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Hematological biomarkers in Ankylosing Spondylitis patients and its relation to disease activity

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Abstract

Background: Some hematological indices can be used in assessment of disease activity in autoimmune diseases as spondyloarthropathy
The aim of this work is to determine hematological changes in ankylosing spondylitis and their relationship to activity.
Patients and methods: The current study included 20 AS patients and 10 as control. The disease activity was evaluated using the Ankylosing Spondylitis Disease Activity Score (ASDAS). Various indices and characteristics related to complete blood counts were evaluated.
Results: A total of 20 AS patients and 10 age- and sex-matched controls were included. The median age of AS patients was 44 years, with 81.3% males, and a median disease duration of 7 years. ASDAS categories revealed 3 patients with inactive disease, 8 with very high disease activity (VHDA), and nearly equal numbers with low disease activity (LDA) and high disease activity (HAD) (5 and 4, respectively). Significant differences were observed between patients and controls in MCV, MCH, MCHC, MPV, RDW, MLR, NLR, and neutrophil and monocyte counts. ASDAS was significantly correlated with Hb, HCT, lymphocyte count, NLR, and PLR. When stratified by ASDAS levels, patients with HAD and VHDA had significantly different Hb, HCT, MCHC, NLR, PLR, ESR, and CRP compared to those with inactive disease or LDA.
Conclusion: The study highlights significant hematological and inflammatory differences between AS patients and controls, emphasizing the utility of markers such as NLR, PLR, and CRP in assessing disease activity. Stratification by ASDAS levels further demonstrates that patients with high and very high disease activity exhibit more pronounced alterations in these parameters, underscoring their potential role in monitoring disease severity and guiding management strategies.

Keywords

Hematological markers, Ankylosing spondylitis, ASDAS.

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Introduction:

Spondyloarthropathy is the immune-mediated condition that is characterized by systemic inflammation and functional affection of multiple organs, including joints (1). Ankylosing spondylitis is most commonly associated with chronic inflammatory arthritis (AS). Globally, the prevalence of ankylosing spondylitis (AS), a chronic inflammatory disease, ranges from 0.1% to 1.4% [1]. Although it can also affect peripheral joints and entheses, AS usually affects the axial spine and sacroiliac joints.
Along with skeletal involvement, AS also associated with extra-articular manifestations such as psoriasis (10%), acute anterior uveitis (25–35% of cases), and inflammatory bowel disease (IBD), which affects about 50% of cases(2)

As the disease progresses, these patients typically alternate between active and remission states. The Disease Activity Score and common laboratory indicators such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are factors that influence disease activity and the inflammatory milieu.(3)
The membership of the SpondyloArthritis International Society (ASAS) determined that the optimum way to assess the activity of AS illness was to use ASDAS with C-reactive protein (CRP), with ASDAS with erythrocyte sedimentation rate (ESR) as the backup option. This indicator has four additional self-reported elements in addition to the CRP or ESR score: back pain, peripheral pain or swelling, morning stiffness duration, and patient overall appraisal of disease activity.(4,5)

While activated platelets can set off a series of physiological and pathological reactions, releasing various platelet-derived proteins, chemokines, and growth factors that may lead to immune-inflammatory disorders, a mass activation of the immune system causes the overproduction of autoantibodies, immune complexes, and the progression of inflammatory cytokines, all of which interact to eventually cause disease onset (6,7).

A complete blood count (CBC) is a low-cost, easy, and relatively sensitive clinical indicator of inflammatory response. There is growing evidence that several autoimmune diseases can be accurately and reliably identified by bioinflammatory markers such as mean platelet volume (MPV), red blood cell distribution width (RDW) (8), and other metrics like neutrophil-to-lymphocyte ratio [NLR] (8). Additionally, the width-to-platelet ratio (RPR) of red blood cell distribution has been proposed as a quick and surrogate indicator.(9)

Patients& methods:

Study design: cross-sectional observational study. A convenient sample of patients was selected from the orthopedic department's outpatient clinic.

Study design and sample size: twenty AS patients participated in the study and a total of 10 healthy age- and sex-matched volunteers were included in this study as controls and All AS patients were diagnosed according to Modified New York criteria(10) ≥18 years of age.

