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ORIGINAL ARTICLES

Neutrophil-to-Lymphocyte ratio may have a role in the differential diagnosis of ischemic stroke

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Abstract

There is increasing evidence that inflammation is involved in the mechanisms of acute ischemic stroke. The utility of neutrophil-to-lymphocyte ratio (NLR) has recently been reported to help in the prognostication of stroke. The utility of NLR in the diagnosis of stroke remains uncertain. In this retrospective study, patients with acute ischemic stroke who presented within the first 24 hours of symptom onset to our clinic from September to December 2019 were studied, involving 93 patients with stroke and 60 control. The mean age in the stroke group was 71.7 ± 12.2 years whereas it was 72.6 ± 5.7 in the control group (p=0.405). The female/male ratio was 47/46 in the stroke group and it was 26/34 in the control group (p=0.411). The NLR level was higher in patients with ischemic stroke as compared to control (p=0.029) whereas hemoglobin levels were lower in the patient group (p= 0.025). The ROC curve analysis revealed that a NLR cutoff point of 2.34 had 61.3 % sensitivity and 60% specificity in detecting patients with ischemic stroke. The regression analyses also revealed that the NLR and hemoglobin levels were predictors for stroke.
In conclusion, NLR and hemoglobin may potentially be paraclinical markers in differential diagnosis of stroke.

Keywords: Neutrophil-to-Lymphocyte Ratio; Stroke; Diagnosis; Inflammation; Biomarker.

INTRODUCTION

There is increasing evidence that inflammation is involved in the mechanisms of acute ischemic stroke (AIS). The inflammatory response following AIS is considered to be an important pathological process in the post-ischemic injury in the brain.[1,2] Inflammatory responses can emerge before ischemic stroke.[3,4] In AIS, the chemokines and cytokines released from ischemic tissues promote the infiltration of peripheral circulating leukocytes to ischemic sites.[5] Among peripheral circulating leukocytes, neutrophils have been regarded as an important mediator in ischemic brain injury and they have been shown to begin to enter the brain as early as 6 hours after transient middle cerebral artery occlusion in rat models.[6] The infiltration of circulating neutrophils into ischemic brain regions leads to poor prognosis.[5,7] The increased free oxygen radicals released by neutrophils, neutrophil deviated matrix metalloproteinase-9 associated blood-brain barrier disruption, edema, and hemorrhagic transformation of AIS may be the main mechanisms leading to poor prognosis.[8] After the ischemic injury, lymphocytes also infiltrate the ischemic regions and mediate ischemic brain injury. It is known that neutrophils and lymphocytes mediate different inflammatory processes and recent studies have shown the importance of the neutrophil-to-lymphocyte ratio (NLR) that reflects the balance between neutrophil and lymphocyte levels and their different immunological activities. The potential utility of NLR in the differential diagnosis and prognosis of many diseases has been investigated.[9-13] In stroke, the association of NLR with mortality and prognosis of patients has been demonstrated.[14-17] However, the potential utility of NLR value in differential diagnosis is rarely been reported in the literature. In this study, we aimed to investigate the differences in NLR and some other hematological parameters between AIS patients and control individuals (CI). We also investigated

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the differences in the NLR levels according to the distinct stroke subtypes and the presence of some comorbidities in stroke.

