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Original Article

Combination of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio as diagnostic biomarker for rheumatoid arthritis

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Abstract: Objective: The outstanding diagnostic role of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) attract more attention recently. The purpose of this study is to evaluate the diagnostic value of the NLR and PLR in Rheumatoid arthritis (RA) patients. Methods: NLR and PLR were compared between 125 RA patients (66 RA patients in disease active stage, 59 RA patients in non-active stage) and 126 healthy individuals; receiver operating characteristic curve (ROC curve) and the area under the ROC curve (AUC) were used to identify their associations with RA; Spearman correlation analysis was used to identify the correlations between NLR (PLR) and clinical characteristics of RA patients. Results: RA patients both in disease active stage and in non-active stage have high levels of NLR and PLR compared to healthy individuals, respectively (P<0.0001; P<0.0001). Results of ROC curve indicated that the combination of NLR and PLR as a panel revealed high diagnostic accuracy for discriminating active RA patients or non-active RA patients from healthy individuals, respectively (AUC, 0.880; 95% Confidence Interval [CI], 0.819-0.940; P<0.0001; AUC, 0.839; 95% CI, 0.772-0.960; P<0.0001). Moreover, the value of PLR and NLR showed positive correlations with erythrocyte sedimentation rate (ESR) (NLR, r=0.5048; P<0.0001; PLR, r=0.3489; P=0.0012). Conclusion: Combination NLR and PLR in peripheral blood had a high diagnostic value to identify RA. Moreover, the value of NLR and PLR positively correlated with ESR can help us to estimate the severity of RA.

Keywords: Neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory autoimmune disease, characterized leukocyte and other inflammatory corpuscle infiltration, leading to destruction of progressive cartilage and bone [1]. Approximately, 75% of untreated patients resulted in disability within three years. Therefore, it is increasingly stringent to explore early and novel diagnostic molecular markers. Currently, anti-citrullinated protein antibody (ACPA) and rheumatoid factor (RF) are used as serologic biomarkers in clinical examination. However, protein and polypeptide composition are complicated in serum, and the high abundance of albumin and globulin is apt to interfere with the examination results. In addition, the level of protein and polypeptide in blood are susceptible to physiological state, lifestyle and physiological environment in vitro, which lead to poor repeatability and reliability of clinical diagnosis.

During the development and progression of the diseases, hematological changes are often occurred prior to morphological changes to reflect the clinical status. Moreover, blood samples are available easily, complete blood count (CBC) is popularized due to its minimally invasive, easy to manipulate in clinical examination. Hematological change is a kind of feedback to the systemic inflammatory. CBC is used to detect the variation of different components in peripheral blood for diagnosing diseases of the haematopoietic system. In recent years, some scholars concluded that different changes of

Table 1. Clinical features of the RA patients and healthy controls enrolled in the study

Characteristics	RA (active)	RA (non-active)	HCs	*P*-value
Sex, male/female	6/60	9/50	10/116	NS[a,b,c]
Age, mean (range)	53.3 (24-75)	51.54 (22-81)	46.67 (26-68)	NS[a,b,c]
RF titer (IU/ml), mean (range)	332.7 (8-1450)	237.2 (4 -2560)	NA	0.0223[c]
ESR (mm/h), mean (range)	52.8 (2-319)	22.0 (4-250)	NA	<0.0001[c]
ACPA (AU/ml), mean (range)	275.28 (20-942.7)	335.44 (8-768)	NA	0.7460[c]
CRP (mg/L), mean (range)	13.6 (1-55.3)	9.2 (1-64.7)	NA	0.0026[c]
DAS28, mean (range)	5.8 (3.51-7.58)	2.1 (1.18-3.18)	NA	<0.0001[c]
SJC, mean (range)	7.8 (2-0)	0.038 (0-1)	NA	<0.0001[c]
TJC, mean (range)	13.1 (2-28)	0.21 (0-2)	NA	<0.0001[c]
Neutrophil (10^9/L), mean (range)	4.78 (1.80-13.1)	4.38 (1.70-13.3)	4.28 (2.21-6.27)	NS[a,b,c]
Lymphocyte (10^9/L), mean (range)	1.74 (0.60-4.30)	1.70 (0.60-3.30)	2.38 (1.24-2.91)	NS[a,b,c]
Platelet (10^9/L), mean (range)	215 (80-548)	215 (80-394)	230 (129-300)	NS[a,b,c]
NLR, mean (range)	3.17 (1.17-12.5)	2.97 (1.00-17.1)	1.86 (1.31-3.31)	<0.0001[a,b]
PLR, mean (range)	142.6 (36.4-386.7)	138.8 (36.3-430)	100.1 (5.8-164.9)	<0.0001[a,b]

RA, rheumatoid arthritis; HC, healthy control; RF, Rheumatoid factor; ESR, erythrocyte sedimentaition ratio; ACPA, anticyclic citrullinated peptide antibody; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; SJC, swollen joint count; TJC, tender joint count; NA, not applicable; NS, no significant. a, comparison between active-RA group and control group; b, comparison between non-active RA group and control group; c, comparison between active-RA group and non-active RA group.