Individuals with diabetes mellitus, liver/kidney diseases, cancer, other autoimmune diseases, infectious diseases, pregnancy or other inflammatory conditions were excluded.
The Local Ethics Committee of faculty of medicine-Tobruk University approved the study protocol. Before enrolling in the study, each participant signed an informed consent form. The Declaration of Helsinki's guiding principles were followed.

The sample size was determined using data from earlier autoimmune disease research. This cross-sectional study's sample size was determined using Epi-calc 2000. To detect odds ratio OR=2.5, assuming 80% power, 0.05 level of significance, and 60% of exposed individuals, the sample size will be 80. Taking into account the dropout rate of

Techniques

A thorough medical history and clinical examination were performed on each patient. Using the Ankylosing Spondylitis Disease Activity Score (ASDAS), disease activity was measured (11).Back pain, peripheral pain/swelling, duration of morning stiffness, and patient global assessment were all assessed on a numerical rating scale (from 0 to 10) according to the following formula:
ASDAS - CRP = 0.12 × Back Pain + 0.07
× Peripheral Pain/Swelling + 0.06 × Duration of Morning Stiffness + 0.11 × Patient Global + 0.58 × Ln (CRP + 1
The 3 cut-offs, selected to separate disease activity states, were as follows:
< 1.3 between "inactive disease" and "low disease activity"
< 2.1 between "low disease activity" and "high disease activity"
and > 3.5 between "high disease activity" and "very high disease activity."

Laboratory tests: Within two hours of being collected, blood samples were extracted and subjected to chemical analysis. The Coulter Counter mode was used to analyze the complete blood cell (CBC), which included the total white blood cell (WBC), platelet counts, MPV, PDW, RDW%, MCV, MCH, and the differential of neutrophil and lymphocyte counts.

Statistical analysis

The Statistical Package for the Social Sciences SPSS 22.0 program (IBM Microsoft) was used to analyze the data. Kolmogorov's test was utilized to assess the normality of quantitative data. Numbers and percentages were used to display the qualitative variables, and the chi-square test was employed for analysis. The Mann-Whitney U test was utilized to compare groups, and numerical variables were presented as medians (IQR). The relationship between clinical factors and laboratory data was assessed using Spearman's correlation analysis. A P-value of less than 0.05 was chosen as the significance level.

Results:

Twenty AS patients and ten persons as controls were included in the current study. The demographical, clinical characteristics and laboratory data of both groups are shown in Tables 1. The median age of the AS patients was 44 years, and the percentage of males in the

AS disease group was 81.3% with age- and sex-matched controls. The median disease duration of the patients was 7 years. As regards ASDAS, 3 patients had inactive disease while 8 had VHDA, with nearly equal numbers of LDA and HAD (5, 4 respectively). Also, Table 1 presents the laboratory findings of the studied groups. There was a significant difference between the patients and controls as regards MCV, MCH, MCHC,MPV, RDW, MLR, NLR, neutrophil & monocyte count. Moreover, there was a statistical correlation between ASDAS on one side and Hb, HTC, lymphocyte count, NLR, PLRon the other side (Table 3). When the patient group was divided into 2 subgroups based on ASDAS, with patients with inactive and LDA in one subgroup and those with HAD and VHDA in another subgroup, there was a significant difference as regards Hb, HTC, MCHC, NLR, PLR, ESR, and CRP levels.

