

Baseline Neutrophil Counts and Neutrophil Ratio May Predict a Poor Clinical Outcome in Minor Stroke Patients with intravenous Thrombolysis

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Background: Evidence about whether neutrophil counts, neutrophil ratio linked with clinical outcome in patients with minor stroke after thrombolysis is scarce. The purpose of this study is to explore the association of neutrophil counts, neutrophil ratio with the 90-day clinical outcome in patients with minor stroke treated with intravenous thrombolysis. *Materials and methods:* A total of 163 patients with minor stroke treated with thrombolysis within window time were recruited from 2013 to 2017. Their total neutrophil and leukocyte counts on admission were measured. Disability or death (defined as a modified Rankin scale score ≥ 2) at 90-day were captured. Multiple logistic regression models were used to estimate the association between neutrophil counts, and neutrophil ratio to clinical outcome. Receiver operating characteristic curves were used to evaluate the predictive value of neutrophil counts or neutrophil ratio. *Results:* At the 90-day follow-up, 36 patients (21%) had disability or death after stroke onset. Compared to the first tertile, the higher neutrophil counts increased the risk of 90-day disability or death (adjusted odds ratio [aOR] for third tertile: 2.93 (1.08-7.96); P for trend = .03). Similarly, higher neutrophil ratio also increased the risk of disability or death (aOR for third tertile: 5.81 (1.7-19.88); P for trend = .005). The comparison of area under the curve for neutrophil ratio versus neutrophils was .1 (P = .04). Thus, neutrophil ratio with the cutoff point of .74 had a better discriminative ability to the outcome (6.11, 2.36-15.86). *Conclusions:* Baseline higher neutrophil counts and neutrophil ratio were associated with an increased risk of 90-day disability or death in patients with minor stroke who received thrombolytic therapy.

Key Words: Neutrophil—neutrophil ratio—minor stroke—intravenous thrombolysis—clinical outcome

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Introduction

Stroke has become the leading cause of mortality and adult disability in China^{1,2} and the second leading cause of mortality in the world.³ Currently, intravenous thrombolysis is universally recommended as the first-line effective reperfusion treatment for nonlarge vessel occlusive ischemic stroke within 4.5 hours from symptom onset. However, there are still about half of the patients who did not benefit from thrombolysis, and achieving a favorable functional outcome. About 7.7% of patients even had symptomatic intracranial hemorrhagic transformation.⁴

Multiple factors contribute to the poor outcome and mortality after stroke and treated with thrombolysis such as the time of onset of treatment,⁴ the severity of symptom or size of stroke,⁵ diabetic history,⁶ etc. Besides these clinical factors, serum biomarkers may also play a pivotal role in the prognosis of stroke after intravenous thrombolysis. Extensive evidence showed that inflammation is not only associated with the increased risk of stroke, but also the worsening of outcome. Experimental and clinical studies have proven that systemic and local inflammatory responses at the acute phase of vascular thromboembolic events are activated and have a sustained impact on the insult of brain, which result in the deterioration of stroke and poor outcome.⁷ Neutrophil, as the most common marker of acute inflammation, is often the first cell to be recruited after stroke to the injured area and contributes to the increased expression of adhesion molecules, cytokines/chemokines, proteases, and reactive oxygen species.⁸ Clinical studies revealed that the increased circulating neutrophils were related to the stroke severity⁹ and infarct size.¹⁰ In patients treated with intravenous thrombolysis, previous study showed that higher neutrophil counts and neutrophil to lymphocyte ratio at baseline increased risk of symptomatic intracranial hemorrhage, death, and poor functional outcome.¹¹ However, this study included stroke patients with moderate degree of stroke (National Institutes of Health Stroke Scale [NIHSS] scores 6-16) and excluded those with minor stroke (NIHSS ≤ 5).

