ORIGINAL ARTICLES

Neutrophil-to-lymphocyte ratio in systemic lupus erythematosus is influenced by steroids and may not completely reflect the disease activity

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Abstract

Objectives: Neutrophil-to-lymphocyte ratio (NLR) has emerged as an important parameter in inflammatory network and could pave the way for newer treatment strategies in systemic lupus erythematosus (SLE). The study evaluated NLR as a predictor of disease activity in SLE and attempted to relate the factors influencing the NLR.

Methods: The study included 117 SLE patients fulfilling the systemic lupus international collaborating clinics/American College of Rheumatology (SLICC/ACR) criteria (2010). The subjects were classified into mild, moderate, and severe systemic lupus erythematosus disease activity index 2000 (SLEDAI 2K) groups and compared. NLR values were classified as ≤2, >2–4 and >4 groups and its relationship with study variables was evaluated by Notched box-and-Whisker plots, Spearman correlation and Mountain plot. ROC and multiple linear regression were used to verify discriminatory ability and factors influencing NLR respectively.

Results: Approximately 24% (n=28) of patients each had mild and moderate SLEDAI disease activities, and 52.14% (n=61) had severe activity. Patients with severe disease activity were significantly younger (31.69±10.09 years) and were on more immunosuppressants/DMARDs. The patients in the >4 NLR group had significantly elevated total leucocyte count (TLC) 5560 (3360-11480) cells/mm^3 and CRP 4.46 (0.3-48.2) mg/L and more patients were on steroid therapy. The >2-4 NLR group had moderate inverse correlation with SLEDAI. NLR, ESR, CRP, and C3 did not show agreement with SLEDAI. The NLR was associated with CRP and steroid usage and could not discriminate disease severity.

Conclusion: The relationship of the NLR with SLEDAI was not consistent. NLR was associated with CRP and steroid use. NLR as a marker of inflammation or as a predictor of SLE disease activity needs further investigation.

Keywords: Neutrophil-to-lymphocyte ratio, systemic lupus erythematosus, SLEDAI, immunosuppressants/DMARDs, steroids, mountain plot

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multiple autoantibodies and varied clinical features including pancytopenia. Quantifying the disease for the purpose of disease assessment is still a challenge. Complement levels, ESR, presence of auto-antibodies and their titers, especially of the anti-ds DNA, are the laboratory parameters that are considered for disease assessment in routine practice.[1] SLE disease activity index (SLEDAI), Safety of estrogens in lupus erythematosus national assessment (SELENA) and British Isles Lupus Assessment Group (BILAG) are the commonly used scores in clinical trials and in certain academic clinical settings. The SLEDAI score is commonly used in clinical practice.[2]

In the recent years, neutrophil-to-lymphocyte ratio (NLR) has emerged as one of the parameters useful for evaluating several inflammatory diseases.[3] Recent studies have highlighted the relationship of NLR with disease activity.[4-7] The NLR has been found to correlate with clinical disease activity in rheumatoid arthritis,

Henoch-Schonlein purpura, malignancy and ischemic heart disease.[8-12] Accumulating evidence suggests that inflammatory markers such as NLR and PLR (platelet-to-lymphocyte ratio) are significantly elevated in SLE patients and they have been often recommended as useful markers for assessing disease activity.[13-15] Literature also indicates positive correlation between NLR and pulse wave velocity, renal involvement and different classes of renal histological staging, and its use as an additive marker for diagnosing infection in SLE patients.[16-18]

The current study evaluated NLR as a predictor of disease activity in SLE. The secondary objectives were to verify the relationship of NLR, ESR, CRP, and C3 with SLEDAI, and to examine the discriminatory ability of NLR and inflammatory parameters in classifying disease severity. The study included only non-renal SLE or renal lupus patients with no active renal disease. Since SLE per se alters the ratio of total count as well as lymphocyte count and the addition of steroid (used in majority of the SLE patients) is known to influence NLR, the study considered use of steroid as one of the parameters to analyse the relationship of NLR and disease activity.[19-20]

Subjects and methodology

The cross-sectional study recruited SLE patients fulfilling the systemic lupus international collaborating clinics/American College of Rheumatology (SLICC/ACR) criteria (2010) during the three-months period starting from December 2017.[21] The study was approved by the institutional ethics committee and the patients were enrolled consecutively on their routine visit to the center after obtaining informed consent. Subjects with active renal disease or suspicious infections were excluded from the study. Data values that are unusually far from the range of values of the variable in the study population were considered as extreme values. Inclusion of such data values would bias the measures of central tendency and test estimates and were excluded from analysis. The disease activity was assessed using SLEDAI-2K and the patients in remission were excluded from analysis.[22] Organ damage was ascertained using SLICC/ACR damage index (SDI).[23]

