



# Dynamic increase in neutrophil levels predicts parenchymal hemorrhage and function outcome of ischemic stroke with r-tPA thrombolysis

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## Abstract

**Background** The higher level of neutrophil on admission has been reported to predict worse 3-month outcomes in ischemic stroke patients. Our study was to explore the dynamic changes of neutrophil and lymphocyte after r-tPA thrombolysis of ischemic stroke and the relationship with parenchymal hemorrhage (PH) and 3-month function outcome.

**Methods** A total of 208 acute ischemic stroke (AIS) patients with intravenous thrombolysis were included and then received 3-month follow-up in the present study. Blood samples for neutrophil and lymphocyte counts were obtained on admission, at 24 h and at 7 days after r-tPA infusion. The associations of increase in neutrophil, lymphocyte, and neutrophil to lymphocyte ratio (NLR) with PH or 3-month poor outcome were examined by logistic regression.

**Results** Increasing trends in the neutrophil and NLR were observed in AIS patients after r-tPA treatment. Increased level of neutrophil at 24 h after r-tPA infusion but not that on admission was associated with PH (OR = 2.86,  $P = 0.029$ ) and 3-month poorer functional outcomes (OR = 2.67,  $P = 0.009$ ). Moreover, patients were divided into four groups according to the percent change in neutrophil within 24 h following r-tPA treatment, and we found that there was a trend of incremental OR when compared higher increase group with lower ones.

**Conclusions** Dynamic increase in neutrophil and NLR after stroke may predict PH and 3-month poor outcome in AIS patients receiving r-tPA treatment. Therefore, neutrophil and NLR may serve as activity markers for PH and 3-month poor prognosis in AIS patients with intravenous thrombolysis.

**Keywords** Ischemic stroke · Thrombolysis · Neutrophil · Lymphocyte · Parenchymal hemorrhage · Outcomes

## Abbreviations

PH Parenchymal hemorrhage  
HT Hemorrhagic transformation

AIS Acute ischemic stroke  
NLR Neutrophil to lymphocyte ratio  
r-tPA Recombinant tissue plasminogen activator  
MMP-9 Matrix metalloproteinase-9  
BBB Blood-brain barrier  
OTT Onset to treatment time  
SBP Systolic blood pressure  
DBP Diastolic blood pressure  
NIHSS National Institutes of Health Stroke Scale  
MRS Modified Rankin scale  
SD Standard deviation  
MCAO Middle cerebral artery occlusion  
CRP C-Reactive protein  
OR Odds ratio  
CI Confidence interval  
IQR Interquartile ranges  
sICH Symptomatic intracerebral hemorrhages

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## Introduction

Stroke becomes one of the most common diseases for mortality and morbidity of human being worldwide nowadays [1]. Most stroke survivors leave functional impairments which dramatically decline quality and independence of life [2]. A number of clinical trials have proved the efficacy and benefit of intravenous thrombolysis with recombinant tissue plasminogen activator (r-tPA) in acute ischemic stroke (AIS) patients within 4.5 h from symptom onset [3, 4]. Intravenous administration of r-tPA is now recommended as the primary choice for AIS patients within the time window; nevertheless, approximately half of the participants are still not independent or even died after 3 months. Therefore, although a few factors related to functional outcome of ischemic stroke patients treated with intravenous alteplase has been identified, detection of new risk factors is still necessary and important. Hemorrhagic transformation (HT) is claimed to be one of the major complications leading to poor functional outcomes after thrombolysis. The increased risk of hemorrhagic transformation following ischemic stroke was most likely attributable to the factor of reperfusion and the toxic effects of r-tPA [5].