So far, only one study reported that neutrophil and neutrophil leukocyte ratio was associated with stroke recurrence in minor stroke patients, but those patients were only on antiplatelets therapy.¹² The association between neutrophil counts or neutrophil ratio in patients with minor stroke receiving thrombolytic therapy has not been well studied. Therefore, we conducted this retrospective cohort study in 2 teaching hospitals to investigate whether neutrophil counts or neutrophil ratio were associated with the poor clinical outcome in minor stroke patients who received thrombolytic therapy.

Materials and Methods

Study Participants

We consecutively enrolled patients with minor ischemic stroke treated with recombinant tissue plasminogen activator (r-tPA) therapy in the First and Second Affiliated Hospital of Soochow University from September 2013 to September 2017. The intravenous dose of r-tPA was .9 mg/kg (maximum 90 mg) within 60 minutes, with initial 10% of dose as a bolus. In addition, all patients also received antithrombotic and other routine medications after r-tPA administration. The inclusion criteria for patients in this study were as follows: age between 18 and 80 years old, NIHSS ≤ 5 , receiving r-tPA within 4.5 hours after symptom onset, pre-morbid modified Rankin scale ≤ 1 . Patients with identified infection (including unexplained fever, pneumonia, and urinary tract infection) at baseline, missing baseline neutrophil and leukocyte counts, stroke mimics, and tumor were excluded. The study protocol has been approved by the Ethics Committee of the First and Second Affiliated Hospital of Soochow University, respectively. Written consent was obtained from all study participants or their surrogate before enrollment.

Data Collection

The baseline characteristic including demographic data, vascular risk factors, and clinical features were obtained at admission. Vascular risk factors included past stroke history, hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, coronary artery disease, current drinking, and smoking. Clinical features included systolic blood pressure and diastolic blood pressure on admission, door to needle time (DNT), symptom onset to r-tPA treatment time (OTT), and baseline NIHSS. Laboratory indexes, such as fasting blood glucose, low-density lipoprotein, high-density lipoprotein (HDL) and creatinine were also collected after r-tPA perfusion. The criteria for current drinking and smoking were prescribed previously.¹³ Stroke severity by NIHSS was assessed by an experienced neurologist at admission.¹⁴ Subtype of ischemic stroke were classified according to the Trial of Org 10172 in Acute Stroke Treatment criteria.¹⁵

Whole blood samples were collected in EDTA-anticoagulated tube from stroke patients at admission before r-tPA infusion. Total leukocyte and neutrophil counts were tested using an automatic biochemical analyzer (XN-3000 Hematology Analyzer, SYSMEX, Inc, Japan). Neutrophil ratio, the ratio of the absolute neutrophil value to the total number of leukocytes, represents the relative increase of neutrophil levels and is often used as an inflammatory parameter in clinical practice.

Follow-Up and Outcomes Assessment

The outcome was disability or death defined by 2-6 on the modified Rankin scale at 90-day follow-up. Records of

death were confirmed by their relatives, and/or by death certificates and hospital records. Follow-up was conducted by the trained neurologists who were blind to the baseline information of patients.

Statistical Analysis

Participants were divided into 3 groups according to the tertile of neutrophils counts (T1, $<3.8 \times 10^9/L$; T2, ≥ 3.8 and $\leq 5.13 \times 10^9/L$; T3, $>5.13 \times 10^9/L$) or neutrophil ratio (T1, $<.61$; T2, $.61-.69$; T3, $>.69$). Continuous variables were presented as mean \pm SD or median with interquartile range (IQR), and were compared using 1-way analysis of variance or the Kruskal-Wallis test. Categorical variables were presented as percentages and compared using χ^2 test or Fisher's exact test. Multiple logistic regression models were used to assess the association of neutrophil counts or neutrophil ratio with poor functional outcome at 90-days. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) of secondary and third tertile and compared to the first

tertile of neutrophil counts or neutrophil ratio. Two models were applied. In the first model, only age and sex were adjusted. In the second model, all of the potential confounders were adjusted. Using receiver operating characteristic (ROC) curves to evaluate the discriminative value of neutrophil counts or neutrophil ratio by calculating area under the curve (AUC). A 2-sided *P* value less than .05 was considered to be statistically significant. Statistical analysis was conducted with SAS software version 9.4.