The demographic, clinical and inflammatory parameters were recorded in a pre-structured proforma at the recruitment visit and all the analysis were performed as part of the study. Age, gender, total leucocyte count (TLC), differential leucocyte count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complement

factor 3 (C3), SLEDAI-2K and SDI were considered for the study based on clinical relevance. Data on immunosuppressants/disease modifying antirheumatic drugs (DMARDs), steroids and biologics received by the patients were documented. Hematological manifestations of leucopenia and lymphopenia were verified. Leucopenia was assessed by classifying patients into 3 TLC groups: leucopenia <3000 cells/mm^3, normal ≥3000 ≤11000 cells/mm^3 and leukocytosis >11000 cells/mm^3. Patients with lymphocyte count <1500 cells/mm^3 were considered as those with lymphopenia and ≥1500 cells/mm^3 as normal. SLEDAI total score and NLR were calculated. NLR was calculated by dividing relative percentage values of neutrophil by lymphocyte. Patients were classified on the basis of SLEDAI total score (SELENA-SLEDAI groupings) into the following groups: minimum disease activity or remission (0-3), mild (4-8), moderate (9-11), and severe (≥12).[22] The patients were reclassified based on NLR values into 3 groups ≤2, >2–4 and >4. The NLR cut-off values were considered based on the previous studies by the same authors on NLR and rheumatoid arthritis disease activity.[24,25] Drug data of the study subjects was classified as single, double and 3 or more according to the types of immunosuppressants/DMARDs received. Steroid therapy was considered as currently on and not currently on and biologics were considered as given or not given.

Statistics

The independent variables were included based on their clinical relevance. The data are presented as mean±sd for normal distribution, median (min-max) for data without normal distribution and counts for categorical variables. Notched box-and-Whisker plots were used to verify the distribution of NLR, ESR, CRP, C3 and SLEDAI. The scales of the parameter were normalized by linear transformation to 0 –100 range scale using the formula: 100 * (observed value - minimum value)/ (maximum value - minimum value). The SLEDAI and NLR groups were compared for demographic, clinical and inflammatory parameters by ANOVA or Kruskal–Wallis test for continuous variable and chi-square test for categorical data. Pairwise comparison and adjusted residual method was performed to interpret variables with significant differences in ANOVA or Kruskal-Wallis test and chi square test respectively.

The relationship of the SLEDAI and NLR with demographic, clinical and inflammatory parameters was analyzed using bivariate Spearman's correlation. Agreement among the inflammatory parameters (NLR, ESR, CRP, C3 and SLEDAI)

was verified by Mountain plot. SLEDAI and NLR sub-group level correlation and agreement were also verified for inflammatory parameters. SLEDAI was considered as standard reference for Mountain plots. The discriminating power of NLR, ESR and C3 was verified by constructing the receiver operating characteristic (ROC) curve using SLEDAI as binary standard. Patients with SLEDAI score ≥12 were classified as having severe disease and 4-11 as mild-moderate disease for ROC analysis. Univariate and multiple linear regressions were used to identify baseline predictors of NLR. To get a parsimonious model, the baseline predictors with P ≤0.15 in univariate regression were included in multivariate model. Sensitivity analysis was performed to verify the influence of steroid therapy on the variables in SLE patients by comparing patients currently on and not on steroid therapy. A two-tailed P <0.05 was taken as statistically significant for all the tests. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY, USA: IBM Corp.) and MedCalc for Windows, version 17.7.2

Table 1: Demographic, clinical and inflammatory parameters in relation to SLEDAI disease activity in SLE patients

