Original Article

Neutrophil to lymphocyte ratio and adverse clinical outcomes in patients with ischemic stroke

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Background: Neutrophils and lymphocytes mediate differential inflammatory responses after ischemic stroke and have different effects on patients' clinical outcomes. Several studies have used the neutrophil to lymphocyte ratio (NLR) as a prognostic indicator for ischemic stroke; however, some limitations remain.

Methods: We derived data from the Third China National Stroke Registry. The NLR is defined as neutrophil count/lymphocyte count. Patients included in the study were classified into four groups by NLR quartiles. Odds ratios (ORs) of adverse clinical outcomes were calculated with the lowest quartile group as the reference category. We plotted receiver operating characteristic (ROC) curves of NLR for adverse clinical outcomes and calculated area under the curve (AUC) values and cutoff values. Under different TOAST classifications, medians of NLR and ORs of adverse clinical outcomes were also calculated. Furthermore, interaction tests between NLR and etiology were performed.

Results: A total of 13,018 patients were enrolled. At both 3- and 12-month follow-ups, higher quartile groups were associated with increased risks of death and poor functional outcomes, even after adjustments. For death, the cutoff values of NLR were 3.872 at 3-month follow-up and 3.180 at 12-month follow-up. For poor functional outcomes, the cutoff value of NLR was 2.846 at both 3- and 12-month follow-ups. The association between NLR and stroke recurrence was significant only at 3-month follow-up before adjustments. There was no correlation between NLR and hemorrhagic transformation during hospitalization. Under different TOAST classifications, the medians of NLR were different; conversely, the correlations of NLR with adverse clinical outcomes had no differences.

Conclusions: High level of NLR within the first 24 h after admission was associated with increased risks of both short- and long-term adverse clinical outcomes in patients with ischemic stroke, regardless of etiology.

Keywords: Neutrophil to lymphocyte ratio (NLR); outcome; ischemic stroke; etiology

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Introduction

Ischemic stroke triggers a robust inflammatory response, resulting in brain injury development and neurological function exacerbation (1-3). However, the post-ischemic inflammatory response is a complicated process involving the induction of multiple inflammatory cell activation (2,3). In the inflammatory process, ischemic tissues release chemokines and cytokines and recruit peripheral circulating leukocytes (4). Among the leukocytes infiltrating into the ischemic regions, neutrophils have been considered to play an important role in brain injury (5). Neutrophils can be recruited rapidly, within hours of ischemic stroke, to cerebral vessels (6). Many studies have shown that the infiltration of neutrophils was associated with larger infarct volumes and deterioration of ischemic brain injury (5,7). Lymphocytes can also infiltrate ischemic regions during the inflammatory response, albeit several days after acute ischemic stroke, which is a later event than that of neutrophils (4,8). Currently, the role of lymphocytes in ischemic brain injury is still controversial (4). Some studies have already demonstrated that lymphocytes could repair the injury via inflammation (9). At the same time, some studies have shown that lymphocytes could cause the release of proinflammatory cytokines and cytotoxic substances, which cause damage to the ischemic brain (8,10). More experimental evidence has indicated that some specific lymphocyte subtypes were important cerebroprotective immunomodulators after acute ischemic stroke (11-13). Thus, neutrophils and lymphocytes might mediate differential inflammatory responses after ischemic stroke and have different effects on patients' clinical outcomes.

The neutrophil to lymphocyte ratio (NLR) is defined as neutrophil count/lymphocyte count. The NLR integrates two subtypes of leukocytes and can simultaneously reflect the balance between neutrophil and lymphocyte levels and their different immune activities. Several studies have used NLR as a prognostic indicator for ischemic stroke and found that increased NLR was associated with early-onset delirium, mortality, or poor functional outcomes defined as modified Rankin Scale (mRS) ≥3 of patients with ischemic stroke (14-19). However, generally, the sample sizes of these studies have been small, follow-up times were short, and the outcome of stroke recurrence or hemorrhagic transformation was minimally reported. Thus, we conducted this study to verify whether NLR was associated with adverse clinical outcomes defined as hemorrhagic transformation during hospitalization and recurrent stroke, all-cause death, poor functional outcomes at 3- and 12-month follow-up in ischemic stroke patients. We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-710).

Methods

Study design and population

We derived data from the Third China National Stroke Registry (CNSR-III). The detailed design and major results of the CNSR-III have been described previously (20). Briefly, the CNSR-III is a large-scale nationwide, multicenter, prospective clinical registry study between August 2015 and March 2018 in China. A total of 15,166 patients were recruited consecutively from 201 hospitals who met the following criteria: (I) age older than 18 years; (II) diagnosis of ischemic stroke or transient ischemic attack (TIA); (III) within 7 days from the onset of symptoms to enrolment; and (IV) informed consent was provided by the patient or legally authorized representative. Acute ischemic stroke was diagnosed according to the World Health Organization (WHO) criteria and confirmed by magnetic resonance imaging (MRI) or brain computed tomography (CT). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The protocol and data collection of the CNSR-III were approved by the ethics committee of Beijing Tiantan Hospital (IRB approval number: KY2015-001-01) and all other participating hospitals. All patients or their legal representatives provided written informed consent before being entered into the study.