Results

In this 2-center cohort study, a total of 163 patients were included in the final analysis after excluding 5 patients who had no neutrophil or/and leukocyte counts and 3 with identified infection. The mean age was 64.1 ± 11.6 years, and 122 patients (74.9%) were men. The median NIHSS score was 3 (IQR 2-4). Patients in the higher neutrophil counts were younger and had shorter time of DNT (Table 1). Patients in the higher neutrophil

Table 1. Baseline features according to the tertiles of neutrophil counts in minor stroke patients with r-tPA therapy

Characteristics	Tertiles of neutrophil counts $\times 10^9/L$			Total patients	<i>P</i> value
	<3.8	3.8-5.13	>5.13		
Age, mean \pm SD	67.1 \pm 10	64.3 \pm 10.3	60.5 \pm 13.4	64.1 \pm 11.6	0.04
Male, n (%)	34 (65.4)	47 (83.9)	41 (74.6)	122 (74.9)	0.09
Vascular risk factors, n (%)					
Hypertension	34 (65.4)	42 (75)	44 (80)	120 (73.6)	0.22
Diabetes mellitus	8 (15.4)	15 (26.8)	19 (34.6)	42 (25.8)	0.08
Hyperlipidemia	18 (34.6)	26 (46.4)	25 (45.5)	69 (42.3)	0.39
Atrial fibrillation	11 (21.2)	5 (8.9)	7 (12.7)	23 (14.1)	0.18
Coronary artery disease	1 (1.9)	3 (5.4)	2 (3.6)	6 (3.7)	0.87
Previous stroke	4 (7.7)	2 (3.6)	3 (5.5)	9 (5.5)	0.57
Current smoking	21 (40.4)	26 (46.4)	23 (41.8)	70 (42.9)	0.80
Current drinking	13 (25)	15 (26.8)	16 (29.1)	44 (27)	0.89
Clinical status					
SBP (mm Hg), mean \pm SD	150.1 \pm 18.9	151.1 \pm 19.8	153 \pm 19.3	151.4 \pm 19.3	0.60
DBP (mm Hg), mean \pm SD	82.8 \pm 10.4	85.2 \pm 11.3	87.8 \pm 12.4	85.3 \pm 11.5	0.13
Baseline NIHSS, median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-4)	0.75
DNT (min), median (IQR)	60 (50-85)	66 (51.5-90)	51 (40-82)	60 (45-86)	0.04
OTT (min), median (IQR)	195 (159-223)	180 (132-223.5)	200 (160-235)	195 (152-225)	0.27
Stroke subtype (TOAST), n (%)					0.23
Large artery atherosclerosis	18 (41.9)	20 (43.5)	31 (62)	69 (49.6)	
Cardioembolism	10 (23.3)	11 (23.9)	8 (16)	29 (20.9)	
Small artery disease	13 (30.2)	11 (23.9)	6 (12)	30 (21.6)	
Stroke of other determined etiology	2 (4.7)	4 (8.7)	5 (10)	11 (7.9)	
Laboratory indexes					
Fasting glycemia, mmol/L	6.4 \pm 1.8	7.4 \pm 3.2	7.4 \pm 3.3	7.2 \pm 2.9	0.20
Leukocyte, $\times 10^9/L$	5.2 \pm 1	6.9 \pm 1	9.8 \pm 2.3	7.3 \pm 2.5	<.001
Neutrophils, $\times 10^9/L$	2.9 \pm 0.6	4.4 \pm 0.4	7.2 \pm 2.2	4.9 \pm 2.2	<.001
High-density lipoprotein, mmol/L	1.2 \pm 0.3	1.2 \pm 0.3	1.2 \pm 0.3	1.2 \pm 0.3	0.61
Low-density lipoprotein, mmol/L	2.7 \pm 0.8	2.9 \pm 0.8	3 \pm 1.2	2.9 \pm 1	0.47
Creatine, mg/dL	73.4 \pm 26.4	70.6 \pm 15.5	72.9 \pm 18.3	71.7 \pm 20.1	0.94
Neutrophil/leukocyte	0.6 (0.5-0.6)	0.6 (0.6-0.7)	0.8 (0.7-0.8)	0.6 (0.6-0.7)	<.001

