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ARTICLE

Neutrophil counts, neutrophil ratio, and new stroke in minor ischemic stroke or TIA

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Abstract

Objective

Evidence about whether neutrophil counts or neutrophil ratio is associated with new stroke is scant. The aim of this study is to assess the association of neutrophil counts or neutrophil ratio with a new stroke in patients with minor stroke or TIA.

Methods

We derived data from the Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events trial. Patients with a minor stroke or TIA were categorized into 4 groups according to the quartile of neutrophil counts or neutrophil ratio. The primary outcome was a new stroke (ischemic or hemorrhagic), and secondary outcomes included a new composite vascular event (stroke, myocardial infarction, or death resulting from cardiovascular causes) and ischemic stroke during the 90-day follow-up. We assessed the association between neutrophil counts, neutrophil ratio, and risk of new stroke.

Results

A total of 4,854 participants were enrolled, among whom 495 had new strokes at 90 days. Compared with the first quartile, the second, third, and fourth quartiles of neutrophil counts were associated with increased risk of new stroke (adjusted hazard ratio 1.40 [95% confidence interval (CI) 1.05–1.87], 1.55 [95% CI 1.17–2.05], and 1.69 [95% CI 1.28–2.23], respectively, p for trend <0.001). Similar results were observed for the endpoint of composite events and ischemic stroke. Parallel results were found for neutrophil ratio.

Conclusion

High levels of both neutrophil counts and neutrophil ratio were associated with an increased risk of new stroke, composite events, and ischemic stroke in patients with a minor ischemic stroke or TIA.

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Coinvestigators are listed at links.lww.com/WNL/A468

Glossary

CAPRIE = Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events; **CHANCE** = Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events; **CI** = confidence interval; **HR** = hazard ratio.

Inflammatory parameters markedly influence stroke outcome according to extensive evidence.[1–4] Stroke provokes not only local inflammatory processes in the ischemic brain but also peripheral immune responses, which cause secondary lesion growth and thus modulate outcome.[5,6] Higher levels of inflammatory markers such as interleukin-6[7] and C-reactive protein[8] were reported to be associated with a worse outcome after ischemic stroke.[9] However, these studies had limitations, chiefly that they were too small and did not assess the clinical utility of the measurements. Leukocyte count is an indicator for acute or chronic inflammation. Neutrophils, which are the most numerous type of leukocyte, play a major role in inflammation. Prior studies have shown that high leukocyte and neutrophil counts were associated with ischemic stroke events.[3,10–12] Furthermore, an elevated leukocyte count in the acute phase of stroke was associated with a poor outcome[2]; however, it is unclear whether this was the result of leukocyte total count or its subtypes.[11] Moreover, it remains unclear whether neutrophil count is a parameter to predict new stroke in patients with acute minor ischemic stroke or TIA. The neutrophil ratio, which is calculated as the ratio of neutrophil absolute value to total leukocyte absolute value, represents the relative increase in neutrophil counts and is usually used as another inflammation parameter in clinical practice. However, little data exist about the relationship between neutrophil ratio and stroke outcome. A previous study indicated that the neutrophil-to-lymphocyte ratio was associated with in-hospital mortality of stroke patients.[13] However, the sample size of this study was small, and it did not report the outcome of stroke recurrence.

Using data from the Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events (CHANCE) trial, we investigated the association of neutrophil count, neutrophil ratio, and risk of stroke after a minor ischemic stroke or TIA.

Methods

Study participants

We derived data from the CHANCE trial.[14] Details on the rationale, design, and major results of the CHANCE trial have been published previously.[14,15] Briefly, CHANCE is a randomized, double-blind, controlled trial conducted at 114 hospitals in China between October 2009 and July 2012 in which 5,170 patients within 24 hours after onset of a minor stroke or high-risk TIA were randomly assigned to the clopidogrel plus aspirin group or aspirin alone group. The primary aim of the trial was to assess the efficacy of the combination of clopidogrel and aspirin in reducing the stroke risk in the first 90 days in patients with TIA or minor stroke. Patients in the trial met the following criteria: age ≥40 years, diagnosis of an acute minor ischemic stroke (NIH Stroke Scale score ≤3) or high-risk TIA (ABCD2 score ≥4), and ability to initiate the study drug within 24 hours after onset. In this analysis, we excluded the patients with proven infection on admission or in hospitalization that was diagnosed on the basis of a standardized protocol to avoid potential influence of infection.

