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The Association Between Neutrophil/Lymphocyte Ratio and Disease Activity in Rheumatoid Arthritis and Ankylosing Spondylitis

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Background: Elevated neutrophil count is associated with poor prognosis and increased mortality in many conditions. Neutrophil to lymphocyte ratio (NLR) has emerged as a marker of inflammation in neoplastic and cardiovascular disorders. Herein, we investigated utility of this simple tool in rheumatoid arthritis (RA) and ankylosing spondylitis (AS). *Methods:* The study consisted of 136 RA and 140 AS patients, along with 117 healthy control subjects. RA and AS activities were determined with Disease Activity Score (DAS) and Bath Ankylosing Spondylitis Disease Activity indices (BASDAI), respectively. The association between NLR and disease activity was analyzed. *Results:* Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and neutrophil counts were significantly higher in RA and AS patients compared to healthy controls. Similarly, NLR was higher compared to control subjects, both in RA (2.53 ± 1.4 vs. 2.16 ± 1.0, $P = 0.019$) and AS (2.43 ± 1.4 vs. 2.16 ± 1.0, $P = 0.077$). NLR correlated well with ESR and CRP, both in RA and AS. Moreover, NLR increased across worsening DAS28 activity groups (2.1 ± 1.0 in patients with remission, 2.5 ± 1.0 in low–moderate, 3.8 ± 2.5 in high disease activity). However, no association was found between NLR and BASDAI. *Conclusion:* NLR is a cheap and readily available marker for the assessment of disease activity in RA. J. Clin. Lab. Anal. **30**:597–601, 2016. © 2015 Wiley Periodicals, Inc.

Key words: neutrophil to lymphocyte ratio; rheumatoid arthritis; ankylosing spondylitis; disease activity, neutrophil

INTRODUCTION

Inflammation is the key determinant and primary underlying mechanism leading to disability and increased mortality in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS) (1). Therefore assessment of inflammation in RA and AS with reliable markers is crucial to predict long-term outcome of a particular patient. The most commonly used markers for this purpose are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in daily practice (2). However, both of these markers have some limitations, such as reflection of short-term inflammatory activity and low discrimination ability with other superimposed inflammatory conditions (3, 4).

Systemic inflammation is associated with alterations in circulating blood cells quantity and composition. Indeed, normochromic anemia, thrombocytosis, neutrophilia, and lymhopenia usually accompany many inflammatory conditions (5). Therefore, the features of circulating blood cell components can be used for the assessment of inflammatory activity (6).

Increased neutrophil count and low lymphocyte count are independent predictors of mortality in longitudinal studies as shown in Baltimore Longitudinal Study of Aging (7, 8). Neutrophils are primed in active RA patients and have potential to aggravate disease by secreting

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proteases, prostaglandins, and reactive oxygen intermediates to synovial space and by activating other cells via secretion of B-lymphocyte stimulator (BLyS), tumor necrosis factor, IL-17, and many other mediators (9–11).

Neutrophil to lymphocyte ratio (NLR) is the proportion of absolute neutrophil count to lymphocyte count, which is derived from routine complete blood count (CBC) test. It has become widely accepted that NLR is a useful tool for the evaluation of inflammatory activity in chronic inflammatory disorders like ulcerative colitis and familial Mediterranean fever (12, 13). Moreover, CBC is carried out routinely in patients with RA and AS for the monitoring of side effects of the medications and possible disease associated alterations. However, the association between NLR and chronic inflammatory arthritides has been scarcely studied. Hence, we aimed to investigate whether NLR altered in RA and AS patients and if it is altered, whether it correlates with disease activity.

METHODS

Patients and Controls

One hundred thirty-six RA and 140 AS patients were enrolled into the study consecutively, and 117 healthy individuals constituted the control group. Diagnosis of RA was made according to 1987 ACR criteria and AS was made using modified New York criteria (14, 15). Subjects with hematologic disorders other than anemia, concomitant infectious or inflammatory diseases such as ulcerative colitis, liver, or kidney disease, diabetes mellitus, known or clinically symptomatic cardiovascular diseases, and those with history of cancers were excluded. All patients underwent a workup including detailed history and physical examination including arthritis history, recent or concurrent infections, medications, disease activity, and disease-related sequelae. The activity of RA was determined with DAS28 score and AS was determined with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (16,17). Following DAS28-CRP cutoffs were used for classification of RA activity: ≤ 2.6 remission ($n = 67$), 2.6 through 5.1 low–moderate activity ($n = 44$), ≥ 5.1 high activity ($n = 25$). The study protocol was approved by local research ethics committee.