Table 2. ROC curves and the corresponding AUCs of the NLR and PLR to discriminate RA patients from healthy controls

			Asymptotic	Asymptotic	
	Test Variable (s)	Area	Sig.	95% Confidence Interval	
				Upper Bound	Lower Bound
Active RA group VS Control group	NLR	0.813	<0.0001	0.739	0.888
	PLR	0.762	<0.0001	0.673	0.85
	NLR-PLR panel	0.88	<0.0001	0.819	0.94
Non-active RA group VS Control group	NLR	0.764	<0.0001	0.678	0.849
	PLR	0.777	<0.0001	0.699	0.855
	NLR-PLR panel	0.839	<0.0001	0.772	0.906

various leukocyte ratios occurred in many different types of diseases. The changes of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have been confirmed as responses of the immune system to surgical stress, systemic inflammation and chronic conditions [2-4] and been treated as novel diagnostic factors for different type of diseases [5-8].

To our knowledge, the reliable biomarker of RA in peripheral blood has not been reported. This study is aimed to discuss the role of NLR and PLR in RA patients, and we sought to identify the combination of NLR and PLR as a panel, which could serve as a novel biomarker for diagnosing RA, and may provide potential clues for further understanding the pathogenesis of RA.

Patients and methods

Study subjects

Patient fulfilled the 2010 ACR/EULAR- or the 1987 ACR-classification criteria were obtained from department of rheumatology and immunology, the affiliated hospital of Jiangsu university, from Mar. 2012 to Dec. 2015. Other systematic diseases were excluded, such as diabetes mellitus, therioma, hematonosis, cardiovascular diseases, chronic inflammatory disorders, and other autoimmune diseases. Healthy donors from the physical examination cen-

ter and met the following criteria: no history of previous autoimmune diseases or chronic inflammatory disorders, and no evidence of current inflammatory diseases. Before commencement of the study, all participants were informed of the study, and the Ethics Committee at the participating centers approved the recruitment protocols.

Sample processing and detection

Approximate 2 ml venous complete blood samples were collected from study subjects and placed in spray-coated K_2-EDTA Tubes, then were detected with blood cell counter, the hematology analyser (Sysmex Model XS, Hamburg, Germany). Complete blood count should be finished in an hour as soon as possible.

Data analysis and statistics

The statistical analyses were performed with SPSS v 16.0 (SPSS, Inc., Chicago, IL). The data were presented as the mean (range). For categorical data Chi-square test was performed to compare differences in variables between two groups, and for continuous data, the nonparametric Mann-Whitney U-test was performed. ROC curve and the area under the ROC curve (AUC) analyses were used to determine sensitivity and specificity of each parameter. Logistic regression was used to combine NLR and PLR as a panel, then ROC curve was used to identify this panel whether could distinguish RA from controls with the high sensitivity and specificity. For NLR and PLR measured in patients with RA and HCs, we derived likelihood ratio chi-squre and *p* value by multivariable logistic regression analysis, we calculated a risk probability score, named RSF (risk score function) for "estimated Probability of RA", where RSF = exp(-x)/(1 + exp(-x)) and x is linear expression of input value of NLR and PLR. Spearman's correlation analysis was constructed to assess relationship between NLR (PLR) and clinical characteristics of patients. *P*-value was considered statistically significant when lower than 0.05.

Results

Clinical characteristics of study population

There were 251 individuals enrolled in the study: 125 RA patients (66 RA patients in disease active stage and 59 RA patients in non-active stage), and 126 healthy donors. Demographic and clinical characteristics and laboratory data are summarized in **Table 1**. There was no difference in gender, age, neutrophil, lymphocyte or platelet counts between patients and healthy donors. However, NLR, PLR values were both significantly higher in active-RA patients and non-active RA patients compared to healthy donors, respectively ($P<0.0001$; $P<0.0001$). Nevertheless, the difference was not found between active-RA patients and non-active RA patients ($P>0.05$).