Standard protocol approvals, registrations, and patient consents

The CHANCE trial is registered at ClinicalTrials.gov (NCT00979589). The protocol and data collection of the CHANCE trial were approved by the ethics committee of Beijing Tiantan Hospital and all other study centers. All participants or their representatives provided written informed consent before being entered into the study.

Baseline data collection

Baseline data on demographics and medical history such as ischemic stroke, TIA, myocardial infarction, coronary heart disease, atrial fibrillation, hypertension, diabetes mellitus, hypercholesterolemia, and smoking status were collected through face-to-face interviews by trained interviewers (neurologists from participating hospitals). Total leukocyte counts and neutrophil counts in EDTA-anticoagulated whole-blood samples from venipuncture were determined with automated particle counters at hematology laboratories in the 114 centers within the first 24 hours after admission.

Outcome assessment

The primary efficacy outcome was a new stroke event (ischemic or hemorrhagic) within 90 days.[14] Ischemic stroke was defined as an acute focal infarction of the brain or retina and not attributable to a nonischemic cause; hemorrhagic stroke was defined as acute extravasation of blood into the subarachnoid space or brain parenchyma with associated neurologic symptoms.[14] The secondary efficacy outcome included composite events (stroke, myocardial infarction, or death resulting from cardiovascular causes) and ischemic stroke.[14] A central adjudication committee that was blinded to the study group assignments confirmed all reported efficacy endpoints.

Statistical analysis

We presented continuous variables as mean ± SD or median with interquartile range and categorical variables as percentages. Baseline variables among different categories of neutrophil counts or neutrophil ratio were compared by 1-way

analysis of variance or the Kruskal-Wallis test for continuous and χ^2 test for categorical variables.

The proportional hazard assumption for the Cox models was examined by including a time-dependent covariate with interaction of the neutrophil counts or neutrophil ratio and a logarithmic function of survival time in the model. To examine the interaction effect of neutrophil counts or neutrophil ratio category by treatment group assignment, we tested the statistical significance of neutrophil counts or neutrophil ratio × treatment group assignment in a multivariable Cox model. We further assessed the associations between neutrophil counts or neutrophil ratio and the prognosis of minor stroke or TIA using multivariable Cox regression models. Adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) were calculated. We used 2 models. In the first model, we adjusted for only age and sex. In the second model, we included all the potential confounding variables. Furthermore, the associations between neutrophil counts or neutrophil ratio and patient outcomes were assessed by propensity score methods in sensitivity analyses. The generalized propensity score for each neutrophil count or neutrophil ratio category was estimated with a nonparsimonious multivariable multinomial logistic regression model including all baseline variables. Then, HRs with their CIs were estimated by Cox regression models with adjustment for propensity score.

C statistics and net reclassification index were calculated to evaluate improvement in risk classification by neutrophil counts and neutrophil ratio over traditional risk factors. The optimal cutoff value of neutrophil counts and neutrophil ratio to predict each outcome was determined by receiver operating characteristics curve and the Youden index.

We further evaluated the pattern and magnitude of associations between neutrophil counts or neutrophil ratio and risk of stroke using a Cox regression model with restricted cubic splines for neutrophil counts or neutrophil ratio (continuous variable), adjusting for covariates.

Two-sided values of $p = 0.05$ were considered to be statistically significant. All analyses were conducted with SAS software version 9.4 (SAS Institute Inc, Cary, NC).

Data Availability

Data are available to researchers on request for purposes of reproducing the results or replicating the procedure by directly contacting the corresponding author.

Results

Study participants and characteristics

A total of 5,170 patients with a minor ischemic stroke or TIA were enrolled in the CHANCE trial. After the exclusion of 20 patients with infection (including respiratory and urinary infection) and 296 patients without an available neutrophil count test on admission, 4,854 patients were included in the analysis. Among the 4,854 patients, the average age was 62.6 ± 10.7 years (range 33–96 years, 5 patients with age <40 years were enrolled in the trial), 1,644 (33.9%) were female, 3,494 (72.0%) had ischemic minor stroke, and 1,360 (28.0%) had TIA.

The median neutrophil count was 4.12 (interquartile range 3.20–5.31) × 10^9/L and median neutrophil ratio was 64.01% (interquartile range 56.82%–71.57%). Baseline characteristics of the patients by quartiles of the neutrophil counts and neutrophil ratio are shown in tables 1 and 2. Participants in the higher neutrophil count quartile were more likely to be male, younger, and smokers; to have a history of hypertension; had an index event of minor stroke; and had higher severity of stroke (table 1). Participants in the highest neutrophil ratio quartile were more likely to be older and nonsmokers and to have a shorter time to randomization, an index event of minor stroke, and a higher severity of stroke (table 2).