In recent years, numerous studies have demonstrated that activated inflammatory process may play an important role in the functional outcomes and hemorrhagic transformation [6, 7]. When ischemic stroke occurs, inflammatory mediators such as chemokines and cytokines are released and then promote peripheral leukocytes infiltration. Among the peripheral circulating leukocytes, the neutrophils are firstly recruited to the ischemic area by binding various adhesion molecules in the early stages of ischemic stroke [8]. Moreover, they release matrix metalloproteinase-9 (MMP-9) and induce the damage of blood-brain barrier (BBB), which may induce the hemorrhagic transformation. In addition, the neutrophils have also been demonstrated to be involved in neurogenesis, angiogenesis, and neuroplasticity in neurological recovery stage [9, 10]. Therefore, the activation of neutrophil has been suggested to increase infarct size [11], hemorrhagic transformation, and worse neurologic outcomes [6]. On the other hand, as another type of leukocytes, the lymphocytes also play an important role in the progression of ischemic stroke. Previous studies have reported that lymphocytes count decreases following AIS and lower level of lymphocytes may contribute to poor functional outcome [8]. For example, as a subset of lymphocyte, specific T cell lymphocyte has been demonstrated to exert a neuroprotective effect on central nervous system [12]. Taken together, the neutrophils and lymphocytes may mediate inflammatory processes in different mechanisms after ischemic stroke.

Recent studies have suggested that the level of neutrophil on admission is associated with increased risk of hemorrhage transformation and can predict 3-month clinical outcome after r-tPA thrombolysis [6, 13]. In AIS patients, MMP-9-positive

neutrophil infiltration results in disruption of the BBB and degradation of basal lamina type IV collagen [14]. All of these may contribute to the hemorrhage transformation and poor outcome in patients with ischemic stroke. Mao and colleagues observed that regulatory T cell lymphocyte abolish the r-tPA-induced elevation of MMP-9 and CCL2 after stroke, which decrease the risk of hemorrhage transformation [15]. More specifically, neutrophil-to-lymphocyte ratio (NLR) is regarded as a stable systemic inflammation biomarker [16, 17]. The ratio has also been reported to be correlated with short-term poor prognosis in ischemic stroke patients with r-tPA thrombolysis [18]. Nevertheless, these studies only examined neutrophil and lymphocyte before thrombolysis, but the comprehensive dynamic changes of neutrophil and lymphocyte and dynamic inflammatory process in patients with r-tPA thrombolysis have not been investigated.

As a particular type of hemorrhage transformation, parenchymal hemorrhage (PH) can be divided into PH-1 and PH-2. PH-2 is regarded as one of the major factors affecting the prognosis. Therefore, there is an increasing concern about the relation between dynamic changes of neutrophil, lymphocyte, as well as NLR, and the risk of PH and 3-month outcome in AIS patients with r-tPA treatment. In the present study, we aimed to explore the dynamic variation of the neutrophil and lymphocyte in ischemic stroke patients treated with intravenous r-tPA. More importantly, the further goal of current study was to evaluate whether these changes were associated with PH and functional outcome of AIS patients treated with intravenous thrombolysis.

## Methods

### Study population

A prospective, hospital-based observational study was operated. The study consecutively included consecutive ischemic stroke patients treated with intravenous thrombolysis from July 2016 to July 2018 in the First Affiliated Hospital of Wenzhou Medical University. Intravenous r-tPA (Boehringer Ingelheim, Germany, from 0.9 mg/kg to a maximum of 90 mg) was used with 10% of the total as a bolus within 4.5 h of stroke onset and the rest 90% by a 60-min infusion. The inclusion criteria for enrollment were as follows: (1) older than 18 years old; (2) diagnosis of acute ischemic stroke by neurologists according to the recommendations from World Health Organization [19]; (3) onset-to-treatment time within 4.5 h. The study exclusion criteria were according to AHA/ASA Guidelines [20] and follows: (1) evidence of infection at admission or any systemic infection that occurred during hospitalization; (2) a bridging therapy consisting of IV r-tPA followed by endovascular therapy (3) a history of cancer or autoimmune disease; (4) incomplete medical records and a

missing of 3-month prognostic information. The present study was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University. All patients or their relatives signed the informed consent.

### Clinical data and blood sample

Some baseline characteristics were collected as follows: age, sex, drinking, smoking, hypertension, diabetes, hyperlipidemia, onset to treatment time (OTT), systolic blood pressure (SBP) before thrombolysis and diastolic blood pressure (DBP) before thrombolysis. Stroke severity was assessed by a certified neurologist using the National Institutes of Health Stroke Scale (NIHSS) at admission and at 24 h, and 7 days after treatment with intravenous r-tPA.