Biochemical and Hematological Measurements

Peripheral venous blood samples were drawn from antecubital vein of patients after overnight fasting. CRP and pertinent blood biochemistry (glucose, liver, and kidney function tests) were analyzed from freshly separated serum with central laboratory of the hospital. Total and differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were determined from EDTA anticoagulated blood samples with use of an automated coulter counter. ESR was determined with Westergren method and CRP was measured with turbidimetric method. As a hospital policy all samples were studied within 2 h after the collection.

Statistical Analysis

Data are expressed as mean $\pm$ SD for continuous variables and as percentage for categorical variables. Normality distribution was assessed by using the Kolmogorov–Smirnov test. Categorical and numerical variables of healthy subjects and disease groups were compared with chi-square test, Student's *t*-test, or analysis of variance tests. Correlation of NLR with other disease activity measures and acute phase reactants were tested with Pearson's correlation test. Linear regression analysis was used to determine contribution of factors on NLR. P values < 0.05 were considered significant throughout the study.

RESULTS

Demographic data of patients and control groups are given in Table 1. Mean age of RA patients was higher than AS patients and control subjects. There are more female patients in RA group. Mean disease duration in RA and AS patients were 9 and 12 years, respectively.

Both ESR and CRP levels were remarkably higher and hemoglobin level was lower in RA patients compared to control subjects (Table 1). Neutrophil count and NLR were significantly higher in RA group. Moreover, NLR increased across worsening DAS28 activity groups (2.1 ± 1.0 in patients with remission, 2.5 ± 1.0 in low–moderate activity, 3.8 ± 2.5 in patients with high disease activity). There was no difference in NLR between control subjects and RA patients in remission as determined with DAS28-CRP.

Similar to RA patients ESR, CRP, and neutrophil counts were higher and hemoglobin was slightly lower in patients with AS. NLR was slightly higher in patients with AS but did not reach a statistical significance.

The correlation of NLR with disease activity measures of RA and AS were depicted in Table 2. Although there was a moderate correlation between NLR and acute phase response, none of these measures correlated with BASDAI. On contrary, all these parameters correlated well with the composite disease activity indices DAS28-CRP and DAS28-ESR in RA patients. Linear regression analysis showed that DAS2-28-CRP is the only determinant of NLR in RA patients ($R = 0.30$, 95% confidence intervals 0.08–0.51) among variables age, sex, hemoglobin levels, and disease-modifying antirheumatic drugs (DMARD) treatment.

TABLE 1. Demographic, Clinical, and Laboratory Characteristics of Control Subjects, Rheumatoid Arthritis, and Ankylosing Spondylitis Patients

	Control subjects $N = 117$	Rheumatoid arthritis $N = 136$	Ankylosing spondylitis $N = 140$	P_1	P_2
Age (years)	37.2 ± 13.2	51.7 ± 13.5	37.1 ± 9.8	<0.001	0.001
Sex (F/M)	75/42	121/15	61/79	<0.001	0.9
ESR (mm/h)	16.8 ± 10.7	31.2 ± 17.6	29.9 ± 19.5	<0.001	<0.001
CRP (mg/l)	3.2 ± 2.0	11.7 ± 14.9	13.8 ± 17.8	<0.001	<0.001
WBC (/μl)	7,315 ± 1,846	7,807 ± 2,619	8,180 ± 2,132	0.09	0.001
Neutrophil count (/μl)	4,440 ± 1,443	4,918 ± 2,204	5,025 ± 1,821	0.04	0.005
Lymphocyte count (/μl)	2,222 ± 590	2,150 ± 907	2,374 ± 910	0.46	0.12
NLR	2.16 ± 1.0	2.53 ± 1.4	2.43 ± 1.4	0.019	0.077
PLT ($\times 10^3$/μl)	270 ± 67	289 ± 76	299 ± 91	0.16	0.03
Hb (g/dl)	13.9 ± 1.4	12.9 ± 1.5	13.4 ± 1.8	<0.001	0.016
DAS28-CRP	-	3.34 ± 1.4	-		
DAS28-ESR	-	4.6 ± 1.3	-		
BASDAI	-	-	3.91 ± 2.32		

P_1: control versus rheumatoid arthritis; P_2: control versus ankylosing spondylitis.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; NLR, neutrophil to lymphocyte ratio; WBC, white blood cell count.