Among the enrolled patients in the CNSR-III, 14,146 were diagnosed with ischemic stroke, and therein 13,018 patients were involved in our analysis after excluding those with infection within 2 weeks before admission (n=422), missing available complete blood count on admission (n=305), and loss to follow-up (n=401) (*Figure 1*).

Baseline data collection and etiology classification

Baseline data, including age, gender, body mass index (BMI), smoking and drinking status, medical history, National Institutes of Health Stroke Scale (NIHSS) score at admission, and time from symptom onset to enrollment and therapy were systematically collected by trained neurologists through face-to-face interviews in

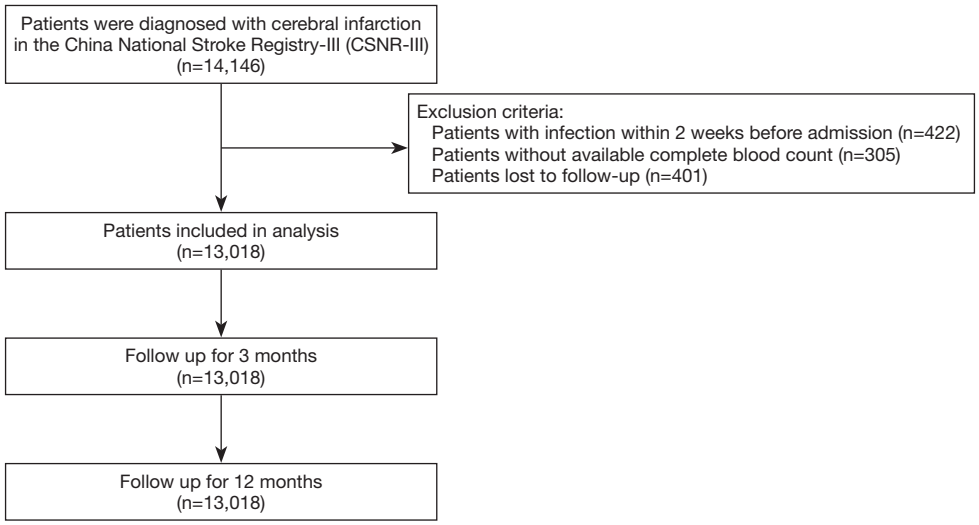

Figure 1 Flow chart.

each participating hospital. Moreover, the etiology of each patient was evaluated using Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification according to clinical manifestations, imaging, and other diagnostic tests by trained neurologists at the discharge visit.

Neutrophil, lymphocyte count testing, and NLR calculation

Fasting whole blood samples from venipuncture were taken into vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) within the first 24 h after admission and kept at room temperature. Afterward, neutrophil and lymphocyte counts were analyzed by automated hematology analyzers at each participating hospital. All measurements were performed by laboratory personnel blinded to patients' clinical situations. The NLRs for all participants were calculated using the below formula.

NLR = neutrophil count/lymphocyte count

Outcome assessment

Hemorrhagic transformation was defined as any non-traumatic extravascular hemorrhage within a known infarct area, and imaging evidence (CT or MRI) was required, regardless of whether it caused a change in symptoms during hospitalization. Neuroradiologists reviewed the CT or MRI images with extensive experience in stroke who were blinded to patients' clinical situations.

Participants were interviewed face-to-face at 3 months and contacted over the telephone at 12 months by trained research coordinators according to the protocol of the CNSR-III. Information including functional status and cerebrovascular events were collected at each follow-up. Any incidents of all-cause death and stroke recurrence during the follow-up periods were recorded. Each case fatality was confirmed on a death certificate from the attended hospital or the local citizen registry. Recurrent stroke included both ischemic and hemorrhagic stroke, which was confirmed from the treating hospital, and suspected events without hospitalization were judged by independent endpoint judgment committees. The mRS was used to assess participants' functional dependence, and poor functional outcomes were defined as $3 \leq$ mRS ≤ 5.

Statistical analysis

Continuous variables were presented as medians with interquartile ranges (IQR) and categorical variables as proportions. Participants were classified into four groups by NLR quartiles. The NLR ranges of quartile groups were NLR <1.848, $1.848 \leq$ NLR <2.548, $2.548 \leq$ NLR <3.728, and NLR ≥ 3.728, respectively. Baseline data were compared across NLR quartile groups using the Fisher's exact test for categorical variables and the Kruskal-Wallis test for continuous variables. Firstly, both crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) of adverse clinical outcomes were calculated using the multivariate logistic regression model with the lowest NLR quartile

group as the reference category in order to investigate the correlations between NLR and adverse clinical outcomes. Then, only adjusted ORs and 95% CIs of adverse clinical outcomes under different TOAST classifications were calculated in the same way. The interaction tests between NLR and etiology were performed to explore the difference in the relationships between NLR and adverse clinical outcomes under different etiology. To adjust for other potential confounding variables, multivariable analyses including age, gender, BMI, drinking, smoking, hypertension, lipid metabolism disorders, diabetes, previous stroke, atrial fibrillation, heart failure, heart valve disease, peripheral arterial disease, intravenous thrombolysis, endovascular therapy, time from symptom onset to enrollment, and the NIHSS score at admission were performed.