Abbreviations: DBP, diastolic blood pressure; DNT, door to needle time; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; OTT, onset to treatment time; SBP, systolic blood pressure; SD, standard deviation.

ratio had higher blood pressure and HDL levels at baseline. The time of OTT was a little different between groups (Table 2).

Association of Neutrophil Counts and 90-Day Clinical Outcome in Patients with Minor Stroke after r-tPA Therapy

A total of 36 (20.7%) patients had a poor functional outcome and the mortality was 2 (1.2%). The association of neutrophil counts and the 90-day clinical outcome of minor stroke with r-tPA therapy was shown in Table 3. Patients in the higher neutrophil counts were associated with poor functional outcome after adjustment for age, sex, time of DNT, as comparison to the patients in the first tertile. The adjusted OR and 95% CI for the second and third tertile was 1.12 (0.38-3.33) and 2.93 (1.08-7.95), respectively. In addition, a linear relationship was observed between neutrophil counts and the poor functional outcome (P for trend = .03).

Association of Neutrophil Ratio and 90-Day Clinical Outcome in Patients with Minor Stroke after r-tPA Therapy

The association of baseline neutrophil ratio and clinical outcome in those with minor stroke after r-tPA therapy at 90-day was shown in Table 4. In detail, after adjusting for model 1 (age, sex) and further adjusting for model 2 (time of OTT, HDL, SBP, and DBP), patients in the second and third tertiles linked with the increased risk of poor functional outcome in comparison to the first tertile. The adjusted OR and 95% CI for the second and third tertile was 3.84 (1.05-14.07) and 5.81 (1.7-19.88), respectively. A similar linear relationship was also detected between neutrophil ratio and poor functional outcome (P for trend = .005).

Discriminative Value of Neutrophil Counts, Neutrophil Ratio for the Poor Clinical Outcome

The discriminative ability of neutrophil counts or neutrophil ratio for the poor clinical outcome was evaluated

Table 2. Baseline features according to the tertiles of neutrophil ratio in minor stroke patients with r-tPA therapy

