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RESEARCH ARTICLE

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Neutrophil-to-C3 ratio and neutrophil-to-lymphocyte ratio were associated with disease activity in patients with systemic lupus erythematosus

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Background: Systemic lupus erythematosus is prone to recurrent attacks, and its treatment is related to disease activities. It is important to accurately assess the patient's disease activity. So, the purpose of this study was to investigate the relation between neutrophil-to-C_3 ratio (NC_3R), neutrophil-to-lymphocyte ratio (NLR), and disease activity in patients with Systemic lupus erythematosus (SLE).

Methods: This was a retrospective study. One hundred and ninety-four patients with SLE and 71 healthy controls were included in this study. We divided the patients into two groups according to the SLE disease activity (SLEDAI). Group 1 included patients with a score of >9 (patients with severe disease activity), and Group 2 included patients with a score of 9 and lower (patients with mild disease activity). Correlations between NC_3R, NLR, and disease activity were analyzed.

Results: NC_3R and NLR in patients with SLE were obviously higher compared to healthy controls ($P < 0.05$). There was an obviously significant difference in NC_3R and NLR between Group 1 and Group 2 ($P < 0.05$). SLEDAI scores were positively correlated with NC_3R ($r = 0.353$, $P < 0.01$) and NLR ($r = 0.237$, $P = 0.01$). Receiver operating characteristic (ROC) curve analysis showed that the cutoff value of NC_3R to identify SLE with high disease activity was 5.935, with sensitivity and specificity being 75.9% and 67.0%, while that of NLR was 2.293, with sensitivity being 68.9% and specificity being 82.8%.

Conclusion: NC_3R and NLR are two useful inflammatory markers for evaluating disease activity in patients with SLE.

KEYWORDS
disease activity, neutrophil-to-C3 ratio, neutrophil-to-lymphocyte ratio, systemic lupus erythematosus

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a clinically common autoimmune disease characterized by abnormal immune response to autologous tissue, eventually resulting in systemic disorders and diverse clinical manifestations of patients.[1] The prevalence of women is significantly higher than that of men.[1] The pathogenesis of SLE remains unclear, but environmental triggers and genetic factors have been reported to contribute to the destruction of immune tolerance systems and the production of immunological lymphocytes, antibodies, and inflammatory cytokines, damaging tissues and organs.[1,2] Components of lymphocytes, antibodies, inflammatory cytokines, and complements in peripheral circulation vary among different

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active stages of SLE.[3-5] Patients with higher disease activity often present severer damage of tissues and organs, many of which even threaten the patients' life.[6] It is of great significance in SLE management to early and accurately determine the disease activity of patients. In this study, we determined neutrophil-to-C_3 ratio (NC_3R), neutrophil-to-lymphocytes ratio (NLR), neutrophil-to-C_4 ratio (NC_4R), CRP-to-C_4 ratio (CC_4R), and other inflammatory markers of 194 patients with SLE, to investigate their correlation with disease activity.

2 | MATERIALS AND METHODS

2.1 | Participants

A total of 220 patients with SLE from the Second Affiliated Hospital between January 2016 and June 2017 were enrolled. One hundred and ninety-four of them were finally included in this study as SLE group, who were diagnosed by 2 attending rheumatologists. All patients with SLE were diagnosed based on the criteria established by Systemic Lupus International Collaborating Clinics.[7] Patients who had the following diseases were excluded from the study: (a) Secondary infection such as fever and sputum-positive, (b) Hematological disease (granulocytic leukemia, macroglobulinemia, multiple myeloma, infectious mononucleosis, etc.), (c) HIV infection, (d) complicated with other autoimmune diseases (sicca syndrome, rheumatoid arthritis, mixed connective tissue disease, etc.), (e) chronic hepatic diseases, and (f) resent antibiotic taking, and 71 healthy individuals being in fine basic examination were enrolled as control group. All participants signed the informed consent, and this study obtained ethical approval from the ethics committee of the Second Affiliated Hospital of Nanchang University. Blood samples were taken in the morning, 2.0 mL was placed in the coagulation tube, and another 2.0 mL was placed in the anticoagulant of EDTA-K2 tube.

2.2 | Specimen processing

Beckman Immage 800 (Beckman Coulter, Inc., Brea, CA, USA) and matching detection reagent were utilized to detect serum levels of IgG, IgM, C_3, C_4, and CRP. Neutrophilic, lymphocyte and monocyte counts in the peripheral blood were analyzed with automatic blood fluid module analyzer (XN-20[AI], Sysmex Corporation, Kobe, Japan) and matching reagents.