Association of neutrophil count and neutrophil ratio with risk of stroke

After 3 months, 495 (10.2%) new strokes occurred, of which 479 (96.8%) were ischemic stroke and 16 (3.2%) were hemorrhagic stroke, and 501 (10.3%) composite events occurred.

Table 3 and table e-1, links.lww.com/WNL/A467, show the association between quartiles of neutrophil counts and clinical outcomes. Patients in the higher neutrophil categories were associated with worse outcome at 3 months, including a higher risk of 3-month new stroke, composite events, and ischemic stroke (p for trend <0.001). All of the proportional hazard assumptions were met (p = 0.46, 0.53, and 0.35 for new stroke, composite events, and ischemic stroke, respectively). Compared with the lowest quartile, the second, third, and fourth quartiles of neutrophil counts were associated with increased risk of the following: new stroke, adjusted HR, 1.40 (95% CI 1.05–1.87), 1.55 (95% CI 1.17–2.05), and 1.69 (95% CI 1.28–2.23), respectively; composite events, 1.36 (95% CI 1.03–1.80), 1.49 (95% CI 1.12–1.97), and 1.64 (95% CI 1.24–2.16), respectively; and ischemic stroke, 1.37 (95% CI 1.02–1.83), 1.57 (95% CI 1.18–2.09), and 1.71 (95% CI 1.29–2.27), respectively. Kaplan-Meier survival curves for stroke are presented in figure e-1A, links.lww.com/WNL/A466.

Using the propensity score for adjustment, we observed similar results. There was no interaction effect of neutrophil count by antiplatelet therapy for the risk of new stroke, composite events, and ischemic stroke (p for interaction = 0.27, 0.20 and 0.26 in the full adjusted models, respectively). Adding neutrophil count to a multivariable model containing main traditional risk factors significantly improved predictive ability (table e-2, links.lww.com/WNL/A467). The optimal cutoff value of neutrophil count to predict each outcome was at 4.47 × 10^9/L.

We found similar associations using the neutrophil ratio instead of the neutrophil count. The risk of recurrent ischemic events

Table 1 Baseline characteristics of the patients by neutrophil count quartiles

Characteristics	Total patients (n = 4,854)	Quartiles of neutrophil count, $\times 10^9$/L				p Value
		Q1, ≤3.19 (n = 1,140)	Q2, 3.20–4.11 (n = 1,281)	Q3, 4.12–5.30 (n = 1,215)	Q4, ≥5.31 (n = 1,218)	
Age, median (IQR), y	62.3 (54.7–71.2)	63.3 (55.7–72.4)	62.8 (54.7–71.3)	61.8 (54.6–70.4)	61.3 (53.7–71.1)	<0.001
Female, n (%)	1,644 (33.9)	441 (38.7)	425 (33.2)	392 (32.3)	386 (31.7)	0.001
Medical history, n (%)						
Ischemic stroke	959 (19.8)	223 (19.6)	256 (20.0)	237 (19.5)	243 (20.0)	0.99
TIA	164 (3.4)	38 (3.3)	42 (3.3)	44 (3.6)	40 (3.3)	0.96
Myocardial infarction	89 (1.8)	25 (2.2)	22 (1.7)	22 (1.8)	20 (1.6)	0.76
Angina	170 (3.5)	38 (3.3)	48 (3.7)	44 (3.6)	40 (3.3)	0.91
Congestive heart failure	76 (1.6)	16 (1.4)	19 (1.5)	19 (1.6)	22 (1.8)	0.87
Known atrial fibrillation or flutter	90 (1.9)	22 (1.9)	28 (2.2)	18 (1.5)	22 (1.8)	0.63
Valvular heart disease	14 (0.3)	3 (0.3)	6 (0.5)	2 (0.2)	3 (0.2)	0.53
Diabetes mellitus	1,026 (21.1)	223 (19.6)	271 (21.2)	263 (21.6)	269 (22.1)	0.47
Hypertension	3,186 (65.6)	717 (62.9)	831 (64.9)	800 (65.8)	838 (68.8)	0.02
Hypercholesterolemia	518 (10.7)	110 (9.6)	128 (10.0)	136 (11.2)	144 (11.8)	0.27
Previous or current smoker, n (%)	2,084 (42.9)	439 (38.5)	544 (42.5)	554 (45.6)	547 (44.9)	0.002
Index event, n (%)						0.004
Minor stroke	3,494 (72.0)	804 (70.5)	885 (69.1)	891 (73.3)	914 (75.0)	
TIA	1,360 (28.0)	336 (29.5)	396 (30.9)	324 (26.7)	304 (25.0)	
NIHSS score on admission, median (IQR)	2 (0–2)	1 (0–2)	1 (0–2)	2 (0–2)	2 (1–3)	<0.001
Mean time to randomization, h	12.6 ± 7.0	12.6 ± 7.0	12.7 ± 7.1	12.9 ± 7.0	12.4 ± 6.8	0.36
Time to randomization, n (%)						0.31
<12 h	2,422 (49.9)	586 (51.4)	630 (49.2)	584 (48.1)	622 (51.1)	
≥12 h	2,432 (50.1)	554 (48.6)	651 (50.8)	631 (51.9)	596 (48.9)	
Antiplatelet therapy, n (%)						0.69
Aspirin only	2,418 (49.8)	579 (50.8)	629 (49.1)	615 (50.6)	595 (48.9)	
Clopidogrel + aspirin	2,436 (50.2)	561 (49.2)	652 (50.9)	600 (49.4)	623 (51.1)	