DISCUSSION

Results of this study have demonstrated that NLR is elevated in RA and AS patients. Furthermore, findings suggest that NLR correlates well with disease activity measures in RA patients.

NLR is a routinely available marker derived from the absolute neutrophil and lymphocyte counts of a CBC. The utility of this simple method in the assessment of systemic inflammation has been clearly demonstrated in various disorders including coronary artery disease, diabetes mellitus, Alzheimer disease, ulcerative colitis, cirrhosis, and cancers (12, 18–23). Furthermore, the NLR has been shown to have prognostic value, independent of other factors in cardiovascular disorders and variety of cancers (21, 23).

ESR and CRP are the most widely used markers for measuring acute phase response due to their reliability, reproducibility, and cost effectiveness. Both of these markers correlate closely with clinical disease activity of RA and to a lesser extent with AS (2–4). Our results are in accordance with these previous observations. ESR and CRP are influenced by several factors unrelated to inflammation such as age, sex, anemia, and renal failure. In our control subjects ($n = 117$), we found that NLR is not affected by age, gender, and hemoglobin level of a subject. Neither these factors nor the used drugs (methotrexate, leflunomide, steroids, sulfasalazine, or biologics) had an impact on NLR in patient groups. The advantages of NLR are its reproducibility, availability, lower cost, and good correlation with inflammatory activity.

TABLE 2. The Correlations Between Disease Activity Measures and Neutrophil to Lymphocyte Ratio (NLR)

Ankylosing spondylitis				
	ESR	CRP	BASDAI	
ESR	-	-	Ns	
CRP	$r = 0.69$ $P \leq 0.001$	-	Ns	
NLR	$r = 0.25$ $P = 0.003$	$r = 0.37$ $P \leq 0.001$	Ns	
Rheumatoid arthritis				
	ESR	CRP	DAS28 CRP	DAS28 ESR
ESR	-	-	$r = 0.50$ $P \leq 0.001$	$r = 0.68$ $P \leq 0.001$
CRP	$r = 0.54$ $P \leq 0.001$	-	$r = 0.50$ $P \leq 0.001$	$r = 0.39$ $P = 0.01$
NLR	$r = 0.21$ $P = 0.01$	$r = 0.27$ $P = 0.001$	$r = 0.31$ $P \leq 0.001$	$r = 0.28$ $P = 0.002$

Ns, nonsignificant; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; NLR, neutrophil to lymphocyte ratio; WBC, white blood cell count.

Neutrophil homeostasis is maintained by excellent balance between granulopoiesis, retention, and release from bone marrow and clearance with apoptosis (24). Granulocyte colony-stimulating factor (G-CSF) is chief cytokine in the regulation of granulopoiesis. G-CSF induces myeloid proliferation, differentiation, and mobilizes neutrophils from the bone marrow (24). IL-17 and IL-23 also have roles in the regulation of neutrophil homeostasis (25, 26). Increased neutrophil count in active RA patients can be explained by increased levels of G-CSF. It has been show that serum G-CSF concentrations correlate well with the disease activity of RA (10). In neoplastic disorders, there was a positive correlation between increased NLR and certain cytokines including; IL-1ra, IL-6, IL-7, IL-8, IL-12, IL-17, monocyte chemotactic protein-1 (MCP-1), and platelet-derived growth factor (PDGF) (27), and some of these are also involved in the pathogenesis of RA and AS (11, 28). Alternatively, increased neutrophil count could be explained by impaired neutrophil apoptosis, which is associated with increased levels of antiapoptotic cytokines, such as IL-2, IL-4, and IL-15 in active RA patients (10). On the other hand, lymphopenia is a well recognized but poorly understood finding of RA and is associated with more severe disease (29). IL-23, a critical cytokine in the pathogenesis of AS may be associated with increased neutrophil count observed in our study (30).

Our results are in accordance with two recently published reports that showed increased NLR in RA (31, 32). NLR also correlated well with disease activity of RA in one of these reports (32). Our primary aim was to investigate whether NLR is altered in two most common arthritic conditions. However, utility of this simple tool in prediction of long-term prognosis merits further investigations.

CONCLUSION

NLR is a cheap and readily available marker of inflammation that is well correlated with other inflammatory markers and disease activity indices in RA. Hence, NLR could be a useful single tool for the assessment of disease activity in RA. However, the possible association between NLR and outcome of inflammatory arthritides remain to be investigated in prospective long-term studies.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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