METHODS

Study population

All patients with AIS who presented within the first 24 hours of symptom onset and were hospitalized at the neurology clinic of the Yozgat City Hospital from September to December 2019 were screened for the study. The diagnosis of AIS was based on the clinical features and neuroimaging results. Diffusion-weighted imaging (DWI, 1.5 Tesla) was performed in all the patients. Patients with transient ischemic attacks (TIA) and without lesions on DWI were excluded from the study. Those with an infectious disease (urinary tract infection, sinusitis, cellulitis, or otitis media), malignancy, heart failure, coronary artery disease, ischemic heart disease, pulmonary disease, or chronic inflammatory disease were also excluded from the study**.** In our routine practice, all AIS patients had computed tomography angiography (CTA) of the head and neck, or carotid/vertebral artery transcranial Doppler ultrasound investigations in those contraindicated for CTA. Cardiological investigations of echocardiography and electrocardiography are routinely conducted, and HOLTER investigation is performed according to the recommendations by the cardiologist. The etiology of stroke was defined according to the TOAST classification. The presence of hypertension (HT), hyperlipidemia (HL) and diabetes mellitus (DM) were also noted. For infarct localization, the vascular territories (anterior circulation stroke, posterior circulation stroke, both of them) and lateralization of the infarctions (right hemisphere stroke, left hemisphere stroke, bilateral hemispheric stroke) were identified. The size of the stroke was classified as small cerebral infarction (affecting <1/3 of a main cerebral artery); middle size cerebral infarction (affecting 1/3-2/3 of a main cerebral artery) and large size cerebral infarction (more than 2/3 of a main cerebral artery). The cerebral arteries involved, whether it was anterior cerebral artery, anterior choroidal artery and its branches; middle cerebral artery; posterior cerebral artery or vertebrobasilar system was also identified. The classification of the infarction size was by adapting a previous classification of the stroke syndromes affecting MCA territory.[18] The degree of the extracranial stenosis was classified as non-significant stenosis, 50% to 70% stenosis, and stenosis more than 70%. We formed control group retrospectively from the individuals who were admitted to the neurology clinic of the Yozgat City Hospital with nonspecific symptoms such as dizziness or vertigo and who had no neurological disease, infectious disease (urinary tract infection, sinusitis, cellulitis, or otitis media), malignancy, heart failure, coronary artery disease, ischemic heart disease, pulmonary disease, or chronic inflammatory disease**.** The CI who had no hemogram investigation at the time of neurology clinic admission were excluded. The presence of the comorbidities in the control group, including HT, HL, and DM, were also noted. The clinical data on the patient and control groups were collected using the Hospital Computerized Data System (AKGUN). The hemogram, performed initially upon admission to the emergency service in the patient were used in the evaluation. In the control group, the hemogram evaluated was that acquired at the admission to the neurology clinic. This study was approved by the institutional review board.

Laboratory analysis

All values in the study were calculated from patients' and CI' complete blood count (CBC) analysis. For all the individuals, the values of hemoglobin, hematocrit, leukocytes, lymphocytes, neutrophils, and platelets were noted, separately. NLRs were calculated from the CBC results as dividing the number of neutrophils by the number of lymphocytes. Routine electronic CBC device was used (Cell-Dyne 3700, Abbott, Abbott Park, IL, USA) for this purpose.

Statistical analyses

All statistical analyses were performed using the SPSS statistics 22 program. Continuous variables are expressed as mean ± standard deviation and median (inter quartile range) according to the type of the data being normal or not normally distributed. The compliance of the variables with normal distribution was assessed by the Kolmogorov-Smirnov test. Inter-group analyses were performed with Student's t-test for normally distributed variables and the Mann-Whitney U test for non-parametric variables. The Kruskal-Wallis test was used to compare the not normally distributed variables among *more than two* independent *groups*. The chi-square test was used for the comparison of qualitative data. Stepwise multivariate logistic regression analysis was done to examine the association between the diagnosis

of stroke and other variables. Variables with P < 0.25 in univariate logistic regression were included in a multivariate logistic regression model. In the logistic regression model, the NLR level was assumed to be a binary variable according to the cutoff point detected in the receiver operating characteristic (ROC) curve analysis. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Ninety three AIS patients and 60 CI were enrolled in this study. The mean age in the stroke group was 71.7 ± 12.2 years whereas it was 72.6 ± 5.7 in the CI group (p=0.405). The female/male ratio was 47/46 in the stroke group and it was 26/34 in the control group (p=0.411). Among patients with AIS, 80% had comorbid HT, 34% had HL and 25% had DM. Other clinical features of the patient with stroke are listed in Table 2. The control group was comparable with the AIS group in terms of these comorbidities (Table 1).