2.3 | Statistical analysis

Analysis was performed using SPSS software (version 22.0, International Business Machines Corp., Beijing, China). The normality of distribution was checked by Kolmogorov-Smirnov test, with normal distributions data expressed as mean ± standard deviation and tested by parametric test, whereas non-normal distributions presented as percentage and tested by nonparametric test. Spearman's correlation coefficient was calculated to examine the association between two continuous variables. Receiver operating characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity of NC_3R and NLR in predicting the severe disease activity. Statistical significance was defined as $P < 0.05$.

TABLE 1 General clinical characteristics of population

Variable	SLE (n = 194)	Control (n = 71)
Age (y)	40.61 ± 12.50	43.24 ± 13.09
Gender (n, %)		
Male	15 (7.73%)	10 (14.08%)
Female	179 (92.27%)	61 (85.92%)
SLEDAI (n, %)		
0 ~ 4	120 (61.85%)	
5 ~ 9	44 (22.68%)	
10 ~ 14	21 (10.82%)	
≥15	9 (4.65%)	
ANA	156 (80.41%)	
dsDNA	78 (40.21%)	

3 | RESULTS

3.1 | The characteristics of SLE patients

A total of 194 eligible patients with SLE were finally included in this study, among which there were 15 males and 179 females, with an average age of 40.61 ± 12.50; among 71 healthy controls, there were 10 males and 61 females and the average age of them was 43.24 ± 13.09. There was no statistically significant difference between the two groups of sex and age. After classifying all patients according to SLE disease activity score (SLEDAI), the number of each score group was illustrated in Table 1. There were 30 patients with SLE in the Group 1 (SLEDAI>9) and 164 patients of Group 2 (SLEDAI ≤ 9). See Table 1 for details.

3.2 | The differences in NLR, CC_4R, NC_3R, NC_4R, and related laboratory indicators between SLE patients and healthy controls

The levels of IgM, C_3, and C_4 in patients with SLE were 1.022, 0.618 , and 0.145 g/L, respectively, and lower than those in healthy group (all $P < 0.05$). The levels of NLR, CC_4R, NC_3R, and NC_4R in patients with SLE were 3.605, 49.236, 6.897, and 34.981, respectively, and higher than those in healthy group (all $P < 0.05$), as shown in Table 2.

3.3 | The differences in C_3, C_4, NLR CC_4R, NC_3R, and NC_4R between Group 1 and Group 2

Group 1 (SLEDAI score> 9) had a higher NC_3R of 9.532 (7.064, 12.000) and NLR of 5.713 (3.187, 8.239) and lower C_3 of 0.521 (0.439, 0.603), while patients in Group 2 (SLEDAI score ≤ 9) had NC_3R of 6.273 (5.576,

TABLE 2 The indicators among SLE and control group

	SLE (n = 194)	Control (n = 71)	*P*-value
Age (y)	40.61 ± 12.50	43.24 ± 13.09	0.182
Neutrophils (×109/L)	3.867 (2.080, 4.838)	3.559 (3.127, 3.992)	0.279
Lymphocytes ($x10^9$/L)	1.389 (0.845, 1.708)	1.538 (1.358, 1.719)	0.164
Monocyte ($x10^9$/L)	0.362 (0.190, 0.470)	0.343 (0.293, 0.393)	0.562
IgA (g/L)	2.71 (1.770, 3.535)	2.362 (1.51, 2.79)	0.102
IgG (g/L)	13.939 (10.00, 17.60)	13.451 (12.295, 14.607)	0.512
IgM (g/L)	1.022 (0.565, 1.368)	1.227 (1.06, 1.395)	0.022
CRP (g/L)	6.818 (1.543, 6.112)	5.288 (3.759, 6.817)	0.320
C_3 (g/L)	0.618 ± 0.22	0.891 ± 0.24	<0.01
C_4 (g/L)	0.145 (0.09, 0.170)	0.193 (0.176, 0.210)	0.002
NLR	3.605 (1.643, 3.878)	2.80 (2.369, 3.231)	0.022
CC_4R	49.236 (12.932, 61.712)	28.049 (20.036, 36.062)	<0.01
NC_3R	6.897 (3.543, 8.693)	4.545 (3.70, 5.389)	<0.01
NC_4R	34.981 (16.574, 44.771)	21.709 (17.772, 25.648)	<0.01

NLR, Neutrophil-to-lymphocytes ratio; CC_4R, CRP-to-C_4 ratio; NC_3R, neutrophil-to-C_3 ratio; NC_4R, neutrophil-to-C_4 ratio.

6.969), C_3 of 0.634 (0.600, 0.668), and NLR of 3.108 (2.682, 3.535). There were obviously significant differences in C_3, NLR, CC_4R, and NC_3R between the two groups (all $P < 0.05$) (Table 3).