Diagnostic value of NLR and PLR

To evaluate the potential role of the NLR and PLR as diagnostic biomarker for RA patients (active RA and non-active RA), as presented in **Table 2**, receiver operating characteristic curve (ROC curve) analysis showed that NLR had an AUC of 0.813 in separating active RA patients from healthy individuals (0.813; 95% Confidence Interval [CI], 0.739-0.888; $P<0.0001$), and an AUC of 0.764 in separating non-active RA patients from healthy individuals (0.764; 95% CI, 0.678-0.849; $P<0.0001$). Moreover, Our analysis also revealed that PLR had an AUC of 0.762 in separating active RA patients from healthy individuals (0.762; 95% CI, 0.673-0.850; $P<0.0001$), and an AUC of 0.777 in separating non-active RA patients from healthy in dividuals (0.777; 95% CI, 0.699-0.855; $P<0.0001$). Subsequently, we combined the NLR and PLR as a panel (NLR-PLR panel) using RSF to evaluate the function of NLR-PLR panel for discriminating RA patients from control samples, as previously described, the ROC curve for NLR-PLR panel showed a higher diagnostic accuracy for discriminating active RA patients and non-active RA patients from healthy individuals, respectively (AUC, 0.880; 95% CI, 0.819-0.940; $P<0.0001$; AUC, 0.839; 95% CI, 0.772-0.960; $P<0.0001$) (**Figure 1**). These results indicated that the combination of NLR-PLR panel reveals higher diagnostic value for discriminating RA patients from healthy individuals than using NLR or PLR alone.

Correlation of complete blood count parameters with patient's clinical variables

We investigated the correlation of NLR and PLR with RA clinical variables, containing erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), swollen joint count (SJC), tender joint count (TJC) and 28-joint Disease Activity Score (DAS28). Both NLR and PLR positively correlat-

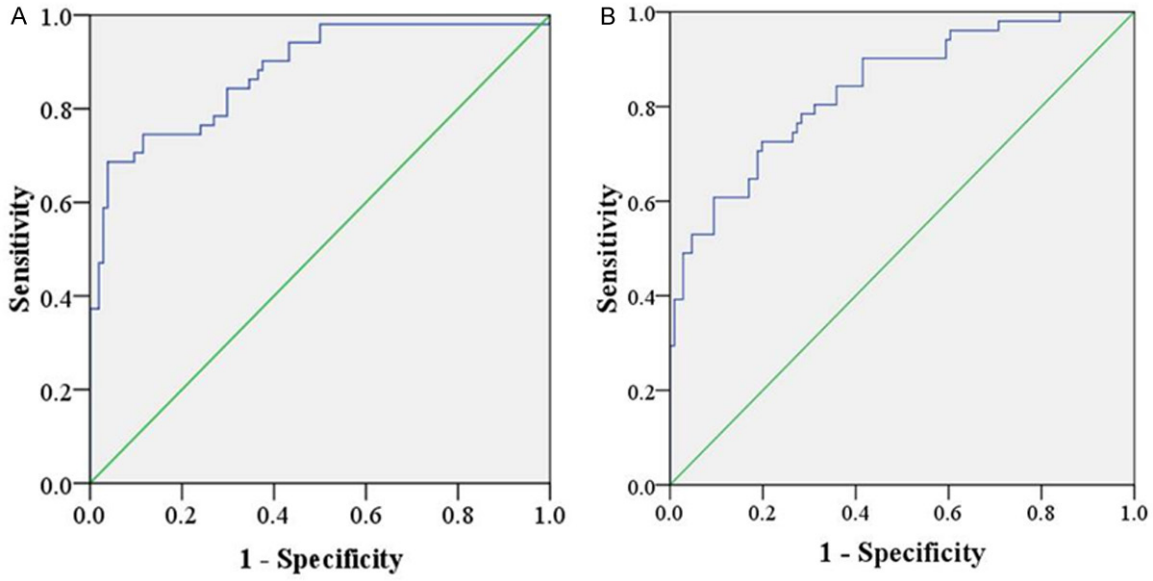

Figure 1. Combination of NLR-PLR panel increased diagnostic. Combination of NLR-PLR panel increased diagnostic. A. ROC curves showing the capacity of NLR-PLR panel to differentiate active RA patients from healthy individuals. B. ROC curves showing the capacity of NLR-PLR panel to differentiate non-active RA patients from healthy individuals.

