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Neutrophil-Lymphocyte Ratio and Platelat-Lymphocyte Ratio as Potential Markers of Disease Activity in Patients with Axial Spondyloarthritis

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ABSTRACT

Background: Both the platelet/lymphocyte ratio (PLR) and the neutrophil/ lymphocyte ratio (NLR) have the ability to serve as inflammatory biomarkers that indicate the presence of various inflammatory disorders.

Aim of the work: This study aimed to ascertain if NLR and PLR are novel biomarkers for axial spondyloarthritis (axSpA) and their connection to illness activity, and to assess how illness activity in axSpA patients relates to NLR and PLR.

Subjects and Methods: This research was carried out on 72 Axial SpA patients who were enrolled from Inpatient and Outpatient Clinics in the Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig university Hospitals' over a six-month period from February to August 2022.

Results: NLR and PLR were statistically substantially greater in active instances compared to non-active instances. Also, in the examined instances, there was a statistically substantial positive connection between NLR and PLR, BASDAI, BASFI, and CRP. ROC analysis and validity of NLR, PLR showed that NLR had significant validity for differentiation between active and non-active cases. PLR had significant validity for differentiation between active and non-active cases at cut off 113.6 with sensitivity 61.1%, specificity 72.2% and accuracy 66.7%.

Conclusion: Our results revealed that NLR and PLR values were linked with disease severity levels, with the median of NLR and PLR being greater in axSpA patients in the active group. Thus, in axSpA patients, they are reliable indicators of inflammation and disease severity.

Key words: Disease activity, Neutrophil/Lymphocyte ratios, Platelet/Lymphocyte ratios, possible indicators.

INTRODUCTION

An autoimmune condition called axial spondyloarthritis (axSpA) affects the major peripheral joints, (sacroiliac, and spine) and extra-articular tissues including the anterior uvea and aorta. Ankylosis of the facet joints and intervertebral discs results from the axial skeleton's creation of new bone, which reduces operational mobility. When the therapy is begun, it is essential to closely monitor its impact. Serial evaluation of an MRI scan is not practical for the objective assessment of inflammation, and this is essentially restricted to the patients' self-reported metrics for the intensity of spinal manifestations and the rates of the most explored as well as insensitive serological indicator C-reactive protein (CRP) [(1)].

Identifying of biomarkers linked to axSpA is a pressing unmet need [(2)]. Low density indicators such as CRP and erythrocyte sedimentation rate (ESR) primarily reflect acute inflammation. Immune cells are essential to the development of axSpA. Key inflammatory indicators of axSpA have been identified as neutrophil, NLR, monocyte, monocyte/lymphocyte ratio (MLR), platelet, and PLR [(3)]. Consequently, this study's objective was to ascertain how NLR and PLR function in axSpA patients and how well they may be used to gauge disease activity.

Because of their dependability and cost-effectiveness, CRP and ESR are often employed as markers to measure acute phase response in axSpA. Nonetheless, they are judged inadequate. In order to guide therapy and learn more about the disease progress. Even though they are often function as acute phase reactants (APR) and show action in axSpA, in around one-third of patients, their levels may still be within normal ranges [(1)].

SUBJECTS AND METHODS

A cross-sectional research, which was performed on 72 Axial SpA patients who were enrolled in the Rheumatology and Rehabilitation Department' Inpatient and Outpatient Clinics, Faculty of Medicine, Zagazig University Hospitals over six-month period from February to August 2022.

Inclusion criteria: Patients having axSpA depending on the ASAS classification criterion [(4)]. This requires a patient with persistent back pain and an age beginning from < 45 years to have either sacroiliitis on imaging plus one SpA feature (scanning arm) or HLA-B27 antigen plus two SpA characteristics (clinical arm). The individuals were above the age of 18, and they were split into active and inactive groups according to a cutoff value of 4, with the active group having a BASDAI of ≥ 4 and the inactive group having a BASDAI of < 4 [(5)].

Exclusion criteria: Those with autoimmune conditions such Systemic lupus erythematosus, Scleroderma, mixed connective tissue disorders, heart disorders, diabetes, kidney and liver illness, liver cirrhosis, cancer, and tuberculosis.

Patients with Ax-SpA had their demographic information, duration of illness, and BASDAI and BASFI scores recorded. Through laboratory analysis, the values of ESR (mm/hour), CRP (mg/dL), and

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albumin (g/dL) were established. Depending on an examination of the whole blood number, the neutrophil (K/μL), lymphocyte (K/μL), and platelets (K/μL) numbers were calculated. Additionally, PLR and NLR levels were determined by spliting, respectively, the neutrophil number by the lymphocyte number and platelet number by the lymphocyte number were calculated.