The NLR level was higher in patients with AIS as compared to the CI (p=0.029). The comparisons of the other laboratory markers including WBC, hemoglobin, platelet, neutrophil, lymphocyte revealed that hemoglobin value was also higher in the AIS group (p=0.025).

The ROC curve analysis revealed that a NLR cutoff point of 2.34 had 61.3 % sensitivity and 60% specificity in detecting patients with ischemic stroke (Figure 1). In multivariate logistic regression analysis, Hb (OR 0.747, CI: 0.594-0.940, P < 0.013) and the NLR (OR 1.990, CI: 1.097-3.973, P = 0.049) were identified as independent predictors of stroke (Table 3).

The etiological classification of the patients revealed that 44 of the patients (47%) had large vessel atherosclerosis, 32 (34%) had small vessel occlusion and 15 (16%) had cardioembolic stroke. Further analyses comparing the NLR between distinct etiologies of stroke did not yield significant differences (p=0.68) (Table 4). The comparative analyses of the NLR values to investigate the significance of etiological classification were repeated after dividing the patient groups as large vessel atherosclerosis or not; small vessel occlusion or not, cardioembolic stroke or not; and additional analyses investigating the pairwise comparisons between distinct stroke etiologies were performed. However, none of the results were statistically significant (Table 5). On the other hand, the overall study group was classified according to the presence of HT, HL, and DM; comparative analyses in terms of the NLR values were performed to evaluate the significance of the presence of these comorbidities which all yielded unremarkable results. Additional analysis according to some clinical parameters including infarction size, vascular territory and the lateralization of the infarction and the degree of the stenosis of the extracranial vessels and the

Table 1: The comparisons of the demographic features and hematological parameters including the neutrophil-to-lymphocyte ratio levels in the patient group and control group

	Patients with ischemic stroke (n=93)	Control group (n=60)	P-value
Age	71.73 ± 12,25	72.66 ± 5,74	0.405
Sex (Male), n (%)	46 (49.5)	34 (56.7)	0.411
Hypertension, n (%)	74 (79.6)	39 (65.0)	0.059
Diabetes mellitus, n (%)	23 (24.7)	9 (15)	0.161
Hyperlipidemia	32 (34.4)	15 (25)	0.282
WBC *(x10^3/ml)*	7.70 ± 2.32	7.43 ± 1.74	0.084
Hemoglobin	13.30 ± 1.55	13.90 ± 1.65	0.025
PLT	226.00 ± 75.21	246.65 ± 64.36	0.223
Neutrophil	4.80 ± 2.48	4.72 ± 1.42	0.056
Lymphocyte	1.70 ± 0.73	2.02 ± 0.88	0.103
NLR	2.82 (1.95-4.44)	2.22 (1.67-2.87)	0.029

WBC: White blood cell, NLR: Neutrophil-to-lymphocyte

Table 2: Some clinical features of the patients with acute stroke

		Patients with ischemic stroke (n=93)
Admission to the intensive care unit, n (%)		25 (26.8)
Therapy	– Antiaggregant or anticoagulant therapy	93 (100)
	– The rate of the patients treated with thrombolysis, n (%)	5 (5.3)
	– The rate of the patients treated with thrombectomy or neurosurgical intervention	0 (0)
Glasgow coma scale		13.56 ± 1.36
AF, n (%)		18 (19.4)
Previous stroke, n (%)		15 (16.1)

variance of the NLR values between the groups were performed. The only significant difference in the NLR values was found between the groups with distinct stenosis degree of extracranial arteries (p=0.018). The results of the pairwise analyses were unremarkable. However, re-analyses after the classification of the patients with <50% stenosis or not revealed that the NLR was higher in the groups with extracranial arterial stenosis more than 50% (p=0.03).