Meanwhile, participants were also grouped according to their TOAST classification, and the median with IQR of NLR in each group was calculated. The Kruskal-Wallis test was used to compare the distribution of NLR under different etiology. Furthermore, we plotted receiver operating characteristic (ROC) curves of NLR for adverse clinical outcomes and calculated area under the curve (AUC) values and cutoff values. A P value <0.05 for two-sided hypothesis testing was considered statistically significant. All statistical analyses were performed with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

A total of 13,018 patients were included in our analysis. Of all participants, the median [IQR] age was 63 [54–70] years, and 4,066 (31.23%) were female. The baseline characteristics of the participants stratified according to NLR are shown in *Table 1*. The medians (IQR) NLR of quartile groups were 1.500 (1.273–1.682), 2.179 (2.016–2.358), 3.021 (2.784–3.338), and 5.178 (4.274–7.171), respectively. From the lowest to the highest quartile groups, the medians (IQR) NIHSS scores at admission were 3 [1–5], 3 [1–5], 3 [2–6], and 4 [2–8], and the medians (IQR) times from symptom onset to enrollment were 2 [1–4], 2 [1–4], 2 [1–4], and 2 [1–3], respectively. Compared with participants with lower NLR, those in higher NLR quartile groups were more likely to be older, male, with lower BMI, shorter time to enrollment, and higher severity of the stroke, and treated with intravenous thrombolysis or endovascular therapy. Moreover, there were differences in smoking, drinking, hypertension, and diabetes history among participants in the NLR quartile groups. Previous stroke, atrial fibrillation, and heart failure were more frequently found in patients with higher NLR, and the other medical histories were comparable in NLR quartile groups (*Table 1*).

Association of NLR with adverse clinical outcomes

A total of 178 (1.37%) participants experienced hemorrhagic transformation during hospitalization. At 3-month follow-up, 195 (1.50%) participants had died, 833 (6.40%) had experienced recurrent stroke, and 1,865 (14.33%) were showing poor functional outcomes. While at 12-month follow-up, 423 (3.25%) participants had died, the recurrent stroke had occurred in 1,286 (9.88%), and there were 1,778 (13.66%) participants with poor functional outcomes. The risks of adverse clinical outcomes in NLR quartile groups are shown in *Table 2*. There was no significant correlation between NLR and hemorrhagic transformation during hospitalization. Both at 3- and 12-month follow-ups, compared with the lowest NLR quartile group taken as reference, higher quartile groups were related to elevated risks of death and poor functional outcomes. The above associations were still significant after adjustments. At 3-month follow up compared to the lowest quartile group of NLR, the other quartile groups had 0.951 (95% CI: 0.765 to 1.183), 1.147 (95% CI: 0.930 to 1.414), and 1.764 (95% CI: 1.453 to 2.142)-fold increased risks of stroke recurrence, respectively (P=0.0479). However, after adjustments, the association between NLR and stroke recurrence was no longer statistically significant. The NLR was not significantly associated with recurrent stroke at 12-month follow-up (*Table 2*).

The ROC curves of NLR for adverse clinical outcomes are shown in *Figure 2*, and the corresponding AUC values and cutoff values are shown in *Table 3*.

NLR and association of NLR with adverse clinical outcomes under different etiology

The median (IQR) of NLR in all participants was 2.548 (1.848–3.729). Under different TOAST classifications, the medians (IQR) of NLR were: 2.747 (1.972–3.981) in large artery atherosclerosis, 2.704 (1.970–4.058) in cardiogenic embolism, 2.391 (1.770–3.295) in small artery occlusion, 2.345 (1.889–3.557) in stroke of other determined cause, and 2.520 (1.818–3.750) in stroke of undetermined cause,