With a score range of 0 to 10, the BASDAI was employed to assess the intensity and duration of disease-specific symptoms including exhaustion, spinal and peripheral joint pain, edema, and morning tightness. Data from the two groups, which were split depending on BASDAI score (BASDAI ≥ 4 = great activity, <4 = poor activity), were compared.

Ethical Approval:

Each research participant provided written informed permission. The Institutional Review Board (IRB) Committee, Zagazig University Ethics Board approved this study. The Declaration of Helsinki, the World Medical Association's code of ethics, was followed while conducting this research on people.

Statistical analysis

The collected data were computerized and statistically analyzed utilizing the Statistical Package for Social Science (SPSS) version 27.0 (IBM, 2020). For the purpose of displaying qualitative data, frequencies and relative proportions were utilized. The chi square test was utilized to assess the variation between qualitative variables. The Mann Whiteny (MW) test was utilized to assess the variation between quantitative measures in two sets of non-normally dispersed data. The Kruskal-Wallis test was utilized to compare quantitative characteristics in more than two groups in non-normally distributed data. Utilizing Spearman's correlation coefficient, one may ascertain the relationship between two quantitative data. P value ≤ 0.05 is significant.

RESULTS

Seventy-two axSpA patients (42 males & 30 females) with mean age of 37.13 ± 9.14 were enrolled in this research. According to the International Society for the Assessment of SpondyloArthritis, axSpA was diagnosed (ASAS). It comprised 72 patients that were separated into two groups depending on the disease activity: Active group (BASDAI ≥ 4) had a median age of 38.72 ± 8.36, 19 (52.8%) females, and 17 (47.2%) males. The median of the illness duration was 7.64 ± 6.06. Although there were 36 patients in the non-active groups (BASDAI < 4), the mean age was 35.53 ± 9.71) and there were 11 women and 25 men, with a mean illness duration of 7.91 ± 5.72.

In terms of back pain, sacroiliitis, skin rash, anterior uveitis, GIT symptoms, or MRI findings, clinical and radiological data indicated that there were no statistically substantial distinctions between the researched groups. However, there was a statistically substantial rise in the frequency of peripheral joints and enthesitis among active cases compared to non-active as shown in table (1):

Table (1): Clinical data among the researched groups

Variable		Group I (Active cases) (*n*=36)		Group II (Non-active cases) (n=36)		χ^2	P
		No	%	No	%		
Back pain:	LBP	32	88.9	36	100		
	Neck pain	2	5.6	0	0	4.24	0.12
	LBP+ Neck pain	2	5.6	0	0		NS
Sacroiliitis:	Unilateral	8	22.2	2	5.6	2.90	0.08
	Bilateral	28	77.8	34	94.4		NS
Peripheral joints:	No	5	13.9	25	69.4	**22.86**	**<0.001**
	Yes	31	86.1	11	30.6		**
Skin rash:	No	35	97.2	35	97.2	0	1
	Yes	1	2.8	1	2.8		NS
Enthesitis:	No	18	50	32	88.9	**12.83**	**<0.001**
	Yes	18	50	4	11.1		**
Anterior Uveitis:	No	33	91.7	32	88.9	0.16	0.69
	Yes	3	8.3	4	11.1		NS
GIT Symptom:	No	34	94.4	35	97.2	0.35	0.56
	Yes	2	5.6	1	2.8		NS
MRI:	Not done	2	5.6	1	2.		
	Uni Sacroiliitis	6	16.7	1	2.8	4.49	0.11
	Bi Sacroiliitis	28	77.8	34	94.4		NS

χ^2: Chi square test NS: Non substantial (P > 0.05).*: substantial (P < 0.05) **: Highly substantial (P<0.001), LBP; low back pain. MRI: Magnetic Resonance Imaging, Uni: Unilateral, Bi: Bilateral.

According to BASDAI and BASFI score analysis we found that there were a statistical substantial higher in mean BASDAI and BASFI score among active subjects compared to non-active cases as shown in table (2).

Table (2): Disease activity score among the researched groups

Variable		Group I (Active cases) (n=36)	Group II (Non-active cases) (n=36)	MW	P
BASDAI:	Mean ± Sd	5.82±1.27	2.49±0.87	**7.31**	**<0.001****
BASFI:	Mean ± Sd	4.78±2.28	2.92±2.08	**3.38**	**0.001***

SD: Standard deviation, MW: Mann Whitney test *: substantial (P<0.05) **: highly substantial (P<0.001)

Neutrophil and platelet counts, as well as NLR and PLR, were statistically substantially higher in active instances of axSpA as compared to non-active instances (BASDAI ≥ 4 vs. BASFI < 4). Between active and non-active cases, there was a statistically substantial rise in mean CRP and fall in average albumin as shown in table (3).