Abbreviations: IQR = interquartile range; NIHSS = NIH Stroke Scale; Q = quartile.

was significantly higher in both upper quartiles of neutrophil ratio than in the bottom quartile (p for trend <0.001). All of the proportional hazard assumptions were met (p = 0.59, 0.46, and 0.37 for new stroke, composite events, and ischemic stroke, respectively). Compared with patients in the first quartile, the third and fourth quartiles of neutrophil ratio were associated with increased risk of the following: new stroke, adjusted HR, 1.38 (95% CI 1.05–1.81) and 1.70 (95% CI 1.30–2.23), respectively; composite events, 1.35 (95% CI 1.03–1.78) and 1.68 (95% CI 1.29–2.20), respectively; and ischemic stroke, 1.39 (95% CI 1.05–1.84) and 1.73 (95% CI 1.32–2.28), respectively. However, the second quartile was not significantly associated with the events (table 4 and table e-3, links.lww.com/WNL/A467). Kaplan-Meier survival curves for stroke are presented in figure e-1B, links.lww.com/WNL/A466. Using the propensity score for adjustment, we observed similar results. There was no interaction effect of neutrophil ratio by antiplatelet therapy for the risk of stroke, composite events, and ischemic stroke (p for interaction = 0.22, 0.20, and 0.22 in the full adjusted models, respectively). Adding neutrophil ratio to a multivariable model containing main traditional risk factors significantly improved predictive ability (table e-2). The optimal cutoff value of neutrophil ratio to predict each outcome was 69.8%.

Table 2 Baseline characteristics of the patients by neutrophil ratio quartiles

Characteristics	Quartiles of neutrophil ratio,[a] %				
	Q1, ≤56.81 (n = 1,215)	Q2, 56.82–64.00 (n = 1,214)	Q3, 64.01–71.56 (n = 1,214)	Q4, ≥71.57 (n = 1,211)	*p* Value
Age, median (IQR), y	61.0 (53.8–69.8)	61.5 (54.7–70.5)	63.1 (55.3–72.2)	63.5 (54.9–72.3)	<0.001
Female, n (%)	435 (35.8)	415 (34.2)	375 (30.9)	419 (34.6)	0.07
Medical history, n (%)					
Ischemic stroke	248 (20.4)	223 (18.4)	241 (19.9)	247 (20.4)	0.55
TIA	40 (3.3)	43 (3.5)	42 (3.5)	39 (3.2)	0.97
Myocardial infarction	22 (1.8)	26 (2.1)	28 (2.3)	13 (1.1)	0.11
Angina	49 (4.0)	42 (3.5)	43 (3.5)	36 (3.0)	0.57
Congestive heart failure	16 (1.3)	19 (1.6)	27 (2.2)	14 (1.2)	0.16
Known atrial fibrillation or flutter	19 (1.6)	25 (2.1)	17 (1.4)	29 (2.4)	0.24
Valvular heart disease	2 (0.2)	8 (0.7)	2 (0.2)	2 (0.2)	0.052
Diabetes mellitus	273 (22.5)	251 (20.7)	257 (21.2)	245 (20.2)	0.56
Hypertension	784 (64.5)	782 (64.4)	809 (66.6)	811 (67.0)	0.40
Hypercholesterolemia	139 (11.4)	138 (11.4)	129 (10.6)	112 (9.2)	0.27
Previous or current smoker, n (%)	543 (44.7)	556 (45.8)	524 (43.2)	461 (38.1)	<0.001
Index event, n (%)					0.002
Minor stroke	831 (68.4)	865 (71.3)	891 (73.4)	907 (74.9)	
TIA	384 (31.6)	349 (28.7)	323 (26.6)	304 (25.1)	
NIHSS score on admission, median (IQR)	1 (0–2)	1 (0–2)	2 (0–2)	2 (0–3)	<0.001
Mean time to randomization, h	12.9 ± 7.0	12.8 ± 7.0	12.8 ± 7.0	12.1 ± 6.9	0.02
Time to randomization, n (%)					0.01
<12 h	584 (48.1)	579 (47.7)	609 (50.2)	650 (53.7)	
≥12 h	631 (51.9)	635 (52.3)	605 (49.8)	561 (46.3)	
Antiplatelet therapy, n (%)					
Aspirin only	616 (50.7)	613 (50.5)	605 (49.8)	584 (48.2)	0.61
Clopidogrel + aspirin	599 (49.3)	601 (49.5)	609 (50.2)	627 (51.8)	

