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Clinical Research

Neutrophil to Lymphocyte Ratio: A Simple and Readily Available Independent Marker of Mortality in Acute Ischemic Stroke

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ABSTRACT

Objective: We performed this study to identify the prognostic value of neutrophil/lymphocyte ratio (NLR) in patients presenting with acute ischemic stroke (AIS).

Material and Method: We retrospectively analyzed 209 consecutive AIS patients and 202 age and sex matched healthy control subjects admitted to neurology clinics of Ankara Numune Training and Research Hospital and Ankara Training and Research Hospital between January and June 2014. Patients were classified into admission National Institute of Health Stroke Scale (NIHSS) score strata, and stroke subtypes were classified using Trial of Org 10172 in Acute Stroke Treatment (TOAST) and Bamford classifications. Mortality and functional outcome using modified Rankin Scale (mRS) was determined at 3 months.

Results: The mean age of patients was 68.5±13.9 years (106 men and 103 women). NLR was significantly higher in AIS group (4.00±3.76) compared to control group (2.18±0.99) (p <0,001). In patients with NIHSS≥16, NLR and mean platelet volume (MPV9) levels were significantly higher compared with moderate (6-15) and mild (≤5) NIHSS score group (p <0.001 and p =0.001, respectively). CRP levels were significantly higher in severe and moderate group (p <0.001). When patients are grouped according to functional outcomes at 3 months, NLR levels were significantly higher in patients who died (p =0.001) and CRP levels were significantly higher in patients who died and those with unfavorable outcome groups (p <0.001).

Conclusion: NLR may be used as a simple and easy-to-measure independent marker for prediction of mortality in acute ischemic stroke patients.

Keywords: *Neutrophil/Lymphocyte Ratio, Acute Ischemic Stroke, Mortality.*

ÖZET

Nötrofil Lenfosit Oranı: Akut İskemik İnmede Mortalitenin Basit, Kolay ve Bağımsız Belirteci

Amaç: Akut iskemik inme nedeniyle başvuran hastalarda nötrofil/lenfosit oranının (NLR) prognostik değerini belirlemek için bu çalışma yapıldı.

Gereç ve Yöntem: Ocak ve Haziran 2014 tarihleri arasında Ankara Numune Eğitim ve Araştırma Hastanesi ve Ankara Eğitim ve Araştırma Hastanesi nöroloji kliniğine başvuran Hastalar geriye dönük olarak incelendi, 209 akut iskemik inme (AIS) hastası ve 202 kontrol grubu incelendi. Sağlık inme ölçeği skoru (NIHSS) ve inme alt tiplerini belirlemek için TOAST ve Bamford sınıflaması kullanıldı. Modifiye Rankin Skalası (mRS) kullanarak fonksiyonel değerlendirme ve ölüm sonuçları 3. ayda değerlendirildi.

Bulgular: Hastaların yaş ortalaması 68.5±13.9 olarak belirlendi (106 erkek ve 103 kadın). AIS grubunda (4.00±3.76) kontrol grubuna (2.18±0.99) göre NLR anlamlı olarak yüksek bulundu (p <0.001).

NIHSS≥16 puan hastalarda, NLR ve Mean Platelet Volüm (MPV) düzeyleri NIHSS≤5 skor grubu ve NIHSS=6-15 ile karşılaştırıldığında anlamlı olarak yüksek bulundu (sırasıyla, p <0.001 ve p =0.001).

CRP düzeyleri NIHSS skoru ağır ve orta olan gurupta anlamlı olarak yüksek bulundu (p <0.001). Hastalar 3 aylık fonksiyonel sonuçlarına göre gruplandırıldığı zaman mortalite oranları NLR düzeyleri yüksek olan hastalarda anlamlı olarak yüksek bulundu (p =0.001).

Sonuç: NLR, akut iskemik inmeli hastalarda mortalite tahmini için basit, kolay ve bağımsız belirteç olarak kullanılabilir.

Anahtar Sözcükler: *Nötrofil/Lenfosit Oranı, Akut İskemik İnme, Ölüm Oranı.*

Inflammatory markers such as erythrocyte sedimentation rate, C-reactive protein (CRP) and interleukin-6 are shown to be significantly associated with development of cardiovascular disease (1, 2). Inflammatory biomarkers such as white blood cell (WBC) count and its subtypes are associated with cardiovascular disease outcomes. Neutrophils play a major role in atherogenesis and atherothrombosis (3). Relative lymphocytopenia is seen among patients with acute coronary syndrome which is thought to be induced by increased cortisol release (4). In acute myocardial infarction, increased neutrophil to lymphocyte ratio (NLR) is correlated with in-hospital mortality and morbidity, and impaired myocardial perfusion after percutaneous coronary angioplasty (5). NLR has recently been used to predict individuals at high risk for upcoming cardiovascular events (6). In contrary to coronary artery disease, the effect of NLR on acute ischemic stroke has been studied in few studies (7, 8). In this study, we aimed to investigate the prognostic role of NLR in acute ischemic stroke patients.