Table (3): Labaratory findings among the studied groups

Variable		Group I (Active cases) (*n*=3	Group II (non-active cases) (n=36)	MW/t	P
Lymphocyte: ($x10^3/mm^3$)	Mean ± SD	2.72±0.03	2.86±0.96	0.84	0.40 NS
Neutrophil: ($x10^3/mm^3$)	Mean ± SD	5.75±0.56	4.72±0.81	**2.41**	**0.02***
Monocyte: ($x10^3/mm^3$)	Mean ± SD	0.87±0.10	0.5±0.17	1.62	0.11 NS
Platelets: ($x10^3/mm^3$)	Mean ± SD	336.28±9.34	288.17±8.95	**2.20**	**0.03***
NLR:	Mean ± SD	2.31±0.12	1.79±0.17	**2.75**	**0.006 ***
MLR:	Mean ± SD	0.35±0.06	0.20±0.01	0.80	0.42 NS
PLR:	Mean ± SD	136.17±5.77	115.24±8.02	**2.35**	**0.02***
ESR: (mm/h)	Mean ± SD	26.36±1.71	21.97±1.88	1.98	0.06 NS
CRP: (mg/dl)	Mean ± SD	14.09±3.05	6.85±0.4	**2.61**	**0.009***
Albumin:(gm/dl)	Mean ± SD	4.18±0.43	4.41±0.4	**2.35**	**0.02***

SD: Standard deviation t: Independent t test MW: Mann Whitney test

The association between NLR, PLR, disease activity, and labaratory markers showed that among the patients under study, there was a statistically substantial +ve connection between NLR, PLR, BASDAI, BASFI, and CRP as shown in table (4).

Table (4): Connection between NLR, PLR and age, duration, disease activity score, inflammatory markers and albumin among the studied cases

Variable	NLR (n=72)		PLR (n=72)	
	r	P	r	P
Age: (years)	0.02	0.88 NS	0.06	0.60 NS
Disease duration: (years)	0.30	0.81 NS	0.18	0.13 NS
BASDAI	**0.28**	**0.02***	**0.24**	**0.04***
BASFI	**0.35**	**0.003***	**0.26**	**0.03***
ESR: (mm/h)	0.07	0.57 NS	0.11	0.35 NS
CRP: (mg/dl)	**0.26**	**0.04***	**0.27**	**0.02***
Albumin: (gm/dl)	0.09	0.41 NS	-0.22	0.06 NS

r: Spearman's correlation coefficient

ROC analysis and validity of NLR, PLR shown that NLR had significant validity for differentiation between active and non-active cases at cut off 1.78 with sensitivity 75%, specificity 61.1% and accuracy 68.1%. PLR had significant validity for differentiation between active and non-active cases at cut off 113.6 with sensitivity 61.1%, specificity 72.2% and accuracy 66.7%.

DISCUSSION

AxSpA is a prevalent autoimmune and inflammatory illness that affects the axial skeleton. The involvement of neutrophils, platelets, monocytes, and lymphocytes in inflammation is becoming more and clearer, and as a result, the combinations of these variables, NLR, PLR, and MLR, have become new inflammation markers in a variety of inflammatory disorders, including axSpA [6, 7]. NLR, PLR, and MLR were reported to be considerably greater in axSpA patients compared to healthy controls in earlier research [8].

The examined groups did not vary statistically considerably in terms of smoking, age, employment, or sex distribution. Our findings are consistent with research of **Pamukcu and Duran**, [1] as they reported that regarding age, sex distribution, and illness duration, there was no substantial variation between the low active group and the elevated active group.

The current research revealed that there were no statistically substantial variations between the researched groups for back pain, skin rash, anterior uveitis, GIT symptoms, or MRI findings when it came to clinical and radiological data. However, there was a statistically significant rise in the frequency of peripheral joints and enthesitis among active cases compared to non-active. There was a statistical substantial rise in mean BASDAI and BASFI scores [(5.82±1,27) & (4.78±2.28) vs (2.49±0.87) & (2.92±2.08) respectively] among active cases compared to non-active cases. Our findings are in agreement with research of **Zhong *et al.*** [9] as they revealed that compared to the group that didn't participate the active group had considerably greater BASDAI and BASFI scores.

In the study in our hands, for active vs non-active patients, there was a statistically substantial rise in median CRP and fall in median albumin. This is in accordance with studies of **Zhong *et al.*** [9] and **Pamukcu and Duran** [1], which demonstrated that in high-active patients compared to low-active cases, there was a statistically substantial rise in median CRP and reduction in median albumin.