Variables	SLEDAI groups			P value*
	Mild (n=28)	Moderate (n=28)	Severe (n=61)	
Age (years)	39.71±11.95	34.14±9.13	31.69±10.09	<0.01
Gender (M/F)	0/28 (0/25)	0/28 (0/25)	5/56 (100/50)	0.14
Duration of illness (months)	63 (8-204)	83 (4-228)	63 (6-217)	0.19
Total leucocyte count (cells/mm^3)	4615 (2230-10220)	5505 (2820-9740)	5100 (2700-11480)	0.14
Leucopenia assessment				
Leucopenia (<3000 cells/mm^3)	3 (60)	1 (20)	1 (20)	0.31
Normal (≥3000≤11000 cells/mm^3)	25 (22.5)	27 (24.3)	59 (53.2)	
Leucocytosis (>11000 cells/mm^3)	0 (0)	0 (0)	1 (100)	
Lymphopenia assessment				
Lymphopenia (<1500 cells/mm^3)	21 (32.3)	13 (20)	31 (47.7)	0.06
Normal (≥1500 cells/mm^3)	7 (13.5)	15 (28.8)	30 (57.7)	
Neutrophil to lymphocyte ratio	2.17 (0.86-10.28)	2.07 (0.77-10.89)	2.16 (0.76-7.01)	0.52
Erythrocyte sedimentation rate (mm/hr)	30 (8-106)	29.5 (10-102)	38 (4-125)	0.52
C- reactive protein (mg/L)	2.78 (0.3-25.7)	1.62 (0.3-48.9)	2.1 (0.3-48.2)	0.18
Complement factor 3 (mg/dl)	80.18±28.26	78.84±26.53	77.84±23.65	0.92
Immunosuppressants/DMARDs				
Single immunosuppressants/DMARDs	7 (20)	14 (40)	14 (40)	0.03
Double immunosuppressants/DMARDs	21 (28.8)	12 (16.4)	40 (54.8)	
3 or more immunosuppressants/ DMARDs	0 (0)	2 (22.2)	7 (77.8)	
Steroids				
Not currently on	17 (25.8)	15 (22.7)	34 (51.5)	0.86
currently on	11 (21.6)	13 (25.5)	27 (52.9)	
Biologics				
Not Given	28 (25.9)	24 (22.2)	56 (51.9)	0.13
Given	0 (0)	4 (44.4)	5 (55.6)	

Variables represented as mean±sd for continuous variables with normal distribution, median (min-max) for continuous values without normal distribution and counts (percentage) for categorical values.

*patients with severe disease (31.69±10.09 yrs) were younger than those with mild disease (39.71±11.95 yrs, P <0.01). Patients with moderate disease activity had greater than expected number of patients on single immunosuppressants/DMARDs (adj. res. 2.7) than those on double immunosuppressants/DMARDs (adj. res. -2.4) compared to mild and severe disease activity groups.

(MedCalc Software, Ostend, Belgium).

Results

A total of 154 SLE patients who fulfilled the SLICC criteria were recruited for the study. The following subjects were excluded: 28 patients with incomplete data (duration of illness (n=15), ESR (n=1), CRP (n=5), C3 (n=6) and immunosuppressants/DMARDs (n=1), 8 with extreme values for the variables in the study (duration of illness (n=2), TLC (n=1), NLR (n=1), ESR (n=1), CRP (n=2), C3 (n=1)) and 1 patient with remission or minimum disease activity. The final sample size considered for the study was 117. The median (min-max) age was 32 (16-61) years with 5 male and 112 female patients. The patients had 67 (4-228) months median duration of illness. The average SLEDAI score was 12 (4-29) median. Based on the disease activity, the patients were classified as follows: mild (n=28, 23.93%), moderate (n=28, 23.93%) and severe (n=61, 52.14%). No organ damage was reported in 71% (n=83) of the patients. The corresponding percentages of patients with organ damage (SDI) one, two, three and four were 20.5% (n=24), 6.84% (n=8) 0.85% (n=1) and 0.85% (n=1) respectively.

The median TLC, NLR, ESR, CRP and C3 noted were 5030 (2230-11480) cells/mm^3, 2.15 (0.76-10.89), 35 (4-125) mm/hr, 2.17 (0.3-48.9) mg/L and 75.6 (24.9-139) mg/dl respectively. A total of 5 (4.27%) patients had leucopenia and 65 (55.56%) had lymphopenia. The patients received mycophenolate mofetil, hydroxychloroquine, azathioprine, methotrexate and leflunomide as monotherapy or in combination. They were treated either with single (29.91%; n=35), two (62.39%; n=73) or 3 or more (7.69%; n=9) immunosuppressants/DMARDs. About 44% (n=51) of patients concomitantly received steroids and 7.69% (n=9) received biologics therapy.