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MATERIAL AND METHOD

Patient Selection: We retrospectively analyzed 209 consecutive acute ischemic stroke (AIS) patients and 202 age and sex matched healthy control subjects admitted to neurology clinics of Ankara Numune Training and Research Hospital and Ankara Training and Research Hospital between January and June 2014.

Patient Classification: Patients were classified according to stroke severity at admission using National Institutes of Health Stroke Scale (NIHSS) as mild (0-5), moderate (6-15), severe (≥16), Etiological subtypes were determined by Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (9). Ischemic stroke was further subdivided according to the topographic of ischemic lesion on CT scan using Bamford classification as total anterior circulation stroke, partial anterior circulation syndrome, posterior circulation syndrome and lacunar syndrome (10). Functional outcome at 3 months was determined using modified Rankin Scale (mRS) and classified as favorable (mRS 0-2), unfavorable (mRS 3-5) and exitus (mRS 6).

Inclusion and Exclusion Criteria: Patients with a diagnosis of acute ischemic stroke on the first day of admission were included in the study. Exclusion criteria included patients who admitted to the hospital beyond 24 hours after AIS, patients with hematologic, inflammatory diseases, severe hepatic and renal insufficiency, immunosuppressant (steroids) or non-steroidal anti-inflammatory drug users, those with an infection history within last 2 weeks, major cardiovascular disease history within 6 months (myocardial infarction, congestive heart failure) and patients with a history of malignancy. The study was approved by the local ethics committee.

Selection of Controls: The control group consists of the subjects that reported to the outpatient Neurology clinic of Ankara Numune Training and Research Hospital and Ankara Training and Research hospital between January and June 2014. Regardless of age, the controls had headache and vertigo as presenting complaint and were admitted to the inpatient care on Neurology floor.

Laboratory Tests: Complete blood count was performed using the peripheral venous blood samples acquired on admission to the emergency department. The blood sample was collected in a calcium ethylenediaminetetra-acetic acid tube. Blood counts were estimated with an auto analyzer (Beckman Coulter, Fullerton, CA, USA). NLR was calculated as the ratio of neutrophils to lymphocytes in peripheral blood. Mean platelet volume (MPV) and red cell distribution width (RDW) values were also studied. The C-reactive protein (CRP) level was determined by the CRP assay (Bayer, Leverkusen, Germany), fibrinogen levels were measured using coagulometric method (Multifibren, Dode Behring/BCS, Marburg, Germany).

Statistical Analysis: Data were analyzed using SPSS software (version 22.0; SPSS Inc, Chicago, IL). Numerical variables were presented as mean±standard deviation or median (min-max) and categorical variables were presented as numbers with percentages. We determined the parametric test assumptions (normality and homogeneity of variances) for numerical variables using Shapiro Wilks test. The variances of the compared groups were analyzed using Levene test. When parametric test assumptions were met, t test was used and when parametric assumptions were not met, Mann Whitney U test was used for analysis. For the comparison of more than two groups, Kruskal Wallis test was used if parametric test assumptions were not met. Binary comparisons were made using Siegel Castellan test. The difference between groups in terms of categorical variables was determined using the Chi-square test. P value<0.05 was considered as significant.

RESULTS

The mean age of 209 AIS patients included in the analysis was 68.5±13.9 years (106 were men). The mean age of 202 healthy control subjects was 67.5±11.2 years (112 men and 90 women). Patient demographic and clinical characteristics were shown in Table 1.

NLR levels were significantly higher in AIS group (4.00±3.76) compared to control group (2.18 ± 0.99) (p <0.001). There was no statistically significant difference between men (3.73±3.30) and women (4.26±4.16) in the AIS group in regards to NLR (p=0.474).