Table 1 Baseline characteristics of the patients stratified by NLR

Characteristics	Overall (n=13,018)	Q1 (NLR <1.848) (n=3,254)	Q2 (1.848≤ NLR <2.548) (n=3,255)	Q3 (2.548≤ NLR <3.728) (n=3,254)	Q4 (NLR ≥3.728) (n=3,255)	P value
Demographic and clinical features						
Age, median (IQR), y	63 [54–70]	61 [54–68]	62 [54–69]	63 [55–70]	64 [56–72]	<0.0001
Female, n (%)	4,066 (31.23)	1,200 (36.88)	993 (30.51)	923 (28.37)	950 (29.19)	<0.0001
BMI, kg/m^2, median (IQR)	24.49 (22.58–26.56)	24.62 (22.86–26.67)	24.61 (22.66–26.67)	24.49 (22.68–26.57)	24.21 (22.05–26.12)	<0.0001
Current smoking, n (%)	4,138 (31.79)	1,092 (33.56)	1,133 (34.81)	1,031 (31.68)	882 (27.10)	<0.0001
Current drinking, n (%)	2,140 (16.44)	514 (15.80)	590 (18.13)	557 (17.12)	479 (14.72)	0.0051
Medical history, n (%)						
Previous stroke	2,896 (22.25)	671 (20.62)	679 (20.86)	757 (23.26)	789 (24.24)	0.0004
Previous transient ischemic attack	286 (2.20)	75 (2.30)	75 (2.30)	65 (2.00)	71 (2.18)	0.8118
Diabetes	3,062 (23.52)	778 (23.91)	760 (23.35)	839 (25.78)	685 (21.04)	0.0001
Coronary heart disease	1,354 (10.40)	331 (10.17)	328 (10.08)	333 (10.23)	362 (11.12)	0.4825
Atrial fibrillation	484 (3.72)	95 (2.92)	92 (2.83)	118 (3.63)	179 (5.50)	<0.0001
Heart failure	84 (0.65)	8 (0.25)	16 (0.49)	24 (0.74)	36 (1.11)	0.0001
Heart valve disease	50 (0.38)	15 (0.46)	8 (0.25)	8 (0.25)	19 (0.58)	0.0674
Hypertension	8,168 (62.74)	1,956 (60.11)	2,037 (62.58)	2,115 (65.00)	2,060 (63.29)	0.0007
Lipid metabolism disorders	978 (7.51)	254 (7.81)	257 (7.90)	252 (7.74)	215 (6.61)	0.1579
Peripheral arterial disease	97 (0.75)	17 (0.52)	23 (0.71)	22 (0.68)	35 (1.08)	0.0642
NIHSS score at admission, median (IQR)	3 [2–6]	3 [1–5]	3 [1–5]	3 [2–6]	4 [2–8]	<0.0001
Time from symptom onset to enrollment, median (IQR), d	2 [1–4]	2 [1–4]	2 [1–4]	2 [1–4]	2 [1–3]	<0.0001
Therapy, n (%)						
Intravenous thrombolysis	1,388 (10.66)	327 (10.05)	291 (8.94)	344 (10.57)	426 (13.09)	<0.0001
Endovascular therapy	87 (0.67)	14 (0.43)	12 (0.37)	20 (0.61)	41 (1.26)	<0.0001

NLR, neutrophil to lymphocyte ratio; BMI, body mass index; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range.

respectively (P<0.0001). Thus, NLRs were significantly different under different etiology, among which NLR was higher in patients with large artery atherosclerosis and cardiogenic embolism. The relationships between NLR and adverse clinical outcomes under different TOAST classifications are shown in *Table 4*. In participants with large artery atherosclerosis, NLR correlated with death and poor functional outcomes at both 3- and 12-month follow-ups. In participants with small artery occlusion, NLR was associated with stroke recurrence at 3-month follow-up and poor functional outcomes at both 3- and 12-month follow-ups. In participants with stroke of other determined cause, NLR was related to poor functional outcomes at 12-month follow-up. A similar correlation was also found between NLR and death at 3-month follow-up in patients with stroke of undetermined cause. However, regardless of adverse clinical outcomes, the interaction P values of NLR and etiology were all >0.05. Therefore, it could be considered that there was no difference in the correlations of NLR with adverse clinical outcomes under different

Table 2 Risks of adverse clinical outcomes stratified by NLR

Outcomes	Overall	Q1 (NLR <1.848)	Q2 (1.848≤ NLR <2.548)	Q3 (2.548≤ NLR <3.728)	Q4 (NLR ≥3.728)	P value
Hemorrhagic transformation, n (%)	178 (1.37)	17 (0.52)	38 (1.17)	38 (1.17)	85 (2.61)	
Crude OR (95% CI)		1	2.249 (1.267–3.993)	2.250 (1.267–3.994)	5.106 (3.026–8.614)	0.1169
Adjusted OR (95% CI)		1	2.297 (1.287–4.099)	2.081 (1.166–3.717)	3.475 (2.026–5.963)	0.4254
3-month follow-up						
Death, n (%)	195 (1.50)	16 (0.49)	32 (0.98)	38 (1.17)	109 (3.35)	
Crude OR (95% CI)		1	2.009 (1.100–3.669)	2.391 (1.331–4.297)	7.012 (4.139–11.877)	0.0028
Adjusted OR (95% CI)		1	1.916 (1.042–3.521)	1.906 (1.051–3.454)	3.676 (2.130–6.343)	0.0021
Stroke recurrence, n (%)	833 (6.40)	174 (5.35)	166 (5.10)	198 (6.08)	295 (9.06)	
Crude OR (95% CI)		1	0.951 (0.765–1.183)	1.147 (0.930–1.414)	1.764 (1.453–2.142)	0.0479
Adjusted OR (95% CI)		1	0.955 (0.767–1.189)	1.114 (0.902–1.378)	1.646 (1.347–2.012)	0.1088
Poor functional outcomes, n (%)	1,865 (14.33)	248 (7.62)	332 (10.20)	491 (15.09)	794 (24.39)	
Crude OR (95% CI)		1	1.377 (1.159–1.635)	2.154 (1.833–2.531)	3.910 (3.358–4.553)	<0.0001
Adjusted OR (95% CI)		1	1.333 (1.109–1.603)	1.805 (1.516–2.149)	2.473 (2.090–2.926)	0.0217
12-month follow-up						
Death, n (%)	423 (3.25)	48 (1.48)	71 (2.18)	99 (3.04)	205 (6.30)	
Crude OR (95% CI)		1	1.489 (1.029–2.155)	2.096 (1.480–2.969)	4.489 (3.266–6.171)	0.0084
Adjusted OR (95% CI)		1	1.428 (0.980–2.082)	1.699 (1.190–2.428)	2.603 (1.864–3.633)	0.0109
Stroke recurrence, n (%)	1,286 (9.88)	272 (8.36)	281 (8.63)	320 (9.83)	413 (12.69)	
Crude OR (95% CI)		1	1.036 (0.870–1.233)	1.196 (1.009–1.417)	1.593 (1.356–1.872)	0.0759
Adjusted OR (95% CI)		1	1.039 (0.872–1.238)	1.157 (0.974–1.373)	1.472 (1.245–1.740)	0.1983
Poor functional outcomes, n (%)	1,778 (13.66)	257 (7.90)	319 (9.80)	459 (14.11)	743 (22.83)	
Crude OR (95% CI)		1	1.267 (1.067–1.505)	1.915 (1.630–2.250)	3.449 (2.965–4.013)	<0.0001
Adjusted OR (95% CI)		1	1.218 (1.014–1.463)	1.578 (1.327–1.877)	2.155 (1.824–2.547)	0.0351