Abbreviations: IQR = interquartile range; NIHSS = NIH Stroke Scale; Q = quartile.
[a] Neutrophil ratio = neutrophil count/total leukocyte count.

Using a Cox regression model with restricted cubic spline, we found that a higher level of neutrophil count or neutrophil ratio was associated with an increased risk of new stroke (figure 1).

Discussion

In this post hoc analysis of the CHANCE trial, we found that both high level of neutrophil count and neutrophil ratio were associated with an increased risk of new stroke in patients with a minor ischemic stroke or TIA. Our study indicated that patients in the highest quartile of neutrophil count and neutrophil ratio, compared with the patients in the lowest quartile, had a 69% and 70%, respectively, increase in risk of new stroke in 90 days.

Traditional risk factors do not explain all epidemiologic features of ischemic vascular diseases, and increasing evidence demonstrates that inflammatory parameters are associated with the risk of future vascular events.[9] It is interesting to note that a black and white community study observed a positive association between elevated leukocyte counts and the risk of ischemic stroke incidence and mortality from cardiovascular disease, and further analysis revealed that granulocyte and monocyte counts have a strong association with ischemic

Table 3 Risk of stroke at 3 months after a minor stroke or TIA by neutrophil count

Outcomes	Quartile of neutrophil count, $\times 10^9$/L	No.	Events, n (%)	Model 1[a]		Model 2[b]		Propensity score adjustment	
				Adjusted HR (95% CI)	*p* Value	Adjusted HR (95% CI)	*p* Value	Adjusted HR (95% CI)	*p* Value
Stroke	Q1, ≤3.19	1,140	81 (7.1)	1		1		1	
	Q2, 3.20–4.11	1,281	123 (9.6)	1.41 (1.06–1.87)	0.02	1.40 (1.05–1.87)	0.02	1.38 (1.04–1.82)	0.03
	Q3, 4.12–5.30	1,215	133 (10.9)	1.57 (1.19–2.08)	0.002	1.55 (1.17–2.05)	0.003	1.56 (1.19–2.06)	0.002
	Q4, ≥5.31	1,218	158 (13.0)	1.83 (1.39–2.41)	<0.001	1.69 (1.28–2.23)	<0.001	1.75 (1.33–2.30)	<0.001
Composite events[c]	Q1, ≤3.19	1,140	84 (7.4)	1		1		1	
	Q2, 3.20–4.11	1,281	124 (9.7)	1.37 (1.04–1.81)	0.03	1.36 (1.03–1.80)	0.03	1.34 (1.01–1.76)	0.04
	Q3, 4.12–5.30	1,215	133 (10.9)	1.51 (1.15–2.00)	0.003	1.49 (1.12–1.97)	0.005	1.51 (1.15–1.98)	0.003
	Q4, ≥5.31	1,218	160 (13.1)	1.78 (1.35–2.34)	<0.001	1.64 (1.24–2.16)	<0.001	1.71 (1.31–2.24)	<0.001
Ischemic stroke	Q1, ≤3.19	1,140	78 (6.8)	1		1		1	
	Q2, 3.20–4.11	1,281	116 (9.1)	1.38 (1.03–1.84)	0.03	1.37 (1.02–1.83)	0.04	1.35 (1.01–1.79)	0.04
	Q3, 4.12–5.30	1,215	130 (10.7)	1.59 (1.20–2.12)	0.001	1.57 (1.18–2.09)	0.002	1.59 (1.20–2.10)	0.001
	Q4, ≥5.31	1,218	155 (12.7)	1.85 (1.40–2.45)	<0.001	1.71 (1.29–2.27)	<0.001	1.78 (1.35–2.34)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; Q = quartile.
[a] Model 1: adjusted for age and sex.
[b] Model 2: adjusted for age, sex, history of ischemic stroke, TIA, myocardial infarction, angina, congestive heart failure, known atrial fibrillation or flutter, valvular heart disease, hypertension, diabetes mellitus, hypercholesterolemia, smoking status, index event and NIH Stroke Scale score on admission, time to randomization, and antiplatelet therapy.
[c] Composite events: stroke, myocardial infarction, or death resulting from cardiovascular causes.