Comparison of variables on SLEDAI disease activity and NLR

The distribution of normalized values of NLR, ESR, CRP, C3 and SLEDAI are presented in Notched box-and-Whisker plots (Fig.1). NLR (skewness= 1.94), ESR (skewness= 1.1) and CRP (skewness= 4.36) were highly skewed. C3 (skewness= 0.47) had symmetric distribution and SLEDAI (skewness= 0.98) had moderately skewed distribution. Comparison of patients classified on SLEDAI score for demographic, clinical and treatment parameters showed age and number of immunosuppressants/DMARDs received differed significantly among mild, moderate and severe disease groups (Table 1). Patients with mild disease activity had percentage wise more number of leucopenia (10.71%) and lymphopenia (75%) patients compared to moderate & severe disease activity groups. However the difference was not statistically significant. Gender, duration

Fig. 1: Notched box-and-whisker plot for normalized values of study variables
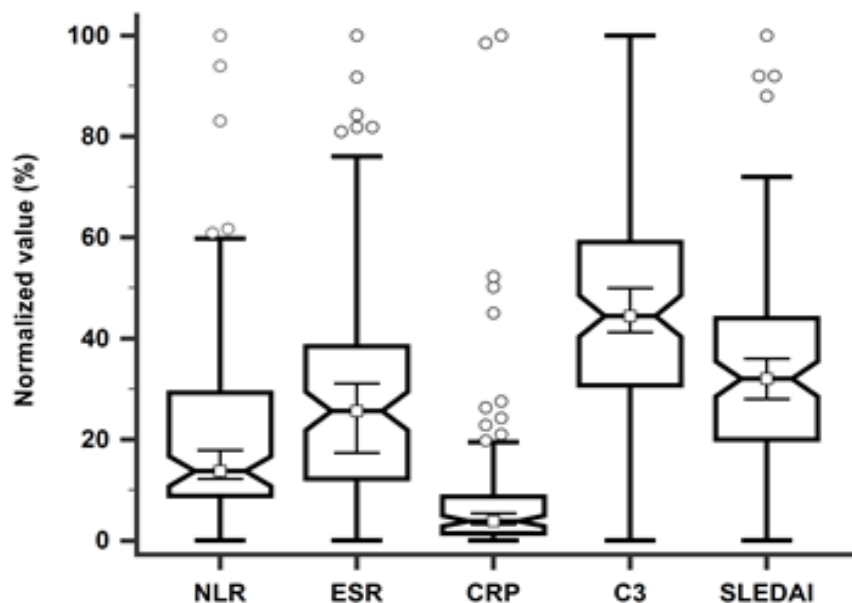
NLR, ESR and CRP were positively highly skewed, SLEDAI was moderately skewed and C3 had symmetric distribution.

of illness, TLC, NLR, ESR, CRP, C3, steroids and biologics did not differ among the SLEDAI disease activity groups.

The sample size of patients reclassified on NLR into 3 groups were as follows: ≤2: n=49, >2–4: n=43 and >4: n=25 (Table 2). The comparison of variables revealed TLC, lymphocyte count, CRP and steroids differed significantly across the groups. Age, gender, duration of illness, ESR, C3, SLEDAI, immunosuppressants and biologics did not differ among the groups.

Correlation and agreement of inflammatory parameters with SLEDAI and NLR

Correlation of demographic, clinical and inflammatory

Table 2: Demographic, clinical and inflammatory parameters in relation to NLR sub-groups in SLE patients

Variables	NLR groups			P value*
	≤2 (n=49)	2-4 (n=43)	>4 (n=25)	
Age (years)	34.53±10.94	32.93±11.15	35.72±9.9	0.57
Gender (M/F)	2/47 (40/42)	2/41 (40/36.6)	1/24 (20/21.4)	1
Duration of illness (months)	71 (6-228)	68 (4-216)	62 (9-217)	0.97
Total leucocyte count (cells/mm^3)	4760 (2230-10220)	5510 (2820-10020)	5560 (3360-11480)	0.02
Leucopenia assessment				
Leucopenia (<3000 cells/mm^3)	4 (80)	1 (20)	0 (0)	0.16
Normal (≥3000≤11000 cells/mm^3)	45 (40.5)	42 (37.8)	24 (21.6)	
Leucocytosis (>11000 cells/mm^3)	0 (0)	0 (0)	1 (100)	
Lymphopenia assessment				
Lymphopenia (<1500 cells/mm^3)	16 (24.6)	27 (41.5)	22 (33.8)	<0.01
Normal (≥1500 cells/mm^3)	33 (63.5)	16 (30.8)	3 (5.8)	
Erythrocyte sedimentation rate (mm/hr)	35 (8-96)	30 (4-125)	38 (9-103)	0.77
C-reactive protein (mg/L)	1.78 (0.3-9.5)	2.2 (0.3-48.9)	4.46 (0.3-48.2)	<0.01
Complement factor 3 (mg/dl)	80.58±25.59	76.53±23.77	78.48±27.91	0.75
SLEDAI	11 (6-29)	12 (4-27)	13 (4-21)	0.34
Immunosuppressants/DMARDs				
Single immunosuppressants/DMARDs	19 (54.3)	10 (28.6)	6 (17.1)	0.22
Double immunosuppressants/DMARDs	28 (38.4)	30 (41.1)	15 (20.5)	
3 or more immunosuppressants/ DMARDs	2 (22.2)	3 (33.3)	4 (44.4)	
Steroids				
Not currently on	34 (51.5)	25 (37.9)	7 (10.6)	<0.01
currently on	15 (29.4)	18 (35.3)	18 (35.3)	
Biologics				
Not Given	47 (43.5)	39 (36.1)	22 (20.4)	0.4
Given	2 (22.2)	4 (44.4)	3 (33.3)	