Adjusted for age, gender, BMI, drinking, smoking, hypertension, lipid metabolism disorders, diabetes, previous stroke, atrial fibrillation, heart failure, heart valve disease, peripheral arterial disease, intravenous thrombolysis, endovascular therapy, time from symptom onset to enrollment, and the NIHSS score at admission. NLR, neutrophil to lymphocyte ratio; OR, odds ratio; CI, confidence interval; BMI, body mass index.

etiology (*Table 4*).

Discussion

In this analysis of the CNSR-III, we demonstrated that among participants with ischemic stroke, a high level of NLR within the first 24 h after admission was correlated with increased risks of both death and poor functional outcomes at 3- and 12-month follow-ups. Moreover, there might also be an association between NLR and stroke recurrence at 3-month follow-up. Although NLR was not related to hemorrhagic transformation during hospitalization, it was still considered a useful predictor for adverse clinical outcomes regardless of etiology in ischemic

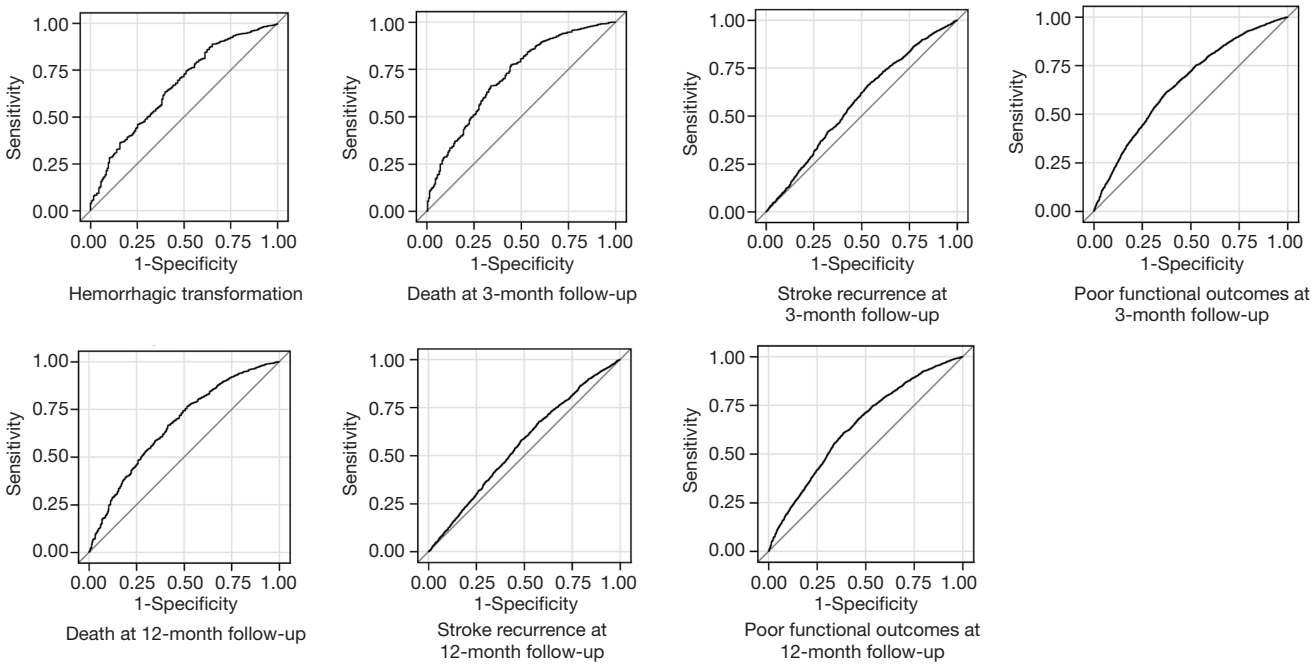

Figure 2 ROC curves of NLR for adverse clinical outcomes. ROC, receiver operating characteristic; NLR, neutrophil to lymphocyte ratio.