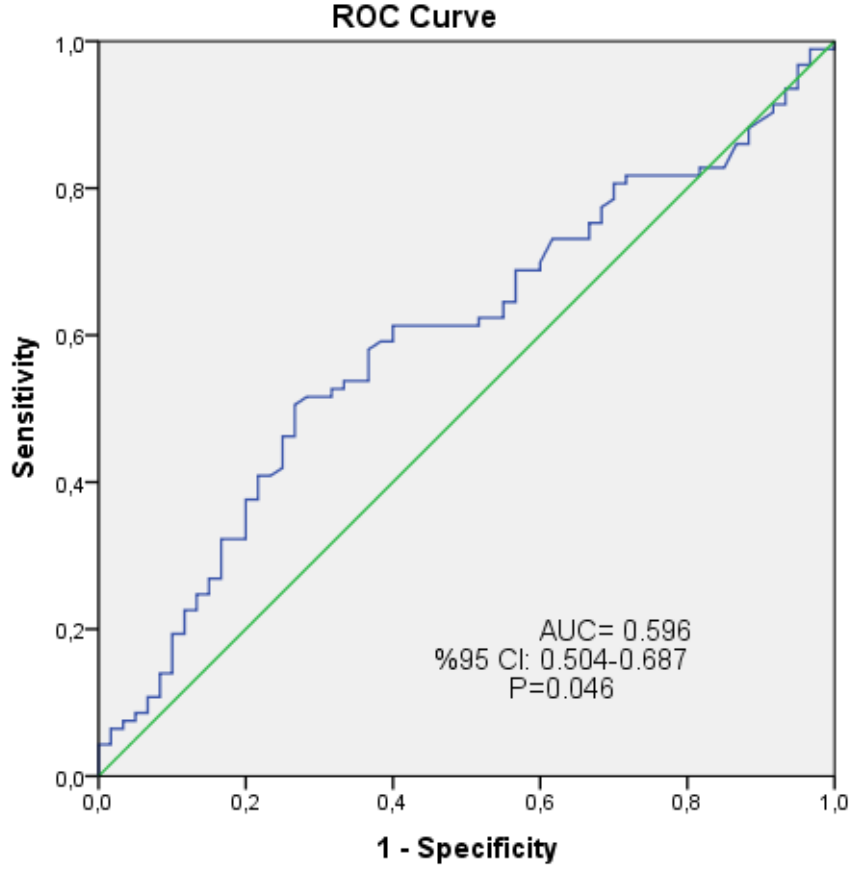

Figure 1. The ROC curve analysis revealed that a NLR cutoff point of 2.34 had 61.3% sensitivity and 60% specificity in detecting patients with ischemic stroke

Table 3: Factors predicting functional significance in multivariate logistic regression analysis

	Univariate		Multivariate	
Variables	OR (95 % CI)	P	OR (95 % CI)	P
Age	0.990 (0.960-1.022)	0.549		
Sex (Male), n (%)	0.748 (0.390-1.437)	0.384		
Hypertension, n (%)	1.825 (0.880-3.787)	0.106	1.485 (0.686-3.215)	0.316
Diabetes mellitus, n (%)	0.645 (0.281-1.481)	0.301		
Hyperlipidemia	1.574 (0.763-3.247)	0.220	1.593 (0.745-3.407)	0.230
WBC *(x10^3/ml)*	1.034 (0.886-1.207)	0.672		
Hemoglobin	0.717 (0.573-0.898)	0.004	0.747 (0.594-0.940)	0.013
NLR >2.34	2.375 (1.223-4.613)	0.011	1.990 (1.097-3.973)	0.049

Table 4: Comparisons of the NLR values between patient groups with distinct etiologies of stroke (Kruskal-Wallis Test)

TOAST classification	N (%)	NLR [Median (IQR)]	P-value
Total number of patients	93 (100)		
Large vessel atherosclerosis	44 (47.3)	2.9500 (1.95-4.11)	0.68
Cardioembolic	15 (16)	3.6364 (2.03-6.36)	
Small vessel occlusion	32 (34)	2.5165 (1.83-3.91)	
Stroke of other determined etiology	0 (0)	-	
Stroke of undetermined etiology	2 (2)	3.3929 (1.50-)	

Table 5: The detailed pairwise comparisons between distinct etiologies of ischemic stroke in terms of the NLR value