Table 1: demographic, clinical, and laboratory data in both studied groups

	Patients group(20)	**Control group(10)**	**P value**
Age	44 (34.5–54)	45 (30.2–54)	0.567
SexFemale **Male**	**6** **14**	**4** **6**	0.814
Duration of disease	**7(4-15)**	**-**	
BMI (kg/m2)	26.90±4.80	27.12±4.94	0.006
CRP (mg/L)	8.03 (3.14–9.80)	2.22 (1.44–2.33)	<0.001
ESR (mm/h)	20 (11.5–29)	8 (5–11)	<0.001
Hb	**12.3 (11.5–12.9)**	**12.5 (11.2–13.5)**	**0.331**
HTC	**38.3 (35.2–42.5)**	**39.3 (35.3–42.4)**	**0.556**
MCV	**80.4 (77.5–88.7)**	**86.6 (82.5–89.9)**	<0.001
MCH	**26.5 (24.0–29.6)**	**28.9 (25.2–28.3)**	<0.001
MCHC	**32.4 (30.7–35.0)**	**34.3 (32.7–33.5)**	**< 0.001**
MPV	**9.9 (9.4–10.8)**	**11.8 (9.6–11.9)**	**< 0.001**
RDW	**13.9 (11.9–15.7)**	**13.2 (10.9–13.3)**	**< 0.001**
RBCs	**4.9 (4.3–5.2)**	**4.6 (4.3–4.9)**	**0.002***
Neutrophil count (´109/L)	3.05±0.89	1.14±0.95	**< 0.001**
Lymphocyte count (´109/L)	2.88±0.39	2.67±0.59	0.749
Monocyte count (´109/L)	0.62±0.29	0.39±0.64	<0.001
Platelet count (´109/L)	277.80±67.55	261.34±54.46	0.015
NLR (´109/L)	2.35±0.79	1.52±0.62	<0.001
PLR (´109/L)	117.64±44.63	123.36±33.97	0.03

Values are presented as number (%) and median (IQR). *BMI* body mass index, *WBCs* white blood cells, *HCT* hematocrit, *RBCs* red blood cells, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *RDW* red cell distribution width, *PLT* platelets, *MPV* mean platelet volume, *PDW* platelet distribution width, *NLR*neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio.

*** Significant at *P* < 0.05**

Table 2 Correlation of laboratory parameters with disease-related variables in patients group:

Laboratory parameters	ASDAS	ESR	CRP
HB	**- 0.332****	**- 0.332****	**0.184**
HTC	**- 0.276***	**- 0.233***	**0.134**
MCV	**- 0.122**	**- 0.085**	**- 0.255***
MCH	**- 0.132**	**- 0.193**	**- 0.234***
MCHC	**- 0.143**	**- 0.222***	**- 0.041**
MPV	**0.098**	**0.193**	**- 0.043**
RDW	**0.111**	**0.132**	**0.156**
RBCs	**- 0.154**	**- 0.143**	**0.220***
Platelet count	**0.154**	**0.154**	**0.155**
Neutrophil count	**0.129**	**0.104**	**0.294****
Lymphocyte count	**- 0.283****	**- 0.155**	**0.132**
Monocyte count	**- 0.044**	**- 0.043**	**0.022**
NLR	**0.393****	**0.176**	**0.085**
PLR	**0.392****	**0.275****	**- 0.092**

Values represent Spearman's rho correlation coefficient
***WBCs* white blood cells, *HCT* hematocrit, *RBCs* red blood cells, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *RDW* red cell distribution width, *PLT* platelets, *MPV* mean platelet volume, *PDW* platelet distribution width, *NLR* neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio.**
*** Significant at $P < 0.05$**
**** Significant at $P < 0.01$**

Table (3): Comparison of laboratory parameters in patients regarding activity score

	<2.1	Between 2.1-3.5	P value
HCT (%)	**37.7(35.4–39.9)**	**37.7 (35.4–39.9)**	0.003*
RBCs(1012/L)	**4.98 (4.49–5.32)**	**4.36 (4.15–5.06**	0.039*
Hb(g/L)	**13.9 (11.4–14.6)**	**12.2 (10.5–13.2)**	<0.001*
MCV (fL)	**82.6 (78.6–87.4)**	**80.9 (75.8–86.1)**	0.192
MCH (pg)	**27.9 (26.0–29.5)**	**26.75 (24.6–28)**	0.017*
RDW%	**13.5 (12.7–15.5)**	**14.4 (13.2–15.8)**	0.135
MPV	**9.2 (8.2–10.6)**	**9.9 (8.6–11.0)**	0.423
WBCS	**6.8 (5.2–9.3)**	**6.1 (5.1–7.8)**	**0.663**
NEUT	**3.5 (2.5–4.8)**	**3.6 (2.8–5.1)**	**0.237**
Lymphocytes	**2.5 (1.8–2.9)**	**1.9 (1.5–2.4)**	**0.033***
PLT	**244.0 (201.0–292.0)**	**264.5 (208.0–322.0)**	**0.239**
NLR	**1.42 (1.02–1.94)**	**1.92 (1.45–2.66)**	**0.004***
PLR	**98.18 (74.15–110.56)**	**137.55 (84.97–171.60)**	**0.005***