Table 3 Predictive values of NLR for adverse clinical outcomes

Outcomes	AUC (95% CI)	NLR cutoff	Sensitivity (%)	Specificity (%)
Hemorrhagic transformation	0.6624 (0.6211–0.7036)	5.371	34.83	88.86
3-month follow-up				
Death	0.7112 (0.6727–0.7496)	3.872	55.39	77.31
Stroke recurrence	0.5689 (0.5478–0.5899)	3.156	46.58	66.19
Poor functional outcomes	0.6538 (0.6402–0.6675)	2.846	62.57	61.30
12-month follow-up				
Death	0.6667 (0.6396–0.6938)	3.180	58.39	66.69
Stroke recurrence	0.5546 (0.5376–0.5716)	3.213	42.54	67.48
Poor functional outcomes	0.6423 (0.6283–0.6564)	2.846	61.31	60.92

NLR, neutrophil to lymphocyte ratio; AUC, area under the curve; CI, confidence interval.

stroke patients in either the short- or long-term.

Similar to our study, most recent studies, which investigated the relationship between NLR and prognosis of ischemic stroke patients, used the level of NLR within the first 24 h after admission. It has been indicated that the NLR calculated at 48–72 h after acute ischemic stroke was identified as the strongest predictor of patients' outcomes (21). The median (IQR) time from symptom onset to enrollment in our study was 2 [1–4] days, and the blood samples were taken within the first 24 h after admission. Thus, the levels of NLR calculated in our study had a stronger predictive value for clinical outcomes in ischemic stroke patients.

Several recent studies have found that NLR was an independent predictor of 3-month mortality in acute ischemic stroke patients (14,15). A meta-analysis that involved 9 studies in 2017 defined poor functional outcomes of ischemic stroke as mRS ≥3 and indicated that for patients

Table 4 Risks of adverse clinical outcomes stratified by NLR under different TOAST classification

Outcomes	Q1 (NLR <1.848)	Q2 (1.848≤ NLR <2.548)	Q3 (2.548≤ NLR <3.728)	Q4 (NLR ≥3.728)	P value	P value interaction
Hemorrhagic transformation						
Large artery atherosclerosis	1	1.802 (0.683–4.757)	1.508 (0.569–3.996)	1.802 (0.695–4.668)	0.1797	0.9871
Cardiogenic embolism	1	>999.999 (<0.001–>999.999)	>999.999 (<0.001–>999.999)	>999.999 (<0.001–>999.999)	0.0075	
Small artery occlusion	1	>999.999 (<0.001–>999.999)	0.719 (<0.001–>999.999)	>999.999 (<0.001–>999.999)	0.7788	
Stroke of other determined cause	1	1.000 (<0.001–>999.999)	1.000 (<0.001–>999.999)	1.000 (<0.001–>999.999)	1.0000	
Stroke of an undetermined cause	1	1.352 (0.597–3.061)	1.517 (0.698–3.299)	2.356 (1.162–4.777)	0.9168	
3-month follow-up						
Death						
Large artery atherosclerosis	1	1.132 (0.392–3.267)	0.979 (0.341–2.814)	3.331 (1.358–8.172)	0.0002	0.7440
Cardiogenic embolism	1	1.227 (0.266–5.656)	1.088 (0.220–5.371)	1.814 (0.451–7.291)	0.3383	
Small artery occlusion	1	>999.999 (<0.001–>999.999)	>999.999 (<0.001–>999.999)	>999.999 (<0.001–>999.999)	0.0593	
Stroke of other determined cause	1	3.872 (<0.001–>999.999)	0.034 (<0.001–>999.999)	2.887 (<0.001–>999.999)	0.9802	
Stroke of an undetermined cause	1	2.455 (0.992–6.077)	2.942 (1.240–6.979)	3.746 (1.637–8.571)	0.0357	
Stroke recurrence						
Large artery atherosclerosis	1	0.943 (0.643–1.382)	0.981 (0.676–1.423)	1.423 (1.001–2.025)	0.0612	0.9095
Cardiogenic embolism	1	0.840 (0.317–2.223)	1.425 (0.583–3.488)	1.876 (0.812–4.336)	0.2359	
Small artery occlusion	1	0.989 (0.590–1.659)	1.012 (0.598–1.710)	1.588 (0.952–2.649)	0.0139	
Stroke of other determined cause	1	1.292 (0.205–8.155)	0.259 (0.017–3.967)	1.512 (0.195–11.736)	0.2233	
Stroke of an undetermined cause	1	0.858 (0.606–1.214)	1.214 (0.878–1.678)	1.673 (1.231–2.274)	0.4625	
Poor functional outcomes						
Large artery atherosclerosis	1	1.490 (1.067–2.080)	1.888 (1.374–2.595)	2.622 (1.925–3.571)	<0.0001	0.5655
Cardiogenic embolism	1	0.706 (0.354–1.410)	1.562 (0.845–2.888)	1.628 (0.903–2.934)	0.1538	
Small artery occlusion	1	1.523 (0.939–2.471)	1.714 (1.064–2.759)	2.640 (1.655–4.210)	<0.0001	
Stroke of other determined cause	1	0.264 (0.038–1.836)	0.085 (0.008–0.955)	0.694 (0.103–4.689)	0.1170	
Stroke of an undetermined cause	1	1.252 (0.947–1.654)	1.773 (1.365–2.303)	2.341 (1.823–3.006)	0.1416	
12-month follow-up						
Death						