Venous blood samples were obtained from all patients at admission, at 24 h, and 7 days after treatment with intravenous r-tPA. Total leukocyte, neutrophil, and lymphocyte counts were analyzed by the automated hematology analyzer (Sysmex Company, XE-2100, Japan). The NLR was calculated as the ratio of the neutrophils count over lymphocytes count, both obtained from the same blood sample.

### Parenchymal hemorrhage and prognosis

All patients received a CT scan within the first 4.5 h of stroke onset. CT was repeated at 24 h, and another CT scan was done immediately in case of rapid neurological deterioration to evaluate the presence of parenchymal hemorrhage. Parenchymal hemorrhage was defined as hemorrhage in the cerebral parenchyma on the CT scan after cerebral ischemic infarction. As a type of hemorrhage transformation, parenchymal hemorrhage can be divided into PH-1 and PH-2. The prognosis after 3 months of AIS was estimated by the Modified Rankin scale (mRS) [21]. According to the mRS scores, the patients were divided into two groups: a good functional outcome for score 0 to 2 and a poor functional outcome for score 3 to 5 or death for the ischemic stroke.

### Statistical analysis

The data managements and analyses were applied with SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was considered statistically significant for all analyses. Categorical variables were presented as counts and percentages, and Fisher's exact test or chi-squared tests were used as appropriate. Continuous variables not met normal distribution were described with median with 25th and 75th percentiles (interquartile ranges (IQR) and compared with Mann-Whitney U test. Normally distributed variables were presented with mean  $\pm$  standard deviation (SD), and Student's *t* test was applied to perform the comparison between two groups. Logistic regression was carried out to find the association

between poor clinical outcome and parameters. Variables with a  $P$  value of  $< 0.1$  in the univariate analysis were chosen into the multivariate analysis. Results were presented as 95% confidence interval (CI) with odds ratio (OR). According to the percentage change of neutrophil, we divided patients into four group and preformed multivariate analysis.

## Results

### Baseline characteristics of patients

Out of 401 AIS patients treated with r-tPA, 193 patients were excluded due to occurrence of infection including lung infection ( $n = 62$ ), urinary tract infection ( $n = 14$ ), and septicemia ( $n = 4$ ) during hospitalization, bridging therapy ( $n = 55$ ), losing to follow up ( $n = 45$ ), and a history of cancer or autoimmune disease ( $n = 13$ ).

Therefore, we included 208 AIS patients receiving r-tPA (mean age 67 years old, 62% men) in the current study. The main baseline characteristics of patients are presented in Table 1, according to the presence or absence of PH, as well as the outcome at 3 months. We observed that patients with the presence of PH were more likely to suffer from neurological deficits and had more atrial fibrillation than those without PH.

### The dynamic changes of neutrophil and lymphocytes following r-tPA treatment

The neutrophil and lymphocytes were obtained at three different time points: at admission, at 24 h, and at 7 days after r-tPA treatment. Figure 1 illustrates the changes in neutrophil, lymphocyte, and NLR at three different time points. Paired Wilcoxon signed-rank test in total participants revealed that neutrophil was significantly increased (median, 4.99 vs. 5.95) whereas lymphocytes were significantly decreased (0.73 vs. 0.71) at 24 h after r-tPA treatment (Fig. 1a, all  $P < 0.05$ ). Similarly, the NLR was also significantly increased at 24 h after r-tPA infusion (6.97 vs. 8.63,  $P < 0.001$ ). Nevertheless, neutrophil and NLR were significantly decreased at 7 days when comparing with those at 24 h (all  $P < 0.001$ ), while lymphocyte was still significantly increased. Moreover, the present study observed that there was no difference in neutrophil count between the good and poor outcome groups at baseline ( $P = 0.887$ ). At 24 h and at 7 days, neutrophil count was significantly higher in the poor outcome group compared to good outcome group (Fig. 1b,  $P < 0.001$ ). As for the lymphocyte, there was no significant difference at admission ( $P = 0.17$ ) and at 24 h ( $P = 0.25$ ) following r-tPA between these two groups (Fig. 1c). At 7 days, lymphocyte count was significantly higher in the good outcome group compared with the poor outcome group ( $P < 0.001$ ). Moreover, NLR at baseline between the good and poor outcome groups was not