			P-value
NLR, Median (IQR)	Large vessel atherosclerosis (n=44) 2.95 (1.96-4.11)	Others (n=49) 2.56 (1.87-5.12)	0.060
NLR, Median (IQR)	Cardioembolic (n=15) 3.63(2.00-6.36)	Others (n=78) 2.75 (1.93-4.09)	0.531
NLR, Median (IQR)	Small vessel occlusion (n=32) (%34) 2.45 (1.83-3.91)	Others (n=61) 3.12 (1.97-5.14)	0.114
NLR, Median (IQR)	Large vessel atherosclerosis (n=44) 2.95 (1.96-4.11)	Small vessel occlusion (n=32) 2.45 (1.83-3.91)	0.201
NLR, Median (IQR)	Cardioembolic (n=15) (%16) 3.63 (2.00-6.36)	Small vessel occlusion (n=32) 2.45 (1.83-3.91)	0.164

Table 6: The comparisons of the NLR values according to the presence of hypertension, hyperlipidemia, and diabetes mellitus, respectively (Mann-Whitney U Test)

		NLR (Median (Range)	P-value
Hypertension	Present (n=74)	2.63 (1.95-4.09)	0.583
	Absent (n=19)	2.16 (1.76-3.77)	
Hyperlipidemia	Present (n=32)	2.82 (1.80-4.08)	0.211
	Absent (n=61)	2.29 (1.82-4.08-)	
Diabetes mellitus	Present (n=32)	2.30 (1.56-3.20)	0.354
	Absent (n=61)	2.44 (1.95-4.40)	

DISCUSSION

The inflammatory acute phase response can emerge both before and after an ischemic event.[3,4] It has been shown that neutrophils may facilitate plaque rupture by releasing proteolytic enzymes, arachidonic acid derivatives, and superoxide radicals.[19] On the other hand, lymphocytes have also a very important role in the regulation of inflammatory response during various stages of the atherosclerotic process.[20] In light of the above, some authors proposed that NLR, being a reflection of the innate and adaptive immune responses, may be associated with worse prognosis in patients undergoing percutaneous coronary intervention or coronary bypass surgery.[12,13] Recently, the value of the NLR in stroke has been investigated in various studies.[14-17,21-25] Nearly all of these studies addressed its association with the severity of the stroke and its prognostic significance.[9-12,18,19] However, the potential value of the NLR for the diagnosisof stroke has rarely been investigated.[23,24] In this report, we found that the NLR levels in the patients with AIS were higher than the CI. The previous report by San *et al*. also found that the NLR values were higher in both patients with AIS and transient ischemic attack

Table 7: The results of the analyses investigating the association between infarction features, extracranial stenosis degree and the NLR levels (Mann-Whitney U Test)

		n (%)	NLR (Median (Range)	P-value
Size of the infarction	< 1/3 of the territory (lacunar)	55 (59.1)	2.62 (1.80-4.47)	p=0.685
	1/3-2/3 of the territory of the artery	26 (27.9)	2.93 (2.02-4.309)	
	>2/3 of the territory of the artery	12 (12.9)	3.50 (2.01-4.84)	
Localization of the infarction	Anterior circulation stroke	64 (68.8)	2.75 (1.90-4.11)	p =0.214
	Posterior circulation stroke	27 (29.0)	2.82 (2.16-5.28)	
	Both anterior and posterior circulation stroke	2 (2.1)	6.09	
Lateralization of the infarction	Right hemisphere	42 (45.1)	3.66 (2.00-5.28)	p = 0.086
	Left hemisphere	41 (44.0)	2.68 (1.88-3.36)	
	Bilateral hemispheric	10 (10.7)	2.30 (1.50-5.99)	
The degree of the stenosis of the extracranial arteries	No significant stenosis	79 (84.9)	2.70 (1.91-4.09)	p=0.018*
	50% to 70% stenosis	6 (6.4)	4.35 (1.89-11.25)	
	>70% stenosis	8 (8.6)	(2.38-6.10)	

*(The pairwise comparisons did not reveal any significant difference. however, re-analyses after the classification of the patients with 50< stenosis or not revealed that the NLR was higher in the group with extracranial arterial stenosis more than %50 (p=0.03)