Table 4 (*continued*)

Table 4 (*continued*)

Outcomes	Q1 (NLR <1.848)	Q2 (1.848≤ NLR <2.548)	Q3 (2.548≤ NLR <3.728)	Q4 (NLR ≥3.728)	P value	P value interaction
Large artery atherosclerosis	1	1.360 (0.669–2.767)	1.499 (0.763–2.943)	2.719 (1.449–5.102)	0.0015	0.7121
Cardiogenic embolism	1	1.214 (0.441–3.343)	0.963 (0.338–2.747)	1.781 (0.718–4.414)	0.2420	
Small artery occlusion	1	1.328 (0.392–4.505)	1.361 (0.412–4.494)	2.624 (0.858–8.023)	0.2607	
Stroke of other determined cause	1	5.419 (0.030–982.300)	3.620 (0.017–756.899)	10.063 (0.100–>999.999)	0.4390	
Stroke of an undetermined cause	1	1.547 (0.871–2.749)	2.333 (1.378–3.951)	2.714 (1.636–4.501)	0.0713	
Stroke recurrence						
Large artery atherosclerosis	1	1.164 (0.841–1.612)	1.201 (0.876–1.649)	1.395 (1.021–1.905)	0.1279	0.7863
Cardiogenic embolism	1	0.612 (0.285–1.317)	1.449 (0.745–2.817)	1.328 (0.700–2.522)	0.1386	
Small artery occlusion	1	1.033 (0.701–1.522)	0.970 (0.652–1.445)	1.119 (0.738–1.699)	0.2958	
Stroke of other determined cause	1	0.878 (0.206–3.739)	0.730 (0.133–4.025)	1.179 (0.234–5.937)	0.2393	
Stroke of an undetermined cause	1	0.973 (0.742–1.277)	1.140 (0.873–1.488)	1.631 (1.268–2.098)	0.5626	
Poor functional outcomes						
Large artery atherosclerosis	1	1.527 (1.091–2.138)	1.747 (1.267–2.410)	2.183 (1.594–2.988)	0.0007	0.8725
Cardiogenic embolism	1	1.107 (0.574–2.134)	1.358 (0.724–2.548)	2.042 (1.144–3.643)	0.1085	
Small artery occlusion	1	1.299 (0.794–2.125)	1.508 (0.934–2.437)	2.153 (1.339–3.461)	0.0045	
Stroke of other determined cause	1	1.838 (0.263–12.863)	0.829 (0.097–7.096)	3.179 (0.408–24.748)	0.0208	
Stroke of an undetermined cause	1	0.983 (0.747–1.295)	1.491 (1.157–1.923)	2.017 (1.584–2.567)	0.1984	

Adjusted for age, gender, BMI, drinking, smoking, hypertension, lipid metabolism disorders, diabetes, previous stroke, atrial fibrillation, heart failure, heart valve disease, peripheral arterial disease, intravenous thrombolysis, endovascular therapy, time from symptom onset to enrollment and the NIHSS score at admission. NLR, neutrophil to lymphocyte ratio; TOAST, Trial of Org 10172 in Acute Stroke Treatment; BMI, body mass index.

with ischemic stroke, higher NLR was correlated with poorer functional outcomes at 3-month follow-up (10). Our study came to a similar conclusion, which was that a high level of NLR was associated with increased risks of both short-term death and poor functional outcomes. The corresponding cutoff values of NLR were 3.872 (sensitivity 55.39%, specificity 77.31%) for death and 2.846 (sensitivity 62.57%, specificity 61.30%) for poor functional outcomes, respectively. Moreover, we extended the follow-up time and found the above association remained significant at 12-month follow-up. NLR ≥3.180 (sensitivity 58.39%, specificity 66.69%) could predict death and NLR ≥2.846 (sensitivity 61.31%, specificity 60.92%) could predict poor functional outcomes. Thus, NLR could be regarded as a predictor for both short- and long-term adverse clinical outcomes in ischemic stroke patients. However, little is known about the immunological mechanisms underlying the above association.

After ischemic stroke, neutrophils migrate immediately, even against blood flow, then transmigrate out of blood vessels and become the first immune cells to invade the injured brain tissue (22). Additionally, the local blood-brain barrier breakdown caused by ischemia promotes neutrophil entry (23). At 48–72 h after ischemic stroke, the neutrophil invasion reaches the zenith, and neutrophils aggravate ischemic injury through a variety of different actions (24). On the one hand, neutrophil aggregation in cerebral vessels might block microvessels, impede perfusion, and eventually lead to prolonged ischemia (6). On the other hand, neutrophils could induce further thrombus formation by releasing molecules to participate in neutrophil extracellular trap formation or interacting with platelets directly (25).