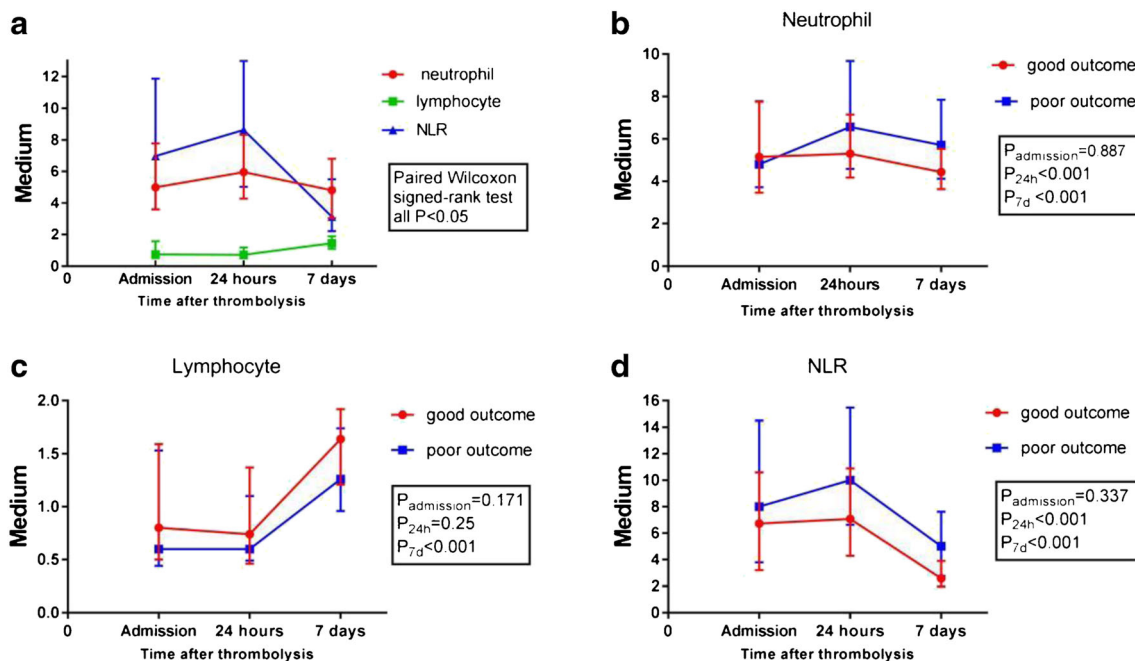
**Table 1** Baseline characteristics of patients according to the presence/absence of parenchymal hemorrhage (PH) or 3-month outcome

	No PH (N= 173)	PH (N= 35)	P	Good outcome (N= 115)	Poor outcome (N= 93)	P
Age, years, mean (SD)	66.7 ± 12.8	70.7 ± 9.9	0.107	64.1 ± 12.7	71.5 ± 11.3	< 0.0001
Female,%	63 (36.4%)	17 (48.6%)	0.826	46 (40%)	34 (36.6%)	0.612
Current drinking,%	41 (23.7%)	6 (17.1%)	0.397	24 (20.9%)	23 (24.7%)	0.508
Current smoking,%	68(39%)	6 (17.1%)	0.012	45 (39.1%)	29 (31.2)	0.234
Hypertension,%	138 (79.8%)	27 (77.1%)	0.726	87 (75.7%)	78 (83.9%)	0.146
Diabetes,%	42(24.3%)	14 (40.0%)	0.056	31 (27%)	25 (26.9%)	0.99
Hyperlipidemia,%	85 (49.1%)	16 (45.7%)	0.622	61 (53%)	44 (47.3%)	0.411
Atrial fibrillation,%	45 (26.0%)	20 (57.1)	< 0.0001	25 (21.7%)	40 (43%)	0.001
Time from stroke onset to rtPA treatment, median (IQR)	150 (47.5, 235)	145 (70,200)	0.771	130 (42.5, 222.5)	165 (56.3, 240)	0.248
NIHSS score, median (IQR)	7(3, 11)	11 (8, 17)	< 0.0001	5(3.8.5)	11(6,15.3)	< 0.0001
Systolic blood pressure, mmHg, mean (SD)	157.5 ± 24.5	153.5 ± 19.9	0.528	159.1 ± 24.9	157.3 ± 23.1	0.737
Diastolic blood pressure, mmHg, mean (SD)	66.7 ± 12.8	81.2 ± 12.3	0.070	87.3 ± 13.9	83.7 ± 15	0.096
Blood glucose, mmol/L, median (IQR)	6.9(6.0, 9.1)	7.8 (6.6, 11.2)	0.092	6.8(5.9, 8.8)	7.5(6.3, 9.9)	0.075
Platelets,109/L, median (IQR)	202 (168,246.7)	198 (151,225)	0.128	206(175,253)	193(157,227)	0.107