stroke incidence.[11,16] Lack of data about granulocyte subtype may weaken the prediction. In addition, large population-based cohorts from England[12] and China[11] showed that elevated neutrophil counts were associated with the incidence of cardiovascular diseases, including unheralded coronary death,[12] heart failure,[12] and stroke.[11] However, clinical meaning may decrease because of the lack of high-risk population. Furlan et al.[17] documented that an elevated leukocyte counts was correlated with poor outcomes after acute ischemic stroke. However, this study did not mention the association between neutrophil and ischemic stroke. Subgroup analysis of the Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial indicated that leukocyte counts, mainly neutrophil counts, were independently associated with recurrent ischemic events in high-risk populations.[3] Different from the definition of high-risk population in CAPRIE, i.e., ischemic stroke, myocardial infarction, or peripheral arterial disease, CHANCE enrolled patients with acute minor ischemic stroke (NIH Stroke Scale score ≤3) or TIA (ABCD2 score ≥4) within 24 hours. Because leukocyte count is based on multiple variables, which reflect the total counts of neutrophils, monocytes, lymphocytes, eosinophils, and basophils, its sensitivity and specificity are low.[18] We also examined the total leukocyte counts for predictive ability of stroke events in our study, but the associations were not as strong. Hao et al.[19] found an association between higher baseline neutrophil ratio and increased risk of symptomatic intracerebral hemorrhage after endovascular treatment in patients with acute ischemic stroke. Unfortunately, little research has been conducted on neutrophil ratio in high-risk ischemic stroke patients until now. Our data showed that elevated neutrophil level on admission was associated with a new stroke in the high-risk population.

The mechanisms linking elevated neutrophil levels on admission to worse 3-month outcome are insufficiently understood. Potential mechanisms include interactions with the endothelium and platelets and overactivity of neutrophil extracellular traps.[20] Neutrophils can release autacoids, which induce vasoconstriction and platelet aggregation[21,22]; once activated, neutrophils release a variety of proteolytic enzymes, including free radicals (direct neurotoxic activity[23]), elastase, and myeloperoxidase (promoting phagocytosis), with potential for tissue destruction. Several reports suggest a detrimental role of brain-infiltrating neutrophils in ischemic tissue damage, for example, by releasing oxygen radicals and inflammatory mediators.[24–27] Neutrophils stimulate thrombogenesis by releasing tissue factors[23,28,29] or inducing other cells to release tissue factors.[30,31] Furthermore, neutrophil granulocytes have been shown to be an important source of matrix metalloproteinase-9, which can cause disruption of the blood-brain barrier in patients with ischemic stroke.[32] In addition, intravascular accumulation of neutrophils impairs local blood flow, leading to the no-reflow phenomenon of the affected microcirculation.[33,34] Because of the limited regenerative capacities of the neural cells, the secondary induced tissue damage may influence the prognosis. While the immune

Table 4 Risk of stroke at 3 months after a minor stroke or TIA by neutrophil ratio