Characteristics	Tertiles of neutrophil ratio			Total patients	P value
	<0.61	0.61-0.69	>0.69		
Age, mean \pm SD	63 \pm 12.4	64.2 \pm 12.3	64.5 \pm 10.1	64.1 \pm 11.6	0.90
Male, n (%)	38 (70.4)	43 (79.6)	41 (74.6)	122 (74.9)	0.54
Vascular risk factors, n (%)					
Hypertension	36 (66.7)	38 (70.4)	46 (83.6)	120 (73.6)	0.11
Diabetes mellitus	12 (22.2)	15 (27.8)	15 (27.3)	42 (25.8)	0.77
Hyperlipidemia	26 (48.2)	16 (29.6)	27 (49.1)	69 (42.3)	0.07
Atrial fibrillation	9 (16.7)	6 (11.1)	8 (14.6)	23 (14.1)	0.70
Coronary artery disease	1 (1.9)	2 (3.7)	3 (5.5)	6 (3.7)	0.87
Previous stroke, n (%)	2 (3.7)	3 (5.6)	4 (7.3)	9 (5.6)	0.91
Current smoking, n (%)	21 (38.9)	26 (48.2)	23 (41.8)	70 (42.9)	0.61
Current drinking, n (%)	13 (24.1)	19 (35.2)	12 (21.8)	44 (27)	0.24
Clinical status					
SBP (mm Hg), mean \pm SD	148.4 \pm 19.5	147.9 \pm 19.8	158 \pm 17.2	151.9 \pm 19.4	0.02
DBP (mm Hg), mean \pm SD	82.2 \pm 11	85.6 \pm 11.7	88.1 \pm 11.3	85.2 \pm 11.7	0.05
Baseline NIHSS, median (IQR)	3 (2-4)	3 (3-4)	3 (2-4)	3 (2-4)	0.64
DNT (min), median (IQR)	60 (49-88)	62.5 (48-98)	55 (40-72)	60 (45-88)	0.06
OTT(min), median (IQR)	210 (162-235)	177.5 (138-205)	198 (155-225)	192.5 (154-225)	0.05
Stroke subtype (TOAST), n (%)					0.68
Large artery atherosclerosis	22 (46.8)	18 (41.9)	29 (59.2)	69 (49.6)	
Cardioembolism	12 (25.5)	10 (23.3)	7 (14.3)	29 (20.9)	
Small artery disease	10 (21.3)	11 (25.6)	9 (18.4)	30 (21.6)	
Stroke of other determined etiology	3 (6.4)	4 (9.3)	4 (8.2)	11 (7.9)	
Laboratory indexes					
Fasting glycemia, mmol/L	6.5 \pm 2	7.2 \pm 2.8	7.5 \pm 3.6	7.2 \pm 2.9	0.30
Leukocyte, $\times 10^9/L$	6.6 \pm 2.2	6.8 \pm 1.9	8.6 \pm 2.7	7.3 \pm 2.4	<.001
Neutrophils, $\times 10^9/L$	3.6 \pm 1.4	4.4 \pm 1.3	6.6 \pm 2.4	4.9 \pm 2.2	<.001
High-density lipoprotein, mmol/L	1.1 \pm 0.3	1.2 \pm 0.2	1.2 \pm 0.3	1.2 \pm 0.3	0.04
Low-density lipoprotein, mmol/L	2.8 \pm 0.8	2.7 \pm 0.7	3.1 \pm 1.2	2.9 \pm 1	0.23
Creatine, mg/dL	73.7 \pm 25	71.6 \pm 17.6	71.4 \pm 18	71.7 \pm 20.1	0.80
Neutrophil/leukocyte	0.6(0.5-0.6)	0.6 (0.6-0.7)	0.8(0.7-0.8)	0.6(0.6-0.7)	\leq .001

Abbreviations: DBP, diastolic blood pressure; DNT, door to needle time; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; OTT, onset to treatment time; SBP, systolic blood pressure; SD, standard deviation.

Table 3. Adjusted odds ratio of 90-day clinical outcome according to tertiles of neutrophil counts

Outcomes	Crude						Model 1*			Model 2†		
	Group of neutrophil counts	N	Events, n (%)	OR (95% CI)	P	P for trend	OR (95% CI)	P	P for trend	OR (95% CI)	P	P for trend
mRS 2-6‡	T1	52	8 (15.4)	1	-	0.05	1	-	0.02	1	-	0.03
	T2	56	8 (14.3)	0.92 (0.31-2.65)	0.87		1.10 (0.37-3.28)	0.86		1.12 (0.38-3.33)	0.84	
	T3	55	17 (30.9)	2.46 (0.96-6.34)	0.06		3.11 (1.16-8.37)	0.02		2.93 (1.08-7.95)	0.04	

Abbreviation: OR, odds ratio.

*Model 1, adjusted for age and sex.

†Model 2, adjusted for age, sex, and door to needle time.

‡mRS 2-6, modified Rankin scale score 2–6.

Table 4. Adjusted odds ratio of 90-day clinical outcome according to tertiles of neutrophil ratio

Outcomes	Crude						Model 1*			Model 2†		
	Group of neutrophil ratio	N	Events, n (%)	OR (95% CI)	P	P for trend	OR (95% CI)	P	P for trend	OR (95% CI)	P	P for trend
mRS 2-6‡	T1	54	4 (7.4)	1	-	0.002	1	-	0.001	1	-	0.005
	T2	54	11 (20.4)	3.20 (0.95-10.78)	0.06		3.51 (1.02-12.09)	0.05		3.84 (1.05-14.07)	0.04	
	T3	55	18 (32.7)	6.08 (1.90-19.47)	0.002		6.52 (1.20-21.31)	0.002		5.81 (1.7-19.88)	0.005	

Abbreviation: OR, odds ratio.