significant difference ( $P = 0.337$ ). Furthermore, NLR was significantly higher in the poor outcome group compared to good outcome group at 24 h and at 7 days (Fig. 1d,  $P < 0.001$ ).

The temporal profiles of the neutrophil and lymphocytes are presented according to the presence or absence of PH in Fig. 2. The neutrophil at admission did not differ between patients with and without PH ( $P = 0.661$ ). An increasing trend of the neutrophil was observed in both groups (Fig. 2a). Nevertheless, the neutrophil in the PH group were

significantly higher than those in the No PH group at 24 h ( $P < 0.001$ ) and at 7 days ( $P = 0.012$ ) after r-tPA. Additionally, there was no significant difference in lymphocyte at admission ( $P = 0.051$ ), at 24 h ( $P = 0.154$ ) and at 7 days ( $P = 0.473$ ) after r-tPA between these two groups (Fig. 2b). Although the NLR at admission did not differ between patients with and without PH ( $P = 0.231$ ), the NLR values in the PH group were significantly higher than those in the no PH group at 24 h ( $P < 0.001$ ) and at 7 days after r-tPA ( $P = 0.041$ , Fig. 2c).

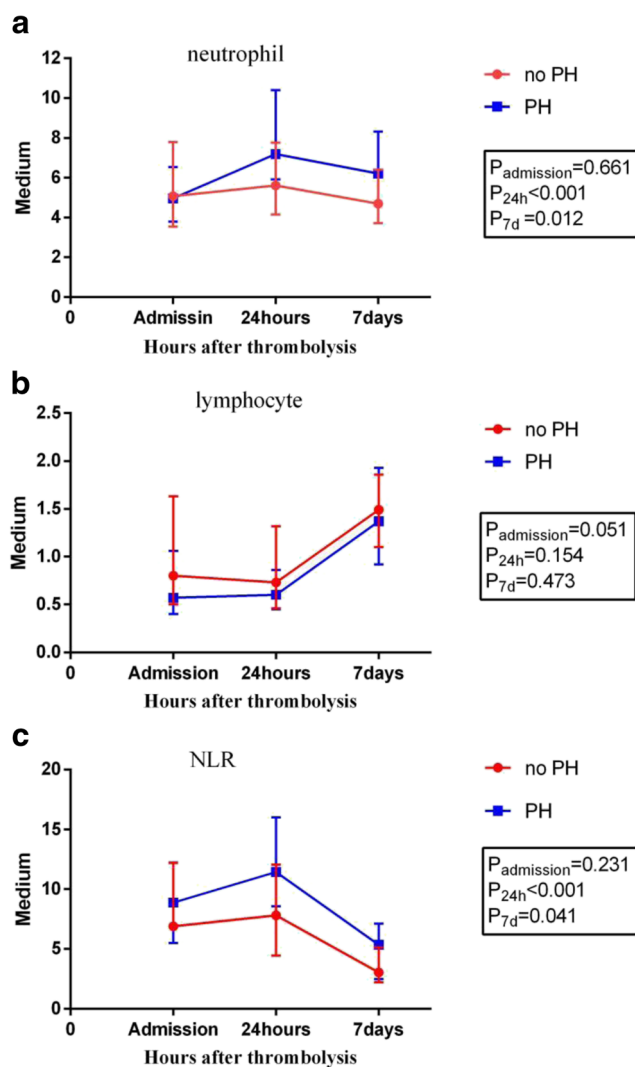


**Fig. 1** Temporal profile (median and interquartile range) of neutrophil, lymphocyte, and NLR in stroke patients treated with r-tPA according to 3-month outcome (**b** neutrophil, **c** lymphocyte, **d** NLR). There was no significant differences in neutrophil, lymphocyte, or NLR on admission