(TIA) in comparison to the healthy controls.[23] Based on their results, the authors suggested that the hematological markers can be used in the differential diagnosis of stroke. Celikbilek *et al*. also found that the NLR levels were higher in patients with AIS when compared to the controls (p=0.001). Nevertheless, they discussed the prognostic role of the NLR and did not discuss its possible value in the diagnosis of stroke. Although the NLR is not a highly specific marker as it can be elevated in various chronic diseases and acute conditions, its diagnostic significance has been investigated in many diseases.[9-11]

Ischemic stroke is a leading cause of severe disability and death, and its timely diagnosis is critical for better outcomes. However, the diagnosis may be challenging as recent studies indicate that stroke is among the most commonly misdiagnosed.[26,27] This is particularly so in trauma patients, pediatric population, and stroke syndromes presenting with seizures.[28-30] It is therefore of value to find non-invasive tests to differentiate from stroke mimics as well as healthy controls. To date, there is no laboratory marker having sufficient sensitivity and specificity to be used in the clinical setting for the diagnosis of stroke.[28] In our study, the NLR level was found to be higher in the AIS group, and that a NLR cutoff point of 2.34 was found to have a 61.3 % sensitivity and 60% specificity in the ROC curve analyses. The multivariate logistic regression analysis also showed that a cutoff point of 2.34 of NLR was found to be a significant predictor for stroke. Taken together, the results of our study suggest the potential value of the NLR level in the differential diagnosis of stroke.

We also investigated the possible association between the NLR levels and the subtypes of stroke which did not reveal a significant association. However, previous study by Tokgoz *et al*. that investigated 255 patients found that the NLR was higher in both the atherosclerotic and cardioembolic stroke subgroups than the lacunar infarct subgroup.[14] Hyun *et al*. found that the NLR was associated with the degree of carotid artery stenosis, and concluded that NLR can predict carotid stenosis in patients with AIS.[16] Another study with 868 patients also shown that the NLR level was significantly higher in the group with great artery atherosclerosis or atherothrombosis compared to other groups.[31] We compared the NLR levels in subgroups of patients with distinct stenosis levels of extracranial arteries which yielded unremarkable results initially. However, after dividing the patients into two subgroups as strokes with non-significant extracranial artery stenosis and strokes with extracranial stenosis more than 50%, higher NLR levels were found in the latter group which was in accordance with the results of the above-mentioned studies.[16,31] We think that the association between the NLR level and stroke subgroups (particularly extracranial atherosclerosis) should be investigated further in larger groups of patients.

Based on the previous reports linking the inflammation with HT and HL, and the utility of the NLR in the investigation of these associations[22,32,33], we also compared the NLR levels in groups according to the presence of HT, HL. However, we have not found any differences in the NLR values between individuals with and without these comorbidities. Our control group was comparable with the stroke group in terms of the presence of these comorbidities. The possible effects of these variables have also been excluded by regression analyses while comparing the stroke group and the control group.

Another major conclusion of our study was that the hemoglobin value was found to be lower in stroke patients. The regression analyses also confirmed the significance of this parameter. The association between anemia and ischemic stroke rather remains an unclear issue in the literature. Sickle cell anemia is known to be highly associated with cerebrovascular disease[34], there are also reports suggesting an association between non-sickle cell anemia and stroke.[35,36] This include the study by Chang *et al*.[35] which involve 51,093 subjects with 153,279 controls. The study reported a significant association between prior anemia and ischemic stroke.

The limitation of this study is first the low number of patients. Second, we have not performed the hemogram investigation at a definite time after stroke which may be crucial when evaluating the results. Asinflammation induced by stroke is a dynamic process with a temporal course, and may vary significantly even during the first 24 hours after stroke. Therefore, the utility of the NLR in the differentiation of stroke may be investigated in future large-scale studies of prospective design in which the blood samples may be studied in different time intervals after stroke.

