Determination of neutrophil- and platelet-to-lymphocyte ratios in correlation with Disease Activity Score-28 in patients with rheumatoid arthritis

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□ ABSTRACT □

Aim: Rheumatoid arthritis (RA) is an inflammatory and autoimmune disease with unknown etiology and systemic involvement. Neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) are two new inflammatory markers used in the assessment of systemic inflammation. The aim here is to study NLR and PLR in patients with RA to investigate their relation with Disease Activity Score of 28 joints (DAS-28).

Methods: The present study was performed in the department of internal medicine-rheumatology, Tishreen university hospital. The study included 62 patients with RA and a control group of 18 age- and gender-matched healthy subjects. We divided the patients into two groups according to the DAS-28 score. Group 1 included patients with a score of lower than 2.6 by the DAS-28 (patients in remission) and Group 2 included patients with a score of 2.6 and higher (patients with active disease).

Results: NLR was 2.44±1.1 in the patient group and 1.61± 0.3 the control group. PLR was 147.9± 78.9 in the patient group and 111.8±19.9 in the control group. There was a statistically significant difference in each of NLR and PLR between the patient and control groups (P = 0.005 and P = 0.02, respectively). Patients in Group 1 had an NLR of 1.55±0.4 and a PLR of 96.1±30.7. Patients in Group 2 had an NLR of 3.59±0.8 and a PLR 211.1±74.5. There was a statistically significant difference in NLR and PLR between the two groups (P = 0.03 and P = 0.001respectively). A correlation was observed between NLR and PLR by DAS-28 ($r = 0.9$, $P \leq 0.0001$ and $r = 0.5$, $P = 0.02$, respectively). For the NLR, the area under the curve (AUC) of the ROC curve was 0.831; at the cut off value of 2.13, the diagnostic sensitivity and specificity were 76.7%, 75.9%, respectively.

Conclusions: The present study showed that NLR and PLR were two new inflammatory markers which could be used to assess disease activity in patients with RA.

Key words: DAS-28, neutrophil-lymphocyte, platelet-lymphocyte, ratio, rheumatoid arthritis.

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تحديد نسبة كل من العدلات والصفيحات إلى اللمفاويات وتقييم علاقتها بفعالية المرض لدى مرضى التهاب المفاصل الرثياني

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□ ملخّص □

مقدمة: الداء الرثياني مرض التهابي مزمن، متطور، مخرب، يصيب المفاصل وهو من أمراض المناعة الذاتية. تعد كل من نسبة العدلات إلى اللمفاويات "NLR" والصفيحات إلى اللمفاويات "PLR" مشعران التهابيان جديدان يستخدمان في تقييم الحالات الالتهابية الجهازية. تهدف دراستنا إلى تقييم النسب"NLR" و"PLR" لدى مرضى الداء الرثياني لتحري علاقتها بمشعر فعالية المرض ل28 مفصل "DAS28".

طريقة الدراسة: شملت الدراسة 62 مريض داء رثياني من مراجعي قسم الأمراض الباطنية – شعبة المفاصل في مشفى تشرين الجامعي، ومجموعة شاهد 18 أصحاء مطابقة بالعمر والجنس. تم تقسيم المرضى إلى مجموعتين وفقا لقيمة DAS28. تضمنت المجموعة الأولى المرضى الذين لديهم قيمة DAS28 < 2.6 (المرض في مرحلة الهداوة) بينما تضمنت المجموعة الثانية المرضى الذين لديهم قيمة DAS28 ≥ 2.6 (المرض في مرحلة فعالية).

النتائج: كانت قيمة NLR=2.44±1.1 لدى مجموعة المرضى و NLR=1.61±0.3 لدى مجموعة الشاهد . وقيمة PLR=147.9±78.9 لدى مجموعة المرضى وPLR=111.8±19.9 لدى مجموعة الشاهد. أظهرت النتائج وجود فرق هام إحصائيا في كل من NLR و PLR بين مجموعتي المرضى والشاهد (p=0.005 و p=0.02، على التوالي). كانت قيمة NLR لدى مرضى المجموعة الأولى NLR=1.55±0.4 و PLR=96.1±30.7. كانت لدى مرضى المجموعة الثانية NLR=3.59±0.8 و PLR=211.1±74.5. ظهر وجود فرق هام إحصائيا في الNLR و PLR بين مرضى المجموعتين (P=0.03 وp=0.0001 على التوالي). تم ملاحظة وجود علاقة ارتباط إيجابية بين كل من NLR و PLR مع الDAS28 (r=0.9, p=0.0001 و r=0.5 p=0.02 على التوالي).

