovascular diseases; (5) exclusion of blood system diseases or recent recipients of blood transfusion. The 195 healthy controls were recruited from the hospital health examination center, with the aforementioned diseases excluded as well. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards.

#### Determination methods

Peripheral venous blood was collected from AS patients and healthy controls, and analyzed within 2 hours. NLR and RDW detection was conducted using a Sysmex XN 3000 automated hematology analyzer (Sysmex Corporation of Japan, Kobe, Japan) using reagents purchased from the manufacturer, and hs-CRP analysis was carried out with a DELAT special protein analyzer (SEAC, Italy), using reagents purchased from Shanghai XIYAKE Diagnosing Necessities Co. (Shanghai, China). ESR was measured using a TEST1 automatic sedimentometer analyzer (Alifax, Polverara, Italy).

#### Statistical analysis

Using SPSS 17.0 software (IBM, Armonk, NY, USA) for statistical analysis, data are presented as mean ± standard deviation. Between-group comparisons were carried out using the Student's t-test; count data were analyzed using chi square test. Pearson correlation analysis was used to analyze the correlation of NLR and RDW with hs-CRP and ESR.  $P < 0.05$  was considered statistically significant.

### Results

#### Comparison of NLR and RDW between AS patients and healthy controls

The neutrophil count in the AS group was significantly higher than the healthy control group ( $P < 0.001$ ), and there was no statistically significant difference in lymphocyte count between the two groups ( $P = 0.554$ ; **Table 1**). The NLR of

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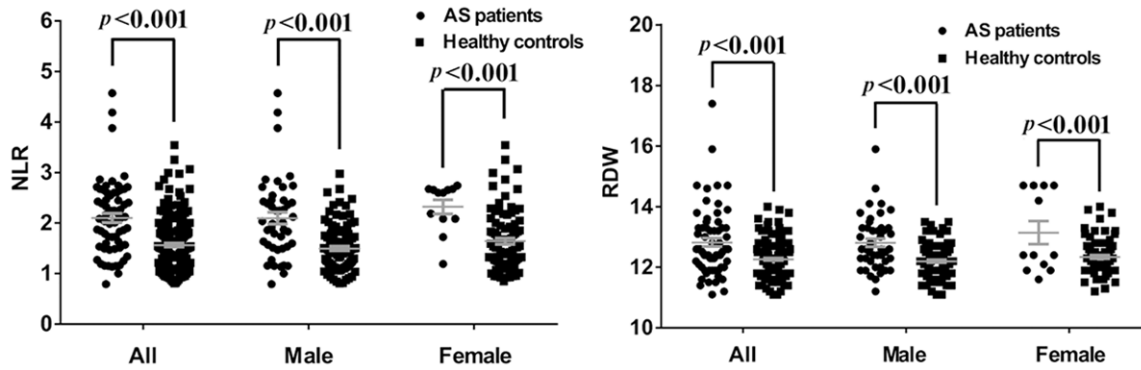


Figure 1. Comparison of NLR and RDW in AS patients and healthy controls.

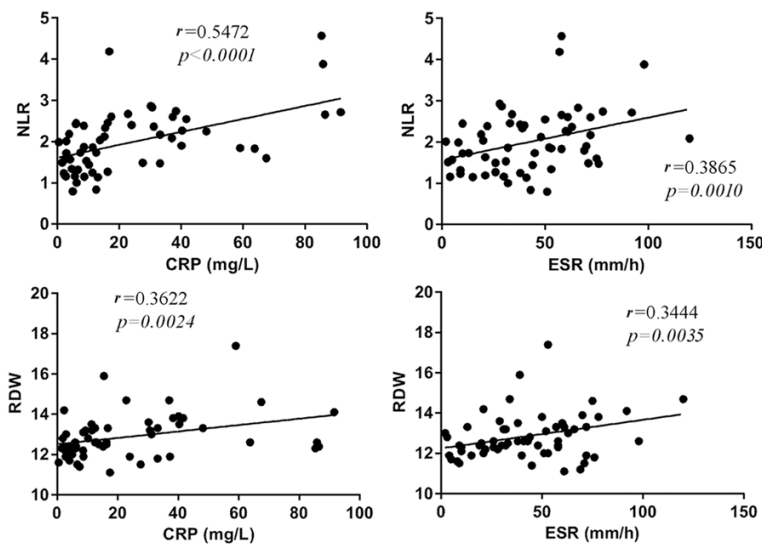


Figure 2. Correlation analysis between NLR and RDW, and hs-CRP and ESR.

the AS group ( $2.04 \pm 0.74$ ) was significantly higher than the healthy control group ( $1.56 \pm 0.51$ ;  $P < 0.001$ ); the RDW in the AS group ( $12.87 \pm 1.14$ ) was significantly higher than in the healthy control group ( $12.26 \pm 0.53$ ;  $P < 0.001$ ; **Figure 1**).