***Table 1**. Clinical and demographic characteristics acute ischemic stroke patients and controls.*

	Patient n:209	**Control n:202**
Age (mean ± SD)	68.5±13.9	51.9±15.2
Sex (F/M) n	103/106	90/112
%	(49.3% - 50,7%)	(44,6% - 55,4%)
Comorbidity		
CAD (%):	20.0	2.4
DM (%):	29.1	9.8
HL (%):	14.8	7.4
HT (%):	63.1	24.7
AF (%):	12.9	4.9
Smoking (%):	25.6	39.6
Stroke type		
Large-artery atherosclerosis	38.7% (n:81)	-
Cardioembolism	27.2% (n:57)	-
Small-vessel occlusion	16.7% (n:35)	-
Stroke of other determined etiology	13.8% (n:29)	-
Stroke of undetermined etiology	3.3% (n:7)	-
LDL, mg/dL (mean ± SD):	126.3±37.6	118±23.4
HDL, mg/dL (mean ± SD):	42.69±10.2	46±13.5
NIHSS, (mean ± SD):	7.2±5.1	-
NLR, (mean ± SD):	4.01±3.7	2.18±0.99
RDW, (mean ± SD):	14.6±2.3	13.8±2.1
CRP, (mean ± SD), (n:170)	6.4±8.3	4.6±2.4

***NIHSS:** National Institute of Health Stroke Scale; **NLR:** Neutrophil/lymphocyte ratio; **RDW:** Red cell distribution width; **CRP:** C-reactive protein; **CAD**: Coronary artery disease; **DM**: Diabetes mellitus; **HL**: Hyperlipidemia; **HT**: Hypertension; **AF**: Atrial fibrillation.*

In patients with NIHSS≥16, NLR and MPV levels were significantly higher compared to patients with NIHSS 6-16 and those with NIHSS≤5 (p<0.001 and p=0.001, respectively). CRP levels were significantly higher in patients with NIHSS≥16 and those with NIHSS 6-16 (p<0.001) (Table 2).

Table 2. *Comparison of NIHSS scores with NLR and other laboratory parameters.*

	NIHSS≤5 (n=105)		**NIHSS 6-15 (n=85)**		**NIHSS≥16 (n=19)**		***p***
	Mean±SD	*Median (Range)*	*Mean± SD*	*Median (Range)*	*Mean± SD*	*Median (Range)*	
NLR	3.3±3.3	2.3 (0-22)	4.3±4.0	3 (1-24)	6.5±4.2	7 (2-18)	<0.001*
CRP	5±7	1.5 (0-36)	20.8±55.1	6,1 (0-329)	28.5±56.6	10.4 (3-214)	<0.001†
RDW	14.6±2.2	14.2 (12-25)	14.5±1.8	14 (11-25)	15.5±4.2	13.9 (13-29)	0.85
MPV	8.7±1.1	8.6 (6-12)	9.6±7.6	8.6 (7-78)	10±1.5	9.8 (8-14)	0.001*
Fibrinogen	46.3±106.9	340 (112-565)	394.6±159.5	339 (139-787)	341.1±149.6	321(122-531)	0.63

NIHSS: *National Institute of Health Stroke Scale;* ***NLR:*** *Neutrophil/lymphocyte ratio;* ***CRP:*** *C-reactive protein;* ***RDW:*** *Red cell distribution width;* ***MPV:*** *Mean platelet volume. Results are expressed as mean ± SD.*NIHSS≥16 group is significantly different than other groups. †NIHSS 0-5 group is significantly different than other groups.*

Among subtypes of TOAST classification, NLR was significantly higher among patients with cardio embolic ischemic stroke (4.99±4.17) compared with those with lacunar infarction (3.47±4.15) (p=0.010). MPV was significantly higher in patients with stroke of other determined etiology and stroke of undetermined etiology group compared to large artery atherosclerosis and lacunar groups (9.6±1.3 and 8.6±1 and 8.3 ± 0.9, respectively, p<0.001). CRP, RDW and fibrinogen levels were not significantly different between groups stratified according to etiologic subtypes.

When patients are analyzed in regards to Bamford classification, NLR and CRP were significantly higher in total anterior circulation compared to other groups (p=0.001), RDW, MPV and fibrinogen levels were not significantly different between groups.

NLR levels were significantly higher exitus group, which was significantly different than other groups. CRP levels, exitus and unfavorable outcome groups were significantly different than favorable outcome group. High NLR has also predicted the 3 months' mortality in AIS patients (Table 3).