الخلاصة: أظهرت الدراسة أن كلاً من NLR و PLR مشعرات التهابية جديدة يمكن استخدامها لتقييم فعالية المرض لدى مرضى الداء الرثياني.

الكلمات المفتاحية: مشعر فعالية المرض، العدلات إلى اللمفاويات، الصفيحات إلى اللمفاويات، نسبة، الداء الرثياني.

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Introduction

Rheumatoid arthritis (RA) is an inflammatory and autoimmune disease with unknown etiology and systemic involvement. Genetic predisposition and environmental factors contribute to the pathogenesis, diversity of clinical findings and activation of the disease.[1] The incidence of RA is approximately 0.5–1% of the general population world-wide, with a higher prevalence in females than males.[2,3] RA is characterized by symmetrical joint involvement, erosion and deformity in the joints as a result of the inflammation.[3,4] The levels of serum Inflammatory markers are usually elevated in the active period of the disease. The disease activity is usually assessed at baseline and during the treatment follow-up period by the Disease Activity Score of 28 joints (DAS-28) system, which is calculated by the number of tender joints, number of swollen joints, visual analogue scale (VAS), C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR).[3,4] Impaired immune system plays an important role in the activation and progression of the disease.[5,6] Parameters of hemogram, especially the immune system elements, have a significant place in the assessment of various diseases and/or hints of diseases. Some of the immune system elements, such as neutrophils, lymphocytes and platelets play a role in the control of inflammation, while also undergoing changes secondary to inflammation.[7,8,9] Recently, neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) came into use as the markers of systemic inflammation and were assessed in a great number of malignancy studies.[10,11,12] This study aims to use NLR and PLR in patients with RA to investigate their relation with DAS-28 used in the assessment of disease activity.

studying the records and archives of the hospital. Disease activity can be assessed by four criteria in DAS-28. Accordingly, the disease can be assessed for severe disease activity (> 5.1), moderate disease activity (3.2 to ≤ 5.1), low disease activity (2.6 to ≤ 3.2) and remission (< 2.6).[13] We divided the patients into two groups according to the DAS-28 system, group1 (patients in remission with DAS28 < 2.6) and group2 (patients with active disease DAS28 ≥ 2.6).

MATERIALS AND METHODS

The present study was performed between November 2018 and March 2020, and designed retrospectively. The study included 62 patients with RA, assessed by the diagnostic criteria of the American College of Rheumatology /European League Against Rheumatism (ACR/ EULAR) 2010 and a control group of 51 age- and gender-matched healthy subjects. The healthy subjects as control group were selected from hospital records. Hemogram, CRP, ESR, were obtained by analysing blood and serum of the patients and the control groups. VAS, DAS-28 and basic clinical characteristics of patients group were obtained by Rheumatology doctors in the department of internal diseases - tishreen university hospital.

We divided the patients into two groups according to the DAS-28 system. Group 1 included patients with a score lower than 2.6 by the DAS-28 system (patients in remission) and Group 2 included patients with a score of 2.6 and higher (patients with active disease). Patients with systemic diseases, such as diabetes mellitus, hypertension, coronary artery disease, chronic obstructive pulmonary disease, as well as patients who had been treated with corticosteroids within the last 3 months, were excluded and not included into the study at the beginning. The study protocol was in accordance with the Declaration of Helsinki.