between patients with and without poor outcome. Comparing with good outcome group, patients in poor outcome group show higher levels of neutrophil and NLR at 24 h and at 7 days

## Prospective associations of neutrophil before and after r-tPA treatment with PH and stroke prognosis

Table 2 summarizes the results of the binary logistic regression analysis of PH and the 3-month outcome. The analysis using cell count before r-tPA treatment as a predictor did not find significant associations of neutrophil, lymphocyte, and NLR with PH or poor functional outcomes after stroke (all  $P > 0.05$ ). In contrast, the analysis using cell count at 24 h after r-tPA treatment as a predictor showed that neutrophil level was obviously associated with parenchymal hemorrhagic (OR = 2.86,  $P = 0.029$ ) and 3-month poor outcome (OR = 2.67,  $P = 0.009$ ). Nevertheless, the changes of lymphocyte were not associated with hemorrhagic transformation and 3-month poor outcome. Moreover, NLR was significantly



**Fig. 2** Temporal profile (median and interquartile range) of neutrophil, lymphocyte, and NLR in stroke patients treated with r-tPA according to PH (**a** neutrophil, **b** lymphocyte, **c** NLR). There was no significant differences in neutrophil, lymphocyte, or NLR on admission between patients with and without PH. Comparing without PH group, patients with PH show higher levels of neutrophil and NLR at 24 h and at 7 days

associated with increased risks for both outcomes ( $P < 0.05$ ). Additionally, NLR at 7 days was also regarded as a predictor of 3-month outcome (OR = 3.64,  $P = 0.001$ ). We found the age and NIHSS score also are independent predictors of the 90-day outcome, besides neutrophil and NLR after thrombolysis.

## Subgroup analysis on the increase in neutrophil at 24 h after r-tPA treatment

In order to investigate the influence of dynamic changes in neutrophil on PH and 3-month unfavorable outcome, we further subdivided patients into four groups according to the percent change in neutrophil within 24 h after r-tPA treatment: decrease or no change reference group;  $0 < \text{increase} \leq 25\%$  group;  $25\% < \text{increase} \leq 50\%$  group; and  $\text{increase} > 50\%$  group. Associations of the percent change in neutrophil within 24 h after r-tPA treatment with PH and stroke prognosis were showed in Table 3. An increase  $> 25\%$  was found to be associated with an increased risk of PH and 3-month poor outcome when compared to the decrease or no change reference group. More importantly, there was a trend of incremental OR when compared higher increase group with lower ones. Patients with a neutrophil increase  $> 50\%$  had numerically higher OR compared with patients with a  $25\% < \text{neutrophil increase} \leq 50\%$ .

## Discussion

To the best of our knowledge, this is the first study to document the prognostic value of the dynamic changes of neutrophil and NLR in ischemic stroke patients treated with intravenous thrombolysis. The present study shows that the neutrophil and NLR are significantly elevated at 24 h after r-tPA thrombolysis. More importantly, these dynamic variations are independently associated with increased risks of PH and 3-month poor functional outcomes after r-tPA treatment in AIS patients. Besides the parenchymal hemorrhage, we also found dynamic changes of neutrophil, and NLR are independently associated with increased risks of hemorrhage transformation. Additionally, neutrophil and NLR on day 7 are also associated with poor outcomes in 3 months. Nevertheless, there is no statistically significant association between dynamic lymphocyte and parenchymal hemorrhage or 3-month poor functional outcomes. It also needs to be mentioned that the age and NIHSS score also are independent predictors of the 90-day outcome, which is consistent with previous studies.

In previous studies, higher neutrophil and NLR