Values are presented as median (IQR)
WBCs white blood cells, *HCT* hematocrit, *RBCs* red blood cells, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *RDW* red cell distribution width,

NEUT neutrophils, *PLT* platelets, *MPV* mean platelet volume, *PDW* platelet distribution width, *NLR* neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio.
* Significant at $P < 0.05$

Discussion:

The combination of genetic, immunoregulatory, and ethnic variables leads to the etiopathogenesis of spondyloarthropathy. A number of important aspects of these multifactorial relationships remain unclear, with the severity of the disease being influenced by the peak of inflammatory status.(12)

Additional innovative biomarkers must be developed in order to track the progression of AS illness. Since CBC makes it simple to gather several hematological indicators, these markers appear potential for assessing disease activity in AS patients to obtain good management of the disease.(13,14)

In several disorders, combined hematological markers of inflammation—in particular, NLR and PLR—are employed extensively with positive outcomes. Additionally, the CBC values and their ratios can be used to predict the severity and prognosis of a number of inflammatory illnesses.(15)

The goal of this study is to evaluate the relationship between the different blood cell indices and hematological changes with the laboratory and clinical data of disease activity.

The results of the study revealed no significant differences between patient group and control, this demonstrated that the patients were correctly matched to the control group. But there was significant differences between both groups regarding laboratory parameters of activity (ESR&CRP), also, there was significant differences regarding MCH, MCV, MCHC, MPV, RDW, NLR with decreased levels of MPV, PLR and red blood cell parameters except for RDW, which was increased in the patient group. Anemia is a common manifestation of the chronic inflammation process and has also been observed in individuals with axial SpA and this may be explained by inhibitory effect of cytokine secretion. However, according to Liang T et al.'s findings [16], the AS group's PLR levels were noticeably greater than those of the non-AS group. The criteria used to choose the patients may be the cause of this disparity. Melek Sezgin et al. [17] observed that RDW was more significant in AS patients than in controls. The impact of anemia rather than a true inflammatory index might be the reason for elevated RDW.

In line with the findings of this study, multiple studies [18–22] have indicated that there is no significant difference in NLR between patients with AS and healthy individuals; similarly, Bozan et al. [23] observed comparable results. This lack of difference could be attributed to the different treatment approaches and criteria used to select AS patients for various research studies.

Also, in our study, there was a statistical correlation ($P< 0.01$) between ASDAS on one side and Hb, HTC, lymphocyte count, NLR and PLR on the other side. Similarly, Liang T. et al. [16] and Sariyildiz A. et al. [24] found a correlation between PLR, activity indices, and disease severity. Additionally, Wu J. et al. [25] as regard NLR, many studies [26, 27] have reported that there was a relationship between it and AS disease activity indices. Others, such as Al-Osami et al. [27]and Inal et al. [28], observed no correlation.

Currently, CBC indices such as NLR and PLR are commonly used as indicators of inflammation.

The current study showed that RBCs parameters, NLR, PLR, ESR, and CRP levels were significantly higher in AS patients with higher disease activity. The persistent inflammation that is seen in individuals with high disease activity can help to explain this observation. Similarly, Liang T et al. [16] found a correlation between PLR and NLR on one side and disease activity grading on the other side. Moreover, they observed that PLR was associated with the severity of AS and may be used independently to diagnose AS. Also, Al Osami et al. [28] found that NLR and PLR were significantly higher in the AS patients with active disease compared to those with inactive disease, even though there was no significant difference in the same parameters between AS patients and healthy controls. Inal et al. [29] and Kucuk et al. [27] also reported the same finding as regards NLR and PLR. Furthermore, Wu J et al. [25] reported that patients with active AS had significantly higher NLRand PLR levels than those in remission.

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