### Correlation analysis between NLR and RDW, and hs-CRP and ESR

The classic inflammation markers hs-CRP and ESR are the current means of measuring inflammation in patients with AS. We further analyzed the correlation between NLR and RDW, and hs-CRP and ESR. The Pearson correlation analysis showed that, in the AS group, NLR had a significant positive correlation with hs-CRP and ESR ( $r = 0.5472$ ,  $P < 0.0001$ ;  $r = 0.3865$ ,  $P = 0.001$ , respectively; **Figure 2**); RDW also showed a significant positive correlation with hs-CRP and

ESR ( $r = 0.3622$ ,  $P = 0.0024$ ;  $r = 0.3444$ ,  $P = 0.0035$ , respectively; **Figure 2**).

### Discussion

AS is a chronic serological negative rheumatic disease primarily affecting the spinal joint that seriously endangers human health. Clinical diagnosis of AS largely depends on abnormal X-ray scans; HLA-B27 can also be used for diagnosis, but more than 10% of AS patients are HLA-B27 negative [12]. ESR and hs-CRP are elevated in patients with many different diseases. We tested whether NLR and RDW could be used as an

index of AS activity in patients. In this study, we included only newly diagnosed AS patients, to exclude the effects of drugs and other diseases. The results showed that the levels of NLR and RDW in AS patients were significantly higher than in healthy controls, and that both had a significant positive correlation with hs-CRP and ESR, which are also indicators of the inflammation status of AS.

White blood cell count is often used as an indicator of inflammation in clinical practice. However, sometimes White blood cell is within the normal range, while the NLR has changed. As an easily assessable laboratory indicator, NLR can be used to assess systemic inflammation, and predict the mortality risk in patients with cardiovascular diseases [13]. As a marker of inflammation, NLR has two major advantag-

es: 1) although various physiological conditions may affect a wide variety of absolute blood count values, the impact on NLR is very limited; 2) as a ratio of two different immune characteristics, changes in NLR are mainly caused by inflammation and lymphopenia [14]. Our study found that in both men and women the NLR in patients with AS was significantly higher than the healthy control group ( $2.04 \pm 0.74$  and  $1.56 \pm 0.51$ , respectively;  $P < 0.001$ ), which was consistent with the results of Gokmen et al [15]. They also found that NLR level in patients receiving anti-inflammatory treatment was significantly lower than those who had not been treated. Thus, NLR can indeed reflect the inflammatory state of AS patients.

RDW reflects the individual differences in red blood cells, presented as the variation coefficient of the size of the red cell, with a greater value suggesting bigger differences. Recently, several studies have found that RDW is associated with inflammatory diseases, such as inflammatory bowel disease, Behcet's syndrome, Sjogren's syndrome, and rheumatoid arthritis [16-19]. RDW is therefore thought to be a potent inflammatory marker. Since the life cycle of a red blood cell is about 120 days, it is less affected by external environmental factors, which may make it more effective in evaluating chronic inflammatory states. We found that the RDW value of AS patients was higher than healthy controls, suggesting that RDW can be used as a laboratory indicator of AS.

Hs-CRP and ESR have become the standard indicators of inflammatory activity for a variety of diseases. Hs-CRP is an acute phase glycoprotein produced by the liver [20] as part of the inflammatory response to plasminogen released from sites of trauma or infection. Therefore, hs-CRP is a marker of acute responses to bacterial infection or injury. In cases of inflammation or tissue necrosis, serum hs-CRP concentrations rise rapidly, but are seldom affected by other factors. In contrast, ESR is a more general indicator of inflammation. In a study with a large sample size of outpatients, Lippi et al [21] found that RDW were positively correlated with hs-CRP and ESR, with similar reports for systemic lupus erythematosus and rheumatoid arthritis [9, 19]. As newly recognized indicators of inflammation, NLR and RDW have the advantage of less interfering factors

and a longer period of reflecting the state of inflammation. We found that, in patients with AS, NLR and RDW were significantly positively correlated with hs-CRP and ESR (**Figure 2**), suggesting that the combined detection of these two markers would effectively reflect the activity status of AS.

There were some limitations in this study. Due to the low incidence of AS in the region, the sample size was relatively small. Compared with the healthy control group, there were significant differences in the gender distribution ( $P = 0.012$ ) of our patient population. Further analysis, after grouping based on gender, found that NLR and RDW in AS patients were significantly different than in the healthy control group (**Figure 1**). In addition, all the RDW values were obtained from a single laboratory, minimizing the effects of the variation in different populations from different regions. NLR and RDW values were studied as representative markers for AS. We have not specifically evaluated whether these markers increase during disease progression. However, our research showed that the increase of NLR and RDW has a certain correlation with AS. An in depth study of the changes of NLR and RDW after individual treatment in AS patients will have important clinical significance for the evaluation of AS activity. In summary, measuring NLR and RDW is simple, economic, and non-invasive, and these two parameters are valuable indicators for monitoring AS activity in combination with hs-CRP and ESR.

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### Disclosure of conflict of interest

None.

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