Table 3. *Comparison of mRS scores with NLR and other laboratory parameters.*

	mRS 0-2 (n=133)		**mRS 3-5 (n=39)**		**Exitus (n=36)**		***p***
	Mean± SD	*Median (Range)*	*Mean± SD*	*Median (Range)*	*Mean± SD*	*Median (Range)*	
NLR	3.3±3.0	2.6 (0-22)	4.4±3.46	3 (1-13)	5.9±5.4	4 (1-24)	0.001*
CRP	5.4±8.3	1.5 (0-53)	16.6±26.8	10 (0-123)	41.4±88.1	7 (0-329)	<0.001†
RDW	14.5±2	14 (12-25)	14.8±3.2	13.8 (12-29)	15.1±2.3	14.4 (12-25)	0.11
MPV	9.2±6.2	8.6 (7-78)	9.0±1.2	8.9 (7-12)	9.3±1.6	8.9 (7-14)	0.15
Fibrinogen	348.1±104.4	339.5 (112-565)	433.8±181.1	432.5 (139-787)	334.7±149.7	333 (122-659)	0.16

mRS: *Modified Rankin Scale;* ***NLR:*** *Neutrophil/lymphocyte ratio;* ***CRP:*** *C-reactive protein;* ***RDW:*** *red cell distribution width;* ***MPV:*** *Mean platelet volume. Results are expressed as mean ± SD.* Exitus group was significantly different than other groups. † Exitus and unfavorable outcome groups were significantly different than favorable outcome group.*

DISCUSSION

We observed that NLR levels were significantly increased in AIS group. NLR levels were shown to increase in high NIHSS score group together with MPV levels. When we compared patients according to the functional outcome status at 3 months, NLR levels were increased significantly in the exitus group where levels of CRP which is another inflammatory marker had increased in the exitus group and also in the severely disabled patients (mRS 3-5). NLR and CRP increase was demonstrated in total anterior circulation strokes. Cardio embolic stroke subtype has significantly increased NLR levels when compared to lacunar stroke subtype.

WBC count and its subtypes were shown as inflammatory markers in cardiovascular diseases (11). Also NLR was indicated as a marker of systemic inflammation (12). Lymphopenia and NLR has been associated with mortality in ST segment elevation myocardial infarction and decompensated heart failure (13-15). Likewise, Azab et al (16), demonstrated that NLR is a strong predictor of poor outcomes in stable and unstable coronary artery disease and an independent predictor of both short and long term mortalities in non ST segment myocardial infarctions. In another study in 2833 acute coronary syndrome patients, admission NLR predicted in-hospital and six months' mortality (17).

Studies in stroke patients are limited in the literature. Celikbilek et al (7), demonstrated a significant increase of NLR in 70 AIS patients (p<0.001). NLR increase was significant in exitus patients (p=0.029). In another study by Tokgoz et al (5), NLR has been shown to be a predictor of short term mortality in acute stroke patients in this study, when NLR is higher than 5, for short term mortality sensitivity was 83.1% and specificity was 62%. There was a strong correlation between NLR and NIHSS score. In another study, mean NLR was significantly higher in 126 acute ischemic stroke patients with non-valvular atrial fibrillation (9).

It is known that inflammatory process develops within 6-24 hours of ischemic stroke and as a response to

ischemic brain injury; neutrophils migrate to the damaged area resulting in accumulation in the ischemic brain tissue. Baseline neutrophil count is reported to increase the severity of ischemic damage and can cause poor neurologic outcome (18). On the other hand, lymphocytes began to increase 1 day after the stroke and peaks in the seventh day and particularly the effect of T cell lymphocytes is reported to have repair effects on inflammation (19). Higher neutrophil counts together with lower lymphocyte counts resulting with high NLR mean large damaged area with less repair effects resulting with poor outcome. Our study has also shown significantly higher NLR in non-survived patients. In the last decade, it is indicated that neutrophilia and lymphopenia are related independently with increased cardiovascular risk. Especially the maximum NLR ratio is stated to be a predictor factor on mortality in myocardial infarction (11, 20).

In our study, NLR levels increased significantly in total anterior circulation strokes where the infarct volume is higher compared to other groups. High NLR has also predicted the 3 months' mortality in AIS patients. NLR calculation which is an easy method to be performed at admission using the routine complete blood count can provide valuable information in addition to other clinical and radiological parameters such as NIHSS score and infarct volume. Also this study can be helpful to encourage experimental studies targeting the effects of inflammatory cells in terms of treatment of acute ischemic stroke as there are conflicting results concerning effects of neutrophils. In animal experiments, decrease of neutrophils has been shown to improve clinical course whereas there are some publications stating anti-neutrophil treatment is ineffective (20-24).

This was a retrospective and non-randomized single center study that included a relatively small number of patients. Therefore, the possibility of selection bias and/or residual confounding from unknown or unmeasured covariates cannot be excluded. Further studies with a larger sample size may be needed. NLR taken in the study was calculated from only complete blood count and consecutive counts taken may have more valuable correlations with stroke subtypes and functional outcomes.

These findings support the role of NLR as a simple, inexpensive and readily available marker of mortality in acute ischemic stroke. As NLR in acute ischemic stroke was studied in only few studies in the literature, the role of neutrophils and lymphocytes is still under debate and has to be explored in future clinical trials.

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