Statistical analysis

The data analysis was performed using SPSS for Windows, version 20 (SPSS Inc., Chicago, IL, USA). The normality of distribution was checked by Kolmogorov–Smirnov test, and parametric or non-parametric tests were used on data according to normal or non-normal distributions. Data were expressed as means standard deviation. The Mann–Whitney U-test was used to compare the parameters and evaluate the correlations. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Patients had a median age of 56 years (range: 30–72), with a gender distribution of 47 women (75.8%) and 15 men (24.2%). The median age was 55 years in the control group (range: 46–60) with a gender distribution of 14 women (77.8%) and 4 men (22.2%). No statistically significant difference was observed in age and gender between the patient and control groups ($P = 0.6$ and $P = 0.8$, respectively). NLR was 2.44 ± 1.1 in the patient group and 1.61±0.3 in the control group. PLR was 147.9± 78.9 in the patient group and 111.8±19.9 in the control group. There was a statistically significant difference in NLR and PLR between the patient and control groups ($P =0.005$ and $P = 0.0001$, respectively). Table 1 summarizes NLR, PLR, CRP, ESR,RF and other parameters of hemogram for the patient and control groups. No statistically significant difference was observed in age and gender between Group 1 and Group 2 (Table 2). Patients in Group 1 had an NLR of 1.55±0.4 and a PLR of 96.1±30.7. Patients in Group 2 had an NLR of 3.59±0.8 and a PLR of 211.1±74.5. There was a statistically significant difference in NLR and PLR between the two groups ($P = 0.03$ and $P = 0.0001$, respectively). Table 2 presents NLR, PLR, ESR,CRP and RF for patients in Group 1 and Group 2. There was a statistically significant difference between Group 1 (patients in remission) and the control group according to NLR ($P = 0.032$). However, there was no statistically significant difference between Group 1 (patients in remission) and the control group. According to DAS-28 NLR and PLR were correlated (Table 3) (Fig. 1). For evaluating the diagnostic efficacy of the NLR for RA we used the Receiver operating characteristic analysis" ROC" curve.

Table 1 NLR, PLR, CRP, ESR and other laboratory parameters of the groups.

	Patient group (n = 62)	Control group (n = 18)	P-value
Hemoglobin, g/dL	12.3±1.01	12.6±0.6	0.2
Platelets, 10^9/L	294.09±219.1	250.55±19.9	0.4
Neutrophils, 10^9/L	4.45±0.8	3.62±0.3	0.0001
Lymphocytes, 10^9/L	2.38±2.7	2.31±0.4	0.9
CRP, mg/L	8.4±5.5	1.4±0.4	0.0001
ESR, mm/h	23.9±7.7	6.6±2.7	0.0001
RF	65.3±22.9	3.8±0.8	0.0001
NLR, %	2.44±1.1	1.61±0.3	0.005
PLR, %	147.9±78.9	111.8±19.9	0.02

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio.

Table 2 Comparison of the NLR and PLR in the groups of rheumatoid arthritis patients.

	Group 1 (n = 27)	Group 2 (n = 35)	P-value
Age (years) Gender (male/female)	47.1 ± 11.2 8 (35%)/19 (65%)	48.4 ± 12.5 7 (25%)/28 (75%)	0.588 0.374
CRP (mg/L)	4.17±2.3	13.9±3.1	0.002
ESR (mm/h)	18.5±2.3	31.04±6.3	0.01
RF	49.7±7.7	85.5±20.2	0.0001
NLR, %	1.55±0.4	3.59±0.8	0.03
PLR, %	96.1±30.7	211.1±74.5	0.0001

Group 1, patients in remission with a DAS-28 score lower than 2.6; Group 2, active patients with a DAS-28 score of 2.6 and higher; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; DAS-28, Disease Activity Score of 28 joints.

Table 3 The correlation between age, gender, duration of disease and NLR, PLR according to DAS-28.

		r	P value
DAS28	NLR	0.9	0.0001
	PLR	0.5	0.02

DAS-28, Disease Activity Score of 28 joints; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio.

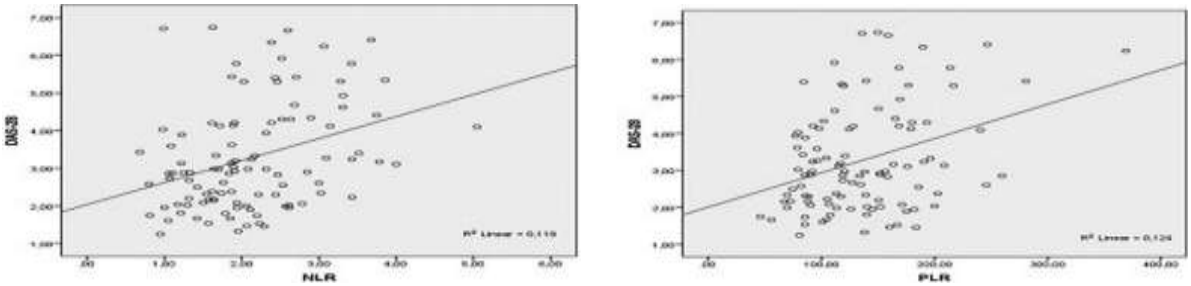

Figure 1 The correlation between neutrophil–lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and Disease Activity Score DAS28.