*Model 1, adjusted for age and sex.

†Model 2, adjusted for age, sex, time from onset to treatment, systolic blood pressure, diastolic blood pressure, and high-density lipoprotein.

‡mRS 2-6, modified Rankin scale score 2–6.

by ROC curves (Fig 1). AUC for neutrophil counts and neutrophil ratio was .62 (95% CI, .51-.74) and .72 (95% CI, .62-.82), respectively. An area difference was found at .1 ($P = .04$) when further comparison of AUC for neutrophil ratio versus neutrophils. An optimal neutrophil ratio cut point level (.74) was obtained from the ROC curve. After adjustment for age, sex, time of OTT, HDL, SBP, and DBP, patients with elevated neutrophil ratio ($\geq .74$) were associated with the 6.11-fold increased risk of poor functional outcome (95% CI 2.36-15.86).

Discussion

In this 2-center retrospective study, we found that baseline higher level of neutrophil counts and neutrophil ratio were associated with an increased risk of 90-day poor clinical outcome in minor stroke patients treated with r-tPA therapy. The linear association was found between the elevated neutrophil counts, neutrophil ratio, and increased risk of poor outcome. Furthermore, the AUC of neutrophil ratio to predict the outcome was superior than the area of neutrophils. The cutoff point of .74 for neutrophil ratio had a better discriminative ability to the

prognosis, which was related with a 6.11-folds increased risk of disability or death.

Neutrophil, as an essential component of inflammation, often aggressively involves in the pathophysiological process of thrombotic events, which might indicate that this biomarker could be a strong predictor for the occurrence of vascular events or the worsening outcome. In general populations, higher neutrophil counts were associated with the increased risk of MI and stroke.^{16,17} In MI patients, baseline elevated neutrophil not only was associated with the severity of coronary artery disease and adverse outcome,¹⁸ but also the increased mortality and heart failure.¹⁹ In stroke patients, several studies provided evidence that admission higher neutrophil to lymphocyte ratio could predict the poor functional outcome,²⁰ even for those received with endovascular therapy.^{21,22} However, in patients with minor ischemic stroke, especially those had intravenous thrombolytic reperfusion, the correlation between baseline neutrophil counts or neutrophil ratio and clinical outcomes has rarely been investigated. Similar to the previous studies, our results found a higher neutrophil count and neutrophil ratio increased worsening outcome in those with minor stroke and r-tPA therapy and even in a gradient