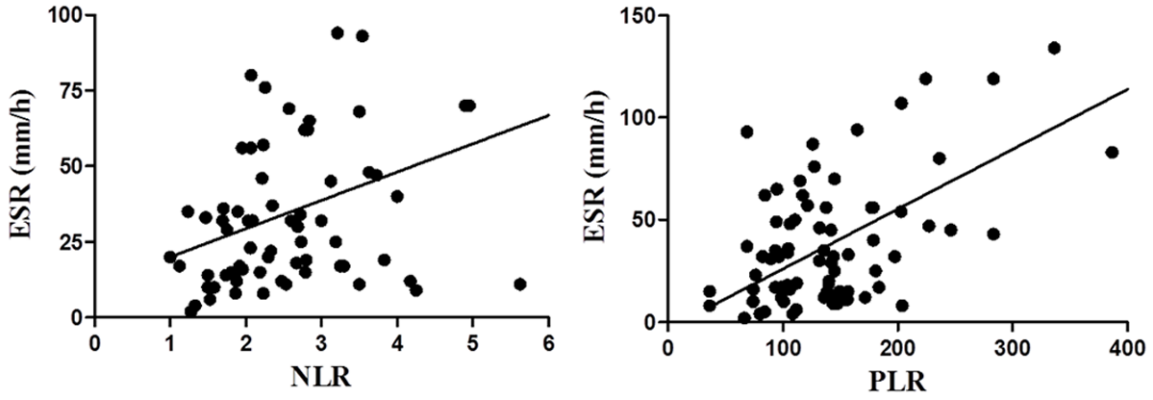

Figure 2. Correlation between NLR (PLR) with ESR in RA patients.

ed with ESR (NLR, r=0.5048; *P*<0.0001. PLR, r=0.3489; *P*=0.0012) (**Figure 2**). However, no significant correlations of NLR and PLR with other clinical parameters were observed in RA patients (the data were not shown).

Discussion

Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disorder. Peripheral immune-mediated reactions are pivotal in the pathogenesis of RA [9], and both the innate and adaptive immune systems are involved in. Neutrophil accounts for 45%-75% of leucocytes in peripheral blood circulation, and is an important reflector of inflammatory condition of body. Neutropenia occurred in autoimmune disease, such as RA, especially in Felty syndrome and RA after treatment [10], but these conditions will induce long-term neutropenia, not the transient condition that we detected. Wright et al. provided a review about neutrophils contributed to disease development of RA. Such as neutrophils release damaging molecules at inflammatory sites, generate and release immunoregulatory cytokines and chemokines which contribute to progress of inflammation. Exposure of these molecules on their cell surface resulted in apoptosis [11]. Lymphocytes are one of the most important cellular immune

response, playing an important role in the pathogenesis, progression and prognosis of RA through releasing inflammatory agents [12, 13]. Recently, neutrophil to lymphocyte ratio (NLR), as the relative difference in the neutrophil and lymphocytes counts, has attracted the interest of investigators as a new systemic inflammatory marker for diagnostic and prognosis role for malignant tumor, diabetic nephropathy, cardiovascular diseases and autoimmune disorders [14-17]. Meanwhile, an increasing number of studies focused on what is the role of platelet to lymphocyte ratio (PLR) played in cardiovascular diseases and cancer [6, 18-20]. At present, some scholars put forward combination platelet count and NLR play a predictive role in esophageal squamous cell carcinoma and postoperative survival in patients undergoing surgery for gastric cancer [16, 21]. But there are no reports demonstrated the relationship between NLR and RA. Therefore, we wondered whether NLR is suitable as a diagnostic marker for RA.

To our knowledge, this study firstly showed that both levels of NLR and PLR were significantly higher in RA patients than healthy controls. Moreover, results showed NLR and PLR had high diagnostic value to discriminate RA from healthy individuals, and combination of NLR and PLR as a panel revealed higher diagnostic value than one of them separately. Therefore, we concluded that NLR-PLR panel is a better independent diagnostic parameter of RA patients. As we known, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were commonly used to indicate inflammation in RA. But these indicators have their limitations, ESR react slowly for inflammatory condition, because of that, some scholars thought CRP can replace ESR [22] but it lack of specificity, our study showed NLR and PLR positively correlated with ESR, indicated NLR and PLR were influenced from disease activity of RA, it may suggested NLR and PLR can serve as factors which suggested the severity of RA, let many RA patients with inflammation have been treated promptly.

There are several weaknesses in our study, the limited number of subjects included in this study and many of they are elderly people may restrict these results be widely applied, and other inflammatory diseases seem unlikely to be entirely excluded, leading to NLR and PLR are not highly specific for RA. Except for these, the results of this study can provide new candidates (NLR and PLR) for the screening of biomarker of RA.

In conclusion, we have identified NLR and PLR from complete blood count of RA patients may serve as biomarkers for RA diagnosis. In addition, NLR and PLR which associated with ESR may serve as factors which assisted us in predicting the severity of RA.

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Disclosure of conflict of interest

None.

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