In conclusion, our results suggest that the NLR value may be a paraclinical marker in differential diagnsosi of stroke. These potential paraclinical markers may be useful in some specific scenarios such as a TIA, diffusion-negative strokes, strokes in trauma patients.

DISCLOSURE

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REFERENCES

1. Fu Y, Liu Q, Anrather J, Shi FD. Immune interventions in stroke. *Nat Rev Neurol* 2015;11(9):524-35.
2. Kim JY, Park J, Chang JY, Kim SH, Lee JE. Inflammation after ischemic stroke: The role of leukocytes and glial cells. *Exp Neurobiol* 2016;25(5):241-51.
3. Grau AJ, Boddy AW, Dukovic DA, *et al*. Leukocyte count as an independent predictor of recurrent ischemic events. *Stroke* 2004;35(5):1147-52.
4. Vila N, Castillo J, Davalos A, Chamorro A. Proinflammatory cytokines and early neurological worsening in ischemic stroke. *Stroke* 2000;31(10):2325-9.
5. Barone FC, Hillegass LM, Price WJ, *et al*. Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. *J Neurosci Res* 1991;29(3):336-45.
6. Zhang RL, Chopp M, Chen H, Garcia JH. Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. *J Neurol Sci* 1994;125(1):3-10.
7. Herz J, Sabellek P, Lane TE, Gunzer M, Hermann DM, Doeppner TR. Role of neutrophils in exacerbation of brain injury after focal cerebral ischemia in hyperlipidemic mice. *Stroke* 2015;46(10):2916-25.
8. Montaner J, Alvarez-Sabin J, Molina CA, *et al*. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. *Stroke* 1 2001;32(12):2762-7.
9. Wada H, Dohi T, Miyauchi K, *et al*. Pre-procedural neutrophil-to-lymphocyte ratio and long-term cardiac outcomes after percutaneous coronary intervention for stable coronary artery disease. *Atherosclerosis* 2017;265:35-40.
10. Kotfis K, Bott-Olejnik M, Szylinska A, Rotter I. Could neutrophil-to-lymphocyte ratio (NLR) serve as a potential marker for delirium prediction in patients with acute ischemic stroke? A prospective observational study. *J Clin Med* 2019;8(7):1075.
11. Hemond CC, Glanz BI, Bakshi R, Chitnis T, Healy BC. The neutrophil-to-lymphocyte and monocyte-to-lymphocyte ratios are independently associated with neurological disability and brain atrophy in multiple sclerosis. *BMC Neurol* 2019;19(1):23.
12. Duffy BK, Gurm HS, Rajagopal V, Gupta R, Ellis SG, Bhatt DL. Usefulness of an elevated neutrophil to lymphocyte ratio in predicting long-term mortality after percutaneous coronary intervention. *Am J Cardiol* 2006;97(7):993-6.
13. Gibson PH, Croal BL, Cuthbertson BH, *et al*. Preoperative neutrophil-lymphocyte ratio and outcome from coronary artery bypass grafting. *Am Heart J* 2007;154(5):995-1002.
14. Tokgoz S, Kayrak M, Akpinar Z, Seyithanoglu A, Guney F, Yuruten B. Neutrophil lymphocyte ratio as a predictor of stroke. *J Stroke Cerebrovasc Dis* 2013;22(7):1169-74.
15. Brooks SD, Spears C, Cummings C, *et al*. Admission neutrophil-lymphocyte ratio predicts 90 day outcome after endovascular stroke therapy. *J Neurointerv Surg* 2014;6(8):578-83.
16. Hyun S, Kwon S, Cho S, *et al*. Can the nutrophil-to-lymphocyte ratio appropriately predict carotid artery stenosis in patients with ischemic stroke?-A retrospective study. *J Stroke Cerebrovasc Dis* 2015;24(11):2646-51.
17. Xue J, Huang W, Chen X, *et al*. Neutrophil-to-lymphocyte ratio is a prognostic marker in acute ischemic stroke. *J Stroke Cerebrovasc Dis* 2017;26(3):650-7.
18. Huang J, Chi H, Chi H, *et al*. Stromal cell-derived factor 1 promotes cell migration to enhance bone regeneration after hypoxic preconditioning. *Tissue Eng Part A* 2019;25(17-18):1300-9.
19. Naruko T, Ueda M, Haze K, *et al*. Neutrophil infiltration of culprit lesions in acute coronary syndromes. *Circulation* 2002;106(23):2894-900.
20. Ait-Oufella H, Salomon BL, Potteaux S, *et al*. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med* 2006;12(2):178-80.
21. Qun S, Tang Y, Sun J, *et al*. Neutrophil-To-Lymphocyte Ratio Predicts 3-month outcome of acute ischemic stroke. *Neurotox Res* 2017;31(3):444-52.
22. Liu X, Zhang Q, Wu H, *et al*. Blood neutrophil to lymphocyte ratio as a predictor of hypertension. *Am J Hypertens* 2015;28(11):1339-46.
23. San I, Yuzbasioglu Y, Otal Y, *et al*. The prognostic relationship between complete blood count parameters and infarct-negative treansient ischemic attack, and ischemic stroke. *Acta Medica Mediterranea* 2016;32:97.
24. Celikbilek A, Ismailogullari S, Zararsiz G. Neutrophil to lymphocyte ratio predicts poor prognosis in ischemic cerebrovascular disease. *J Clin Lab Anal* 2014;28(1):27-31.
25. Song SY, Zhao XX, Rajah G, *et al*. Clinical significance of baseline neutrophil-to-lymphocyte ratio in patients with ischemic stroke or hemorrhagic stroke: An updated meta-analysis. *Front Neurol* 2019;10:1032.
26. Newman-Toker DE, Moy E, Valente E, Coffey R, Hines AL. Missed diagnosis of stroke in the emergency department: a cross-sectional analysis of a large population-based sample. *Diagnosis (Berl)* 2014;1(2):155-66.
27. Kachalia A, Gandhi TK, Puopolo AL, *et al*. Missed and delayed diagnoses in the emergency department: a study of closed malpractice claims from 4 liability insurers. *Ann Emerg Med* 2007;49(2):196-205.
28. Makris K, Haliassos A, Chondrogianni M, Tsivgoulis G. Blood biomarkers in ischemic stroke: potential role and challenges in clinical practice and research. *Crit Rev Clin Lab Sci* 2018;55(5):294-328.
29. Yeboah K, Bodhit A, Al Balushi A, Krause E, Kumar A. Acute ischemic stroke in a trauma cohort: Incidence and diagnostic challenges. *Am J Emerg Med* 2019;37(2):308-11.