Our study revealed a statistically significant increase in neutrophil and platelets count and also in NLR and PLR among active compared to non-active cases. These outcomes support the conclusion of **Al-Osami *et al.*** [10] and **Pamukcu and Duran** [1] where they reported substantial variation in the neutrophil and platelet numbers, NLR and PLR, between the two groups of AS patients (BASDAI ≥ 4 vs. BASDAI < 4; P=0.002, p<0.001, p=0.001, p<0.001, respectively).

The current investigation demonstrated that NLR and the BASDAI, BASFI, and CRP, respectively, had statistically substantial positive correlations (r=0.28, p=0.02, r=0.35, p=0.003, and r=0.26, p=0.04) among the patients under study. PLR and BASDAI, BASFI, and CRP all showed statistically substantial positive connections (r=0.24, p=0.04, r=0.26, p=0.03, and r=0.27, p=0.02) among the patients under study. Our results are supported by study of **Inal *et al***. [11] where they found that in comparison of both healthy controls and patients with BASDAI ≥ 4, NLR and PLR were considerably greater in BASDAI < 4 patients (p <0.05). They also came to the conclusion that NLR and PLR may indicate disease severity in AS patients. The increased cytokine production in patients with greater levels of disease severity may be used to explain this. Since IL-17, IL-23, and TNF are known to tip the scales from neutrophils to lymphocytes, elevated NLR and PLR with high disease intensity may be the result.

Neutrophil count and platelet count, respectively, have an impact on NLR and PLR. On the one hand, acute inflammation causes a rise in neutrophil and platelet counts as part of the APR and in response to stress. As opposed to that, physiological stress causes a drop in the lymphocyte count. This offers a potential explanation for the elevated NLR, PLR, and ratios that were found to rise steadily with increasing degrees of severity. Additionally, it has been shown that IL-17 and IL-23 (important cytokines in the pathophysiology of AS) play a significant role in maintaining neutrophil homeostasis [3].

In the study of **Zeb *et al.*** [12] the active disease group's median NLR and PLR were noticeably greater (i.e., BASDAI score > 4; p< 0.05). No correlation between illness activity and ESR was found. In the research of **Al-Osami *et al.*** [10] comparing patients with active and inactive ankylosing spondylitis, there was a statistically substantial variation in the NLR and PLR (2.31 ± 1.23vs 1.77 ± 0.73, p = 0.002), (142.04 ± 70.98 vs 119.24 ± 32.49, p < 0.001 respectively). Additionally, the PLR was considerably greater in the active and inactive groups compared to the healthy control group (142.04 ± 70.98 vs 99.32 ± 33.97, p=0.014; 119.24 ± 32.49 vs 99.32 ± 33.97, p=0.019). The PLR and NLR were favorably linked with the BASDAI scores (r=0.219, p=0.012 and r=0.170, p=0.051, respectively), however the latter was not statistically substantial.

Also we found that NLR had significant validity for differentiation between active and non-active cases at cut off 1.78 with sensitivity 75%, specificity 61.1% and accuracy 68.1%. The AUC of NLR was 0.67, 95% & CI: 0.55-0.80. PLR had significant validity for differentiation between active and non-active cases at cut off 113.6 with sensitivity 61.1%, specificity 72.2% and accuracy 66.7%. The AUC of PLR was 0.65, 95% & CI: 0.52-0.78. **Al Osami *et al.*** [10] reported that the best PLR cut-off level was 95.9, with a sensitivity of 70.9% and a specificity of 55.5%. The best NLR cut-off value for detecting ankylosing spondylitis patients with significant illness activities was 1.66, with a sensitivity of 61.8% and a specificity of 50.6%.

This study had some limitations: This study's first drawback is that it was conducted at a single center

with a small sample size of patients. Second, since the research excluded other significant indices utilized in the assessment of illness activity in Ax-SpA (such as the ankylosing spondylitis disease activity score (ASDAS), ASDAS - ESR, and ASDAS - CRP), correlation calculations could not be performed. Finally, the treatment's impact on the NLR and PLR levels was not assessed. Additional comprehensive study must be conducted to assess the efficacy of NLR and PLR as an initial detection indicator of inflammation in Ax-SpA.

CONCLUSION

The active group of axSpA patients had greater median NLR and PLR values, and NLR and PLR values were linked with disease activity levels, according to our results. As a result, in axSpA patients, they are reliable indicators of inflammation and disease severity. **Conflicts of Interest:** no conflicts of interest.

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