Variables represented as mean±sd for continuous variables with normal distribution, median (min-max) for continuous values without normal distribution and counts (percentage) for categorical values.

*patients with >4 NLR had increased TLC (p= 0.03) and CRP (p< 0.01) compared to ≤2 NLR group. Patients with ≤2 NLR had less than expected patients on steroid therapy (adj. res. -2.4) than those currently not on steroid therapy (adj. res. 2.4) and patients with >4 NLR had greater than expected patients on steroid therapy (adj. res. 3.2) than those current not on steroid therapy (adj.res. -3.2). Patients with ≤2 NLR had less than expected lymphopenia (adj. res. -4.2) than those with normal lymphocyte counts (adj. res. 4.2) and >4 NLR group had greater than expected lymphopenia (adj. res. 3.7) than those with normal lymphocyte counts (adj. res. -3.7).

Fig. 2: Mountain plot for agreement among inflammatory variables classified group-wise on SLE-DAI-2K into three groups in SLE patients
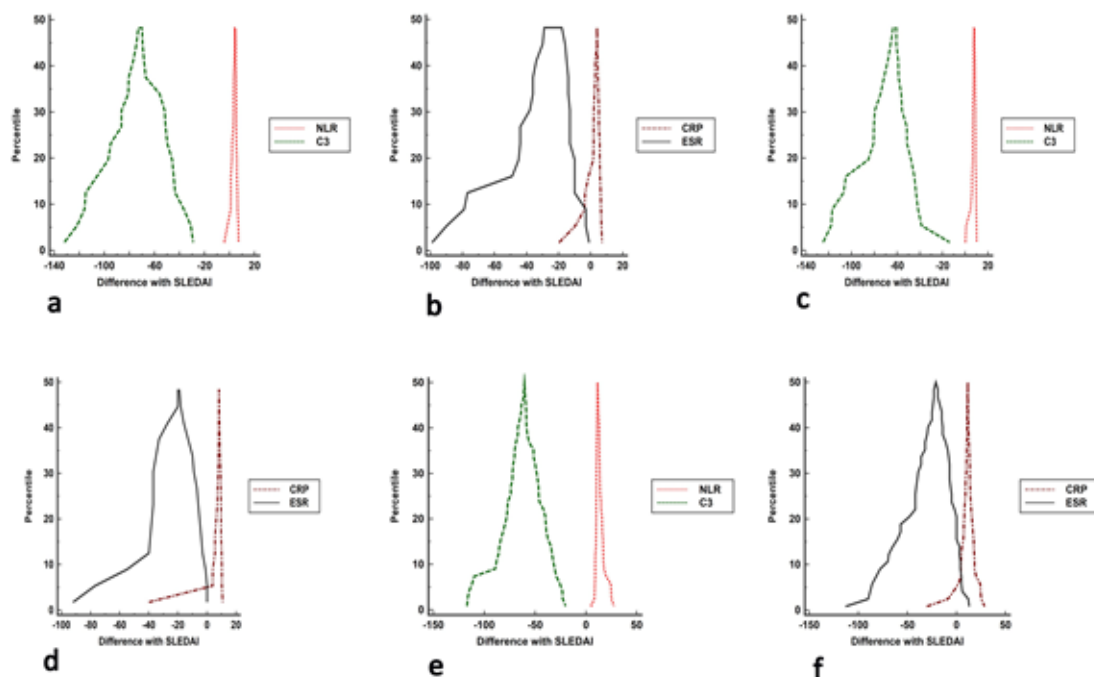
(a) NLR & C3 versus SLEDAI sub-group 1, (b) CRP & ESR versus SLEDAI sub-group 1, (c) NLR & C3 versus SLEDAI sub-group 2, (d) CRP & ESR versus SLEDAI sub-group 2, (e) NLR & C3 versus SLEDAI sub-group 3 (f) CRP & ESR versus SLEDAI sub-group 3. CRP had least bias for mild disease activity group, NLR had least bias for moderate disease activity group and both NLR with CRP had least bias for severe disease activity group.