Outcomes	Quartiles of neutrophil ratio, %	No.	Events, n (%)	Model 1[a]		Model 2[b]		Propensity score adjustment	
				Adjusted HR (95% CI)	*p* Value	Adjusted HR (95% CI)	*p* Value	Adjusted HR (95% CI)	*p* Value
Stroke	Q1, ≤56.81	1,215	93 (7.7)	1		1		1	
	Q2, 56.82–64.00	1,214	104 (8.6)	1.15 (0.87–1.53)	0.33	1.12 (0.84–1.50)	0.43	1.12 (0.85–1.48)	0.42
	Q3, 64.01–71.56	1,214	131 (10.8)	1.42 (1.08–1.87)	0.01	1.38 (1.05–1.81)	0.02	1.37 (1.05–1.79)	0.02
	Q4, ≥71.57	1,211	167 (13.8)	1.81 (1.39–2.36)	<0.001	1.70 (1.30–2.23)	<0.001	1.70 (1.31–2.20)	<0.001
Composite events[c]	Q1, ≤56.81	1,215	95 (7.8)	1		1		1	
	Q2, 56.82–64.00	1,214	105 (8.6)	1.14 (0.86–1.51)	0.37	1.11 (0.83–1.47)	0.48	1.11 (0.84–1.46)	0.47
	Q3, 64.01–71.56	1,214	132 (10.9)	1.40 (1.07–1.84)	0.01	1.35 (1.03–1.78)	0.03	1.35 (1.04–1.76)	0.03
	Q4, ≥71.57	1,211	169 (14.0)	1.79 (1.37–2.33)	<0.001	1.68 (1.29–2.20)	<0.001	1.69 (1.31–2.18)	<0.001
Ischemic stroke	Q1, ≤56.81	1,215	89 (7.3)	1		1		1	
	Q2, 56.82–64.00	1,214	100 (8.2)	1.15 (0.86–1.54)	0.34	1.13 (0.84–1.51)	0.42	1.13 (0.85–1.50)	0.41
	Q3, 64.01–71.56	1,214	127 (10.5)	1.44 (1.09–1.89)	0.01	1.39 (1.05–1.84)	0.02	1.39 (1.06–1.83)	0.02
	Q4, ≥71.57	1,211	163 (13.5)	1.84 (1.40–2.41)	<0.001	1.73 (1.32–2.28)	<0.001	1.73 (1.33–2.25)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; Q = quartile.
Neutrophil ratio = neutrophil count/total leukocyte count.
[a] Model 1: adjusted for age and sex.
[b] Model 2: adjusted for age, sex, history of ischemic stroke, TIA, myocardial infarction, angina, congestive heart failure, known atrial fibrillation or flutter, valvular heart disease, hypertension, diabetes mellitus, hypercholesterolemia, smoking status, index event and NIH Stroke Scale score on admission, time to randomization, and antiplatelet therapy.
[c] Composite events: stroke, myocardial infarction, or death resulting from cardiovascular causes.

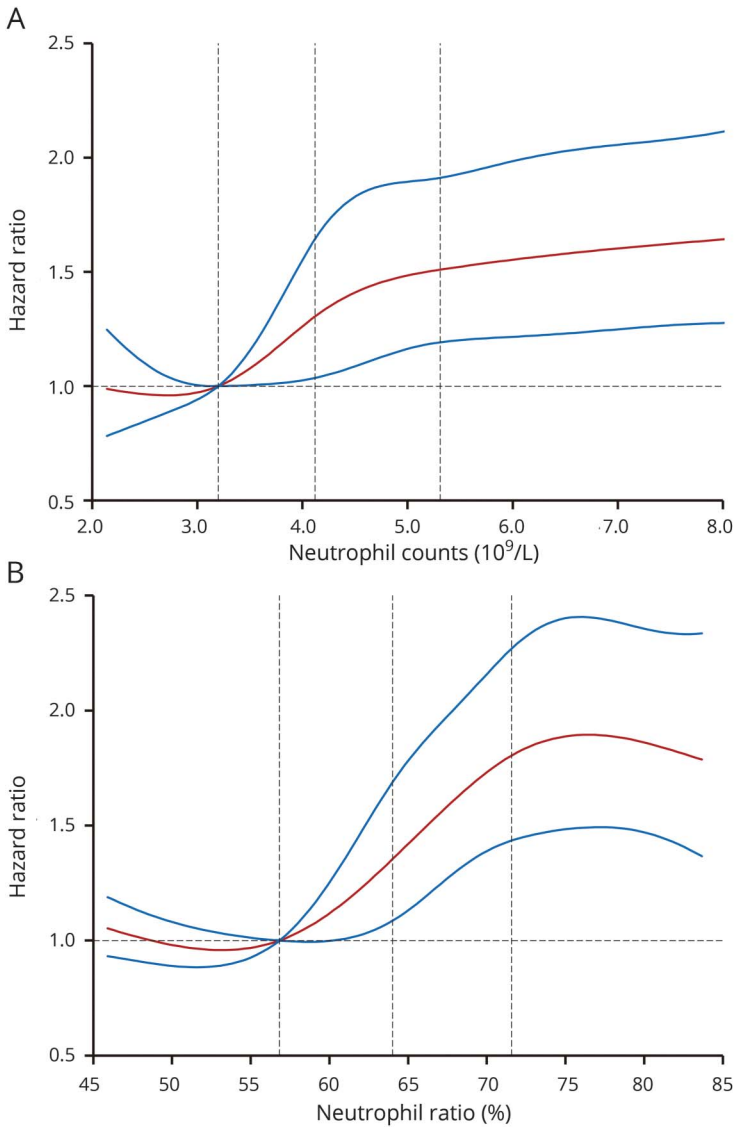
system is essential to clear necrotic tissue and to initiate regenerative processes after stroke, the intensity of the initial immune response may also cause tissue damage.[35,36]