Besides, neutrophils also lead to the release of deleterious substances or inflammatory mediators, thus potentiating ischemic damage (25). In experimental brain ischemia models, various therapeutic interventions, by blocking pro-inflammatory cytokines to inhibit neutrophil infiltration or blocking the neutrophil pro-inflammatory function, successfully reduced the infarct volume and improved neurological outcomes (25,26).

Lymphocytes also accumulate in the post-ischemic brain but more slowly than neutrophils. Different subtypes of lymphocytes exert different effects on cerebral ischemia (8,27). Pro-inflammatory lymphocytes, such as T helper type 1 (TH1) and T helper type 17 (TH17), have been thought to worsen stroke outcomes (28-30). In contrast, regulatory T cells (Treg) have been characterized as disease-limiting protective cells, and their percentage within the T helper cell population is increased in brains after stroke (31). It is acknowledged that Tregs can modulate various immunologic pathways and maintain immune homeostasis by interacting with other cells directly and producing anti-inflammatory cytokines (32). In an experimental stroke model, the selective depletion of Tregs had a link with an increase in infarct volume and behavioral deficits after brain ischemia (11). Besides, regulatory B cells also showed the capacity to limit central nervous system inflammation and neurological deficits in murine experimental stroke (13).

In summary, neutrophils and lymphocytes affect acute brain ischemic injury through different actions, and their functional role might be much more complicated and diversified than currently appreciated. As an inflammatory marker that integrates both neutrophils and lymphocytes, the NLR could reflect the imbalance of post-stroke immunization more accurately than leukocyte or neutrophil counts. Moreover, NLR is much easier to evaluate than many other indicators.

Currently, limited studies are investigating the relationship between NLR and stroke recurrence in ischemic stroke patients. It has previously been indicated that high levels of neutrophil ratio were associated with an increased risk of new stroke in patients with a minor ischemic stroke or TIA (33). Our study showed there might also be a correlation between the level of NLR above 3.156 (sensitivity 46.58%, specificity 66.19%) and elevated risks of stroke recurrence at 3-month follow-up; however, further research is required to validate this finding.

Studies by Maestrini and Guo demonstrated that NLR was independently correlated with symptomatic intracranial hemorrhage after intravenous thrombolysis in patients with ischemic stroke (34,35). At the same time, Goyal and Duan also reported that higher NLR was an independent predictor of symptomatic intracranial hemorrhage after endovascular treatment in patients with ischemic stroke (36,37). A recent meta-analysis involving 7 studies showed that an NLR with a cutoff value of 7.5–11 could predict hemorrhagic transformation in acute ischemic stroke patients (38). However, we found that NLR was not related to ischemic stroke patients' hemorrhagic transformation during hospitalization. The studies mentioned above were based on the observation of ischemic stroke patients under reperfusion therapy whose risks of hemorrhagic transformation were higher, which we thought might explain the difference between our study and those mentioned above.

Our study detected differences in NLR under different etiology. In particular, among patients with large artery atherosclerosis and cardiogenic embolism, NLR was significantly higher. This might be because post-ischemic inflammatory responses are different in patients with different TOAST classifications. Atherosclerosis is a chronic inflammatory process. In patients with large artery atherosclerosis, ischemic stroke might trigger a much stronger inflammatory response based on this chronic inflammatory process. In patients with cardiogenic embolism, the neurological deficit always peaks rapidly, and the infarct volume is usually large, causing a strong, rapid inflammatory response. These factors might explain the higher NLR in patients with large artery atherosclerosis and cardiogenic embolism. Our results were consistent with those of most previous studies; however, NLR has also been reported to not vary significantly among the stroke subtypes (39). Furthermore, our study indicated that the relationships between NLR and adverse clinical outcomes did not differ between patients with different TOAST classifications, which suggested that the different post-ischemic inflammatory responses might have similar impacts on the clinical outcomes of patients with different etiology. However, more recently, studies focused on NLR associations with adverse clinical outcomes in patients with ischemic stroke under different etiology have been limited, and our results need to be confirmed through future research.

There were several limitations to our study. Firstly, heterogeneity of equipment in participating hospitals may have led to biased estimates of association, but this might have had little impact on results due to daily practice and strict quality control in every participating hospital.

Secondly, our study only collected the level of NLR at baseline and did not have dynamic data about NLR or evaluate the change of NLR during hospitalization. However, the dynamic change of NLR over time might provide valuable information to understand the underlying mechanism of inflammatory response after ischemic stroke. Thirdly, the median (IQR) of the NIHSS score at the admission of all participants in our study was 3 [2–6]. The severity of ischemic stroke may have been mild, and this could have limited the ability to estimate the association of NLR with clinical outcomes in more severe ischemic stroke patients. Finally, residual bias might have remained because of the influence of comorbidities or environmental factors such as tumor, trauma, and acute toxicosis.

Conclusions

The NLR within the first 24 h after admission was significantly higher in patients with large artery atherosclerosis and cardiogenic embolism. Regardless of etiology, a high level of NLR was associated with increased risks of both short- and long-term adverse clinical outcomes in patients with ischemic stroke.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The ethics committee at Beijing Tiantan Hospital (IRB approval number: KY2015-001-01) and all study centers gave ethical approval of the CNSR-III study protocol. Written consent was provided by all participants or their legal representatives.

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