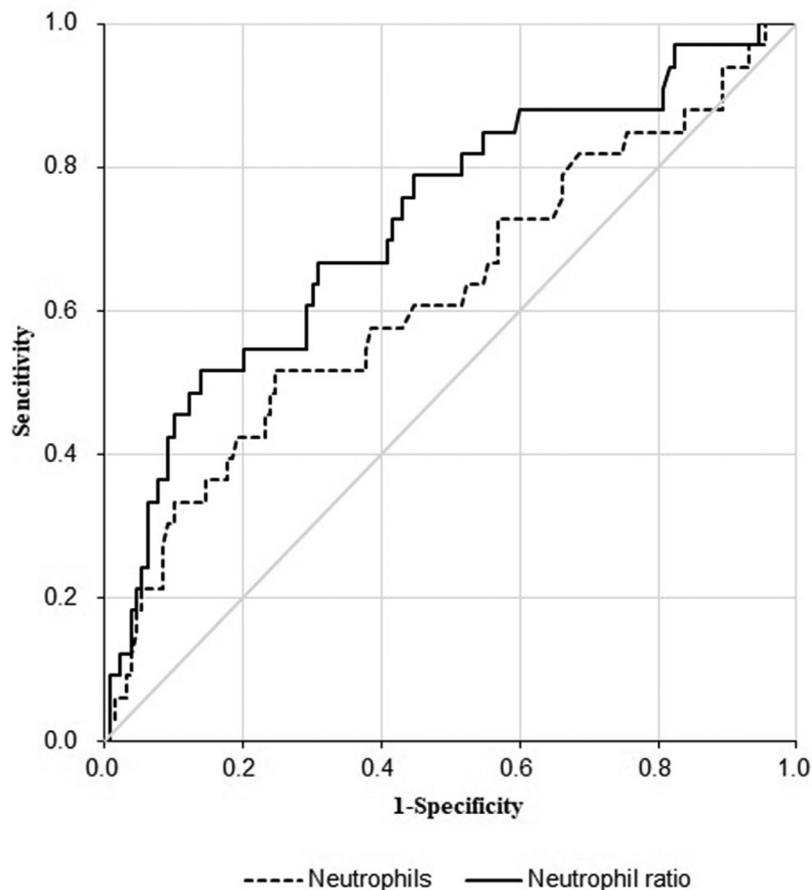


Figure 1. Area under receiver operating characteristic curve of neutrophil counts, neutrophil ratio for the clinical outcome.

manner. In addition, neutrophil ratio, representing as the relative elevation of neutrophil counts, had a better discriminative value than its absolute elevation. This indicator might be more accurate and appropriate to predict the increased risk of poor clinical outcome.

The underlying mechanism for this association between the relative and absolute elevated neutrophils and poor outcome remains unclear. At the onset of stroke, neutrophils are the first blood cells to accumulate into the brain ischemic area and take part in regional blood-brain barrier breakdown, hence the brain injury.²³ The breakdown of blood-brain barrier plays a key role in the brain edema or hemorrhagic transformation. Meanwhile, activated neutrophils enhance the expression of proinflammatory mediators, reactive oxygen species, and cytokines, which contribute to the aggravation of the brain ischemia and the systematic inflammation response.⁸ Human MRI study vividly demonstrated that neutrophil was recruited within hours after stroke onset and related to the infarct growth.²⁴ In addition, neutrophil could promote further thrombus formation after stroke. Multiple mechanisms are involved in neutrophil-related thrombus formation, such as interaction with platelets, release of neutrophil extracellular traps and release of tissue factors and proteases that act on coagulation factors.^{25,26} Therefore, neutrophils play a pivotal role in the initial stage of stroke as shown in experimental and clinical studies. The specific inhibition of neutrophil activation as well as the release of the proinflammatory or prothrombotic mediators may be the novel therapy during stroke treatment. Some approach targeting the specific mediators are under investigation.²⁷

The Strength and Limitations of the Study

The strength of our studies included 2-center cohort study, neutrophils, and leukocytes measured before r-tPA infusion. Meanwhile, a variety of statistical analyses were used to display the correlation of neutrophil counts, neutrophil ratio, and clinical outcome after adjustment for the potential confounding factors. However, this study also had some limitations. First, it is a retrospective study with a small sample size. The generalization of our results to other clinical practice is uncertain. A prospective, large-scale cohort study need to be conducted to validate this association. Second, the causality between neutrophil counts, neutrophil ratio, and the poor outcome in minor stroke with thrombolysis remains undefined. Third, the lower incidence of death in this study was difficult to discriminate the predicative value on mortality. Lastly, the residual bias was unavoidable because the neutrophil counts or ratio may be influenced by some comorbidities, like potential cancer and uncertain inflammation.

Conclusion

Higher neutrophil counts and neutrophil ratio on admission increased the risk of 90-day worsening of

clinical outcome in patients with minor stroke after they had been treated with r-tPA therapy. In addition, elevated neutrophil ratio had a better predictive ability than elevated neutrophil counts.

Declaration of Competing Interest

The authors do not have any conflicts of interest to declare.

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