TABLE 3 The indicators among different activity of SLE

	SLEDAI ≤ 9 (n = 164)	SLEDAI>9 (n = 30)	*P*-value
Age (y)	40.98 ± 12.07	38.52 ± 15.19	0.329
C_3 (g/L)	0.634 (0.600, 0.668)	0.521 (0.439, 0.603)	0.010
C_4 (g/L)	0.138 (0.1227, 0.149)	0.129 (0.102, 0.157)	0.572
NLR	3.108 (2.682, 3.535)	5.713 (3.187, 8.239)	0.046
CC_4R	43.475 (35.427, 51.524)	74.915 (48.059, 101.77)	0.028
NC_3R	6.273 (5.576, 6.969)	9.532 (7.064, 12.00)	0.014
NC_4R	33.145 (29.193, 37.097)	42.801 (31.354, 54.248)	0.070

3.4 | The relationship between the NLR, NC_3R, CC_4R, and disease activity in patients with SLE

The relationship between the NLR, NC_3R, CC_4R, and disease activity was tested by linear regression analysis. SLEDAI scores were positively correlated with NC_3R ($r = 0.353$, $P < 0.01$), NLR ($r = 0.237$, $P = 0.01$), and CC_4R ($r = 0.263$, $P < 0.01$), negatively associated with lymphocyte counts ($r = -0.256$, $P < 0.01$) (Table 4).

3.5 | The receiver operating characteristic (ROC) curves of NC_3R, NLR, and CC_4R for the recognition of severe disease activity

ROC curves of NC_3R, NLR, and CC_4R in identifying higher SLEDAI scores were presented in Table 5 and Figure 1. The optimal threshold for NC_3R, NLR, and CC_4R in identifying higher SLEDAI scores was 5.935, 2.298, and 37.988, respectively. According to ROC curve analysis, the sensitivity of NC_3R, NLR and CC_4R in diagnosing the disease activity of SLE patients was 75.9%, 82.8% and 58.6%, and the specificity was 60.7%, 49.7% and 67.5%, respectively.

4 | DISCUSSION

Systemic lupus erythematosus is a chronic autoimmune disease characterized by a broad spectrum of clinical manifestations, but its course and organ involvement are unpredictable.[8,9] Timely treatment adjustment according to disease activity is of great importance in the management of SLE.[6] SLEDAI, which containing 21 scoring items, is clinically used to evaluate SLE disease activity.[10] However, disease activity evaluated via SLEDAI score is of partial subjectivity, such as feeling disorder, insomnia, or daytime sleepiness. To quickly and correctly determine the activity level and get patients treated timely and effectively, therefore, we design the current study to explore new correlation indicators to well reflect patients' disease activity degree.

Neutrophil-to-lymphocyte ratio (NLR), calculated as neutrophil counts divided by lymphocyte counts, is considered as a marker for general immune responses to various stress stimuli.[11] It was considered as a good diagnostic marker with autoimmune diseases, such as adult-onset Still's disease.[12] It was associated with disease activity in patients with systemic lupus erythematosus.[13] Complement system activation, production and partial deposition of complement fragments, and subsequent inflammation all play critical roles in the pathogenesis of SLE, and during the complement activation pathway, C_3 and C_4 were at the core position.[9] Besides, the compounds and antimicrobial peptides released from neutrophils of patients with SLE caused inflammation and damaged tissues and organs.[14] Inflammatory cells (such as neutrophils, lymphocytes, and

TABLE 4 Correlations of SLEDAI scores with NLR, NC3R, and CC4R in patients with SLE

	SLEDAI scores		NLR		NC3R		CC4R	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
C_3 (g/L)	−0.339	<0.01	−0.06	0.408	−0.374	<0.01	−0.043	0.558
C_4 (g/L)	−0.066	0.361	0.06	0.409	−0.180	0.013	−0.028	0.70
CRP (g/L)	0.120	0.096	0.157	0.03	0.01	0.004	0.794	<0.01
Neutrophils ($\times10^9$/L)	0.068	0.349	0.460	<0.01	0.659	<0.01	0.091	0.207
Lymphocyte ($\times10^9$/L)	−0.256	<0.01	−0.387	<0.01	0.014	0.851	−0.228	0.001
SLEDAI scores	-	-	0.237	0.01	0.353	<0.01	0.263	<0.01

TABLE 5 Receiver operating characteristic curves of the CC4R, NLR, and NC3R for differentiating severe patients with SLE