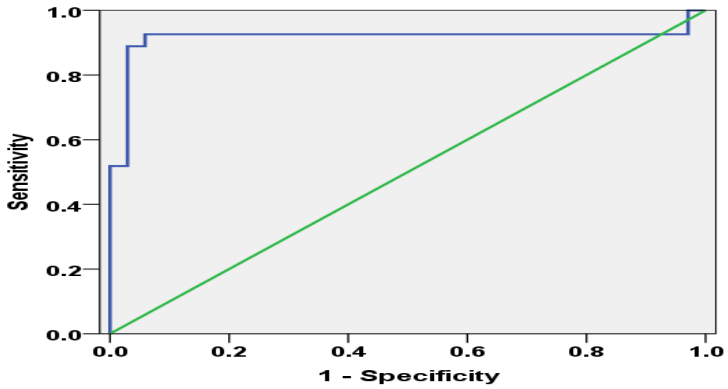

Figure2 The ROC curve of the diagnostic value of the NLR for RA. At the cutoff value of 1.9 and an AUC of 0.91, the diagnostic sensitivity, specificity were 92%, and 83%,respectively.

DISCUSSION

The present study assessed the relation between the disease activity and NLR and PLR in patients with RA. The findings showed that NLR and PLR were higher in patients with RA compared to the control group. According to DAS-28, NLR and PLR were detected to be lower in patients in remission with a score of less than 2.6 compared to patients with active disease and a score of 2.6 and higher. A correlation was observed between the DAS-28 score used to assess disease activity and NLR and PLR.

Neutrophils, more than 50% of which are produced by bone marrow, are at the front line of the defense system. They are responsible for the production of many lytic enzymes, free oxygen radicals, and cytokines.[14] Cytokines have a very significant role in the pathogenesis of a great number of inflammatory diseases. Neutrophils and platelets are involved in the production of these cytokines, which, in turn, contribute to the activation of these neutrophils and platelets. [15,16] Studies show that platelets also play an active role in the inflammation, while having regulatory effects on the immune system as well.[9,17] Cytokines released by tumor cells and other mediators result in neutrophilia, thrombocytosis and reactive lymphopenia. Neutrophils, platelets and lymphocytes are thought to play an important role in tumor immunology and inflammation.[16] Inflammation plays a significant role in the proliferation, angiogenesis and metastasis of cancer cells and is important in the development and progression of the disease.[18,19] Inflammatory markers such as NLR and PLR were reported to be associated with survival, usable as a prognostic factor and guide for the follow-up of the disease and differential diagnosis.[16] Acmaz *et al*.[20] showed that NLR was higher in patients with endometrial cancer compared to patients with hyperplasia and the control group. They also showed that PLR was higher in patients with endometrial cancer compared with hyperplasia and in patients with hyperplasia compared with the control group. The same study reported that these inflammatory markers could also be used in diagnosis. The synovial infiltration of neutrophils and lymphocytes in RA suggest that they may contribute to the development and progression of the disease.[5,6] Although available data suggest that platelets do not have any effect on the disease, they also show that cytokines which are important in the pathogenesis and some mediators impact the activation of platelets.[21] While neutrophils are highly important for the control of inflammation, any abnormalities in their activation may cause autoimmunity and tissue damage.[5] Irregular apoptosis and impaired regulatory mechanisms are considered significant in lymphocytes. Moodley *et al*.[6] showed that early apoptotic markers are high in lymphocytes, reporting that this might be associated with autoimmunity and result in lymphopenia. Gian Luca *et al.*[22] demonstrated that high levels of NLR and/or PLR are consistently associated with the presence of Rheumatoid Arthritis. The present study assessed NLR and PLR in patients with RA and investigating their relation with DAS-28. When considering neutrophils, platelets and lymphocytes in RA, inflammatory markers such as NLR and PLR, which are made up of these components, are predicted to cause some changes in the disease. The higher levels of NLR and PLR in the patient group compared with the control group and in patients with active disease compared with patients in remission, as well as their correlation with DAS-28 in the patient group, suggested that they might be used as markers in the follow-up of disease activity. However, the present study has some limitations. It is a retrospective study, is based on values of patients whether under treatment or not, and does not assess the effects of treatment on bone marrow. As we know, some drugs (i.e., methotrexate, sulfasalazine) that are commonly

used in RA treatment schemas may have different effects on hematological parameters. In conclusion, the present study showed that NLR and PLR could be two new inflammatory markers indicating disease activity in patients with RA. If these results are supported by various prospective studies assessing NLR and PLR in patients with RA, we think that they can be classified among the other inflammatory markers used in the assessment of disease activity.

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