30. Rajani NK, Pearce K, Campion T, *et al*. Pediatric stroke: current diagnostic and management challenges. *Quant Imaging Med Surg* 2018;8(10):984-91.
31. Gokhan S, Ozhasenekler A, Mansur Durgun H, Akil E, Ustundag M, Orak M. Neutrophil lymphocyte ratios in stroke subtypes and transient ischemic attack. *Eur Rev Med Pharmacol Sci* 2013;17(5):653-7.
32. Polonia J. Neutrophil-to-lymphocyte ratio and ambulatory blood pressure: Exploring the link between inflammation and hypertension. *Rev Port Cardiol* 2017;36(2):107-9.
33. Liu CC, Ko HJ, Liu WS, *et al*. Neutrophil-to-lymphocyte ratio as a predictive marker of metabolic syndrome. *Medicine (Baltimore)* 2019;98(43):e17537.
34. Driscoll MC, Hurlet A, Styles L, *et al*. Stroke risk in siblings with sickle cell anemia. *Blood* 2003;101(6):2401-4.
35. Chang YL, Hung SH, Ling W, Lin HC, Li HC, Chung SD. Association between ischemic stroke and iron-deficiency anemia: a population-based study. *PLoS One* 2013;8(12):e82952.
36. Khan MF, Shamael I, Zaman Q, Mahmood A, Siddiqui M. Association of anemia with stroke severity in acute ischemic stroke patients. *Cureus* 2018;10(6):e2870.