variables is presented in supplementary table 1. Correlation was verified for inflammatory parameters of SLEDAI, NLR, ESR, CRP and C3 in both SLEDAI and NLR sub-groups. Values classified in SLEDAI sub-group 1 showed that CRP was strongly positively correlated with corresponding values of NLR (Supplementary table 2). Both NLR and ESR showed moderate correlation with CRP upon classification on the basis of SLEDAI sub-group 3. Inflammatory variables classified on NLR sub-group 1 showed moderate correlation between NLR and C3. Similar results were observed between ESR and CRP (Supplementary table 3). NLR sub-group 2 values had moderate inverse correlation with corresponding SLEDAI score. None of the variables showed correlation in NLR sub-group 3.

The estimates of Mountain plot were >3 median (bias) for all the variables. Mountain plot for differences and agreement of NLR, ESR, CRP and C3 with SLEDAI showed that the bias was least for CRP: 8.6 (2.5th and 97.5th percentiles, -15.45 and 24.37) (Supplementary fig. 1). Mountain plot of SLEDAI subgroups are presented in figure 2. Curves of CRP had the least bias of 3.95 (-17.7 and 7.02) for the

SLEDAI score of mild disease activity, NLR 7.87 (0.25 and 9.94) for moderate disease activity and NLR 11.49 (8.29 and 24.95) and CRP 11.58 (-8.56 and 25.28) for severe disease activity.

CRP had least median (bias) for all the three NLR sub-groups with reference to SLEDAI (Fig. 3). The differences for NLR sub-group 1 with SLEDAI showed a rightward shift for CRP 9.2 (0.31 and 25.63). The plots for the NLR sub-group 2 for SLEDAI was similar to NLR sub-group 1 for CRP 8.4 (-22.54 and 24.67). Mountain plots of NLR sub-group 3 showed slight decrease in median (bias) of 5.5 (-28.89 and 17.89) for CRP with respect to SLEDAI. NLR and CRP showed almost similar bias for the whole values, SLEDAI sub-group scores and NLR sub-group values. However, the spread indicated by 25th and 97.5th percentile, was more for CRP values and narrow for NLR values.

Discriminatory ability of NLR and evaluating factors associated with NLR
NLR, ESR and C3 failed to discriminate the severe disease activity group from mild-moderate disease activity group

Table 3: Linear regression estimates of factors influencing NLR ratio in SLE patients

Variables	Standardized Regression co-efficient	95% CI (Lower, Upper)	P value
Univariate linear regression			
Age (years)	0.05	-0.02, 0.04	0.56
Gender (Male, Female)	-0.07	-2.30, 1.10	0.48
Duration of illness (months)	-0.04	-0.02, 0.04	0.65
Total leucocyte count (cells/mm^3)	0.17	< -0.01, <0.01	0.07
Erythrocyte sedimentation rate (mm/hr)	0.12	<-0.01, 0.02	0.20
C- reactive protein (mg/L)	0.26	0.02, 0.11	0.01
Complement factor 3 (mg/dl)	-0.04	-0.02, 0.01	0.69
SLEDAI	-0.04	-0.09, 0.06	0.69
Immunosuppressants/DMARDs (single, double & 3 or more)	0.21	0.09, 1.27	0.03
Steroids (Not currently on, currently on)	0.29	0.44, 1.77	<0.01
Biologics (Not given, given)	0.14	-0.29, 2.27	0.13
Multiple linear regression			
Total leucocyte count (cells/mm^3)	0.08	<-0.01, <0.01	0.41
C- reactive protein (mg/L)	0.25	0.02, 0.11	<0.01
Immunosuppressants (single, double & 3 or more)	0.16	-0.02, 1.09	0.06
Steroids (Not currently on, currently on)	0.27	0.37, 1.67	<0.01
Biologics (Not given, Given)	0.12	-0.36, 2.06	0.17

Model statistics: F= 5.83, df= 5 and P <0.01

(Supplementary fig. 2). The area under the curves were as follows: NLR 0.554 (95% confidence interval (CI): 0.459, 0.646), ESR 0.559 (95% CI: 0.464, 0.650) and C3 0.510 (95% CI: 0.416, 0.604).