Our study suggested that early inflammation may be detrimental to the stroke outcome. The neutrophil absolute increase or relative increase (elevated neutrophil ratio) could be the result of the acute-phase reaction and the marker of the severity of disease and tissue inflammation.[37] Elevated neutrophil count may represent a marker of severity of tissue inflammation due to ischemic injury and perhaps a lower probability of successful reperfusion or impaired microvascular perfusion.[38] Elevated neutrophil count or neutrophil ratio represented a readily available prognostic parameter. The intriguing result may call for further trials focusing on immunomodulation therapy targeting neutrophils[4] in patients with minor ischemic stroke or TIA.

In fact, the current clinical practice of labeling the range of neutrophil count of 2 to 7 $\times 10^9$/L and neutrophil ratio of 50% to 70% as normal is well accepted by neurologists. However, debate exists on whether it is right to judge the normal range while ignoring any risk information conveyed by the actual value.[12] In our study, the second and third quartiles of neutrophil counts and most of the third quartile of neutrophil ratio were in the normal range. In addition, it still has clinical meaning when absolute neutrophil counts are in the normal range coupled with a high neutrophil ratio. An advantage of using the neutrophil count or neutrophil ratio over other inflammatory parameters is that it is already measured and no additional costs for testing would be incurred. The determination of neutrophil counts and neutrophil ratio is economic, rapid, and universally available and might improve our ability to stratify the risk of minor ischemic stroke or TIA in clinical practice.

The main strength of this study is the large, multicenter, double-blind, and high-risk patient–based design, allowing subgroup analysis. In addition, potential confounders were in consideration by extensive adjustment. However, important limitations should not be ignored. First, heterogeneity of equipment at 114 centers may have led to biased estimates of association, but this may have had little influence on the results because of daily practice and strict quality control in every center. Second, we have no data about hematocrit in this study, which reflects blood concentration. Third, the measurement of neutrophil counts or neutrophil ratio was collected only at baseline. A previous study showed that a continuously persistent increase in leukocyte counts over 3 months was associated with an increased risk of ischemic events.[3] Fourth, the etiology classification of stroke was not collected in the trial, and we could not investigate the associations between neutrophil counts and neutrophil ratio and stroke prognosis by stroke mechanism. Furthermore, the observed findings could also be affected by different time

Figure 1 Adjusted hazard ratios of new stroke according to (A) neutrophil count and (B) neutrophil ratio



Red line indicates adjusted hazard ratio, and blue lines indicate the 95% confidence interval bands. Reference is neutrophil count of 3.19 ×10^9/L and neutrophil ratio of 56.81%. Data were fitted with a Cox regression model of restricted cubic spline with 5 knots (5th, 25th, 50th, 75th, and 95th percentiles) for neutrophil count and neutrophil ratio, with adjustment for potential covariates. The lowest 5% and highest 5% of participants are not shown because of small sample sizes.

points of venipuncture used for the assessment of the analyzed blood sample. Finally, residual bias may still exist because leukocyte level may also be influenced by comorbidities or environmental factors such as tumor, trauma, and acute toxicosis. We may need to concentrate more on these limitations for our further study design and recruitment.

Inflammatory parameters, including neutrophil count and the neutrophil ratio, were associated with an increased risk of new stroke, composite events, and ischemic stroke in patients with a minor ischemic stroke or TIA.

Author contributions

Bihong Zhu: study concept and design, analysis and interpretation of data, drafting of the manuscript. Yuesong Pan: acquisition of data, drafting of the manuscript. Jing Jing and Xia Meng: acquisition of data. Xingquan Zhao: acquisition of data, study supervision or coordination. Liping Liu: acquisition of data. David Wang and S. Claiborne Johnston: revision of the drafting of the manuscript. Hao Li: analysis and interpretation of data. Yilong Wang and Zhimin Wang: study concept and design, acquisition of data, analysis and interpretation of data. Yongjun Wang: study concept and design, obtaining funding, study supervision or coordination.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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Neutrophil counts, neutrophil ratio, and new stroke in minor ischemic stroke or TIA

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