					95% CI	
	Cutoff value	Area	Sensitivity	Specificity	Lower bound	Upper bound
CC_4R	37.988	0.644	0.586	0.675	0.530	0.757
NLR	2.298	0.689	0.828	0.497	0.578	0.800
NC_3R	5.935	0.670	0.759	0.607	0.546	0.794

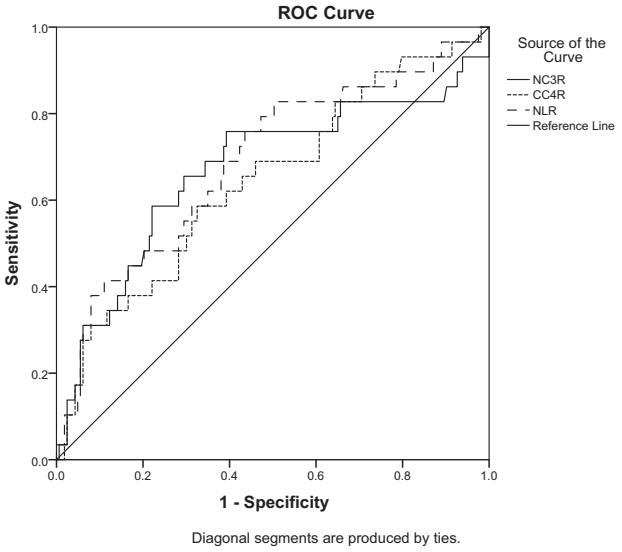

FIGURE 1 Receiver operating characteristic curves of the CC4R, NLR, and NC3R for differentiating severe patients with SLE

monocytes) play the same important role as complement systems in the progression of SLE disease.[4,8,9,15] It reported that patients with SLE had lower complement levels than healthy people,[6,9,16] with inflammatory cells higher than which in healthy people.[14,15] So, NLR, CC_4R, NC_3R, NC_4R, and related inflammatory markers were selected in the study to study its correlation with SLE activity.

There were significant differences in IgM, C_3, C_4, NLR, CC_4R, NC_3R, and NC_4R between SLE and healthy population: Levels of IgM, C_3, and C_4 were lower, and NLR, CC_4R, NC_3R, and NC_4R were higher compared to healthy controls. The immune and complement systems were more active compared to healthy population, which formed circulating immune complexes (ICs) depositing in tissues and organs and causing corresponding damage. The complement system was activated by a classical approach, which causing C_3 and C_4 degradation[9,16] and decreased levels of C_3 and C_4 in SLE population. NC_3R and NC_4R in SLE patients were higher than those in normal patients, because the number of immune-related cells increased and the levels of C_3 and C_4 decreased. Previous study has shown that mature B lymphocytes were generated in mouse model of systemic lupus erythematosus, which did not secrete antibodies yet.[17] Therefore, the quantities of IgM in patient with SLE were lower than controls.

Levels of C_3, NLR, CC_4R, and NC_3R were obviously different between SLE and healthy groups (both $P < 0.05$). The complement system of patients with SLE in Group 1 was more active, and tissue damaged was more serious compared with Group 2. In addition, the degradation of C_3 was faster and the number of inflammatory cells was higher in Group 1. Therefore, C_3, NLR, CC_4R, and NC_3R may clearly distinguish the degree of SLE activity. Linear regression analysis showed that SLEDAI scores were correlated with CC_4R ($r = 0.236$), NC_3R ($r = 0.353$), and NLR ($r = 0.237$) (P values < 0.05). It was reported that CRP, C_3, and C_4 were indicators for autoimmune disease diagnosis and related closely to disease activity.[18,19] Our study found that CC_4R, NC_3R, and NLR correlated with CRP, C_3, C_4, and inflammatory cells. Therefore, CC_4R, NC_3R, and NLR can be served as new indicators of inflammation to evaluate SLE activity.

Furthermore, CC_4R, NC_3R, and NLR had good sensitivity and specificity to evaluate the activity of SLE. The area under the curve of CC_4R, NC_3R, and NLR, respectively, was 0.644, 0.670, and 0.689 by ROC curve analysis. CC_4R was excluded, because this study was focused on the diagnosis of systemic lupus erythematosus with high disease activity, while the sensitivity of it was 0.586. The diagnostic performance of NC_3R and NLR for severe SLE patients was higher than other indicators, and the best NC_3R cutoff value was 5.935,

with 75.9% sensitivity and 67.0% specificity, while the best NLR cut-off value was 2.293, with 68.9% sensitivity and 82.8% specificity. Therefore, NC_3R and NLR, as the new inflammatory markers of SLE, can be used to reflect the activity of SLE, which can simplify the clinical workflow, allow patients with SLE to get reasonable treatment in time and more importantly, and reduce patients' additional cost for medical checkups.

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