The univariate regression estimates demonstrated CRP, immunosuppressants/DMARDs and steroids were statistically significant (P <0.05) factors associated with NLR (Table 3). TLC and biologics were within the P ≤0.15. Age, gender, duration of illness, ESR, C3 and SLEDAI were not significant. Multiple linear regression was performed with TLC, CRP, immunosuppressants/ DMARDs, steroids and biologics as predictors of NLR. The analysis demonstrated that CRP and steroids were predictors of NLR with weak association (Table 3). The regression model was significant (F= 5.83, df= 5 and P <0.01). The model explained 20.8% of the variance (R2= 0.208). Multicollinearity and homoscedasticity diagnostics showed no violation of assumptions and the dataset did not include influential cases or outliers. The residuals were normally distributed. Sensitivity analysis showed patients on steroid therapy had significantly increased NLR than patients not on steroid therapy (Supplementary table 4). C3 levels were significantly lower in patients on steroid therapy.

Discussion

The present study demonstrated that NLR, ESR and C3 does not discriminate the patients with severe disease activity from those with mild-moderate disease activity. The study demonstrated that CRP and steroids were influencing the NLR and the association was weak. The NLR correlated only in the range of 2 to 4 with SLEDAI scores of moderate disease activity. The relationship of the NLR with SLEDAI was not consistent and this was reflected in Mountain plot analysis.

The study conducted by Wu et al. (2016) on SLE patients (n=116) has demonstrated a statistically significant positive correlation between SLEDAI and NLR.[26] The observations were in line with that noted by Yu et al. (2018).[27] Yu et al., based on a retrospective study involving 194 SLE patients

Fig. 3: Mountain plot of inflammatory variables classified group-wise on NLR values into three groups for agreement with SLEDAI scores in SLE patients
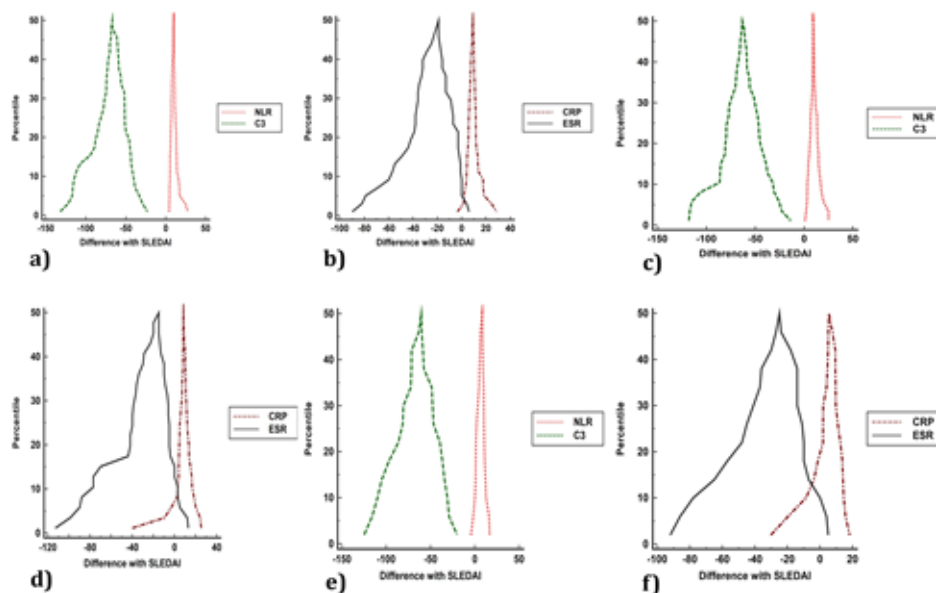
(a) NLR & C3 versus SLEDAI (NLR sub-group 1), (b) CRP & ESR versus SLEDAI (NLR sub-group 1), (c) NLR & C3 versus SLEDAI (NLR sub-group 2) (d) CRP & ESR versus SLEDAI (NLR sub-group 2) (e) NLR & C3 versus SLEDAI (NLR sub-group 3), (f) CRP & ESR versus SLEDAI (NLR sub-group 3). CRP had least bias for SLEDAI score in all NLR sub-groups compared to NLR, ESR, and C3.

and 71 healthy controls, have reported a significant positive correlation of SLEDAI with NLR, and a statistically significantly higher NLR in subjects with severe disease activity (>9 SLEDAI score) than mild disease activity (≤9 SLEDAI score). The current study has shown a significant correlation between SLEDAI and NLR. The correlation was inverse and presented in a narrow SLEDAI disease activity range for the NLR values 2-4.

Moreover, Qin *et al.* (2016) have reported a positive correlation of NLR with SLEDAI scores, CRP and ESR.[14] Haitao *et al.* (2018) have revealed that SLEDAI-2K, ESR and CRP had a significant positive correlation with NLR. The study noted that C3 or C4 was not correlated with NLR.[13] Similarly, Soliman *et al.* (2018) and Yu *et al.* (2018) have also reported a positive correlation of NLR with SLEDAI, ESR and CRP, and a negative correlation with C4.[17,27] The present study has also noted a strong positive correlation between NLR with CRP in mild SLEDAI disease activity group. However, a moderate correlation was noted between NLR and C3, among subjects belonging to the ≤2

NLR sub-groups. The metanalysis by Young Ho Lee *et al.* has suggested direct correlation between SLEDAI and NLR, indicating the possibility of lupus nephritis.[28] The current study suggests that the increased SLEDAI may not always be predicted by the elevated NLR. NLR was associated with CRP and an elevation in CRP was often considered as a marker in the background of SLE to reflect infection, serositis and synovitis. This suggests that the factors other than SLE activity would be influencing the NLR. The study by Kim *et al* has found that NLR was higher in the SLE with infection compared to patients with SLE with flare (14.2 ± 15.4 versus 3.3 ± 2.2, P <0.001).[18] Combination of CRP and NLR could enhance the prediction of infection over the flare, but estimates were not significant. That was one of the reasons for exclusion of patients with recent infections in the present study. The steroid and immune suppression, as expected, had influenced the NLR value in the present work.

 The current study evaluated whether NLR was comparable to SLEDAI and for different subgroups with respect to

disease activity ranges. The correlation of a measure suggests linear association but does not necessarily, quantify similarity or bias. Methods comparing distribution of various measures indicate location of differences.[29] The Mountain-plot analysis, carried out to assess the agreement of NLR and CRP with SLEDAI, demonstrated CRP to be superior to NLR.

Patient's age and number of immunosuppressant differed significantly among the mild (4-8), moderate (9-11) and severe (≥12) SLEDAI disease groups, whereas NLR, CRP, ESR, C3 and steroid usage did not differ significantly. The dose of steroid, if considered, could have differed among the groups. However, sensitivity analysis of patients on and not on steroid therapy did not demonstrate significant difference in SLEDAI. Even by ROC analysis, the inflammatory markers such as NLR, ESR and C3 failed to discriminate severe and mild-moderate disease activity groups.

When subgrouped based on NLR, patients with >4 NLR had elevated TLC and CRP levels and reduced lymphocyte counts. Moreover, among subjects with >4 NLR, steroid usage was significantly higher compared to other NLR subgroups. This substantiates the fact that steroid has a stronger influence on the NLR. Influence of CRP and steroids on NLR was corroborated in regression analysis. The sensitivity analysis revealed patients on steroid therapy had significantly higher NLR. Chronic use of steroid influences NLR. Corticosteroids cause demargination of neutrophils contributing to increase in circulating neutrophil counts. It also causes depletion of lymphocytes. Increase in neutrophil counts and depletion of lymphocytes leads to skewing of the NLR.[30] In the current study SLEDAI was not significantly different among the NLR sub-groups.

The major limitation is the number of patients and even lesser number in the sub-groups. SLE being a heterogeneous disease, the organ involvement and treatment were heterogeneous. The single-centre study limits the generalizability of the results and recommends evaluation in a larger sample. The number of male patients was less to draw meaningful conclusions about influence of gender on SLEDAI and NLR. SLEDAI as a classified variable could have provided varied result. Penalized likelihood regression method provides more reliable estimates. The obtained findings could vary in treatment naïve SLE patients and needs to be validated separately. However, the parameters were analysed by different analytical approaches and the findings from these methods is the strength of the study. Each method excludes the bias of different nature.[31]

In conclusion, NLR as a marker of inflammation or as a predictor of SLE disease activity was not consistent and needs further investigation. SLEDAI was not associated with NLR, whereas CRP was a predictor of NLR. It is important to consider the influence of steroids and immune-suppressive drugs, while interpreting the NLR. A better understanding of various measures of disease process could explain different aspects of disease and may be important in additional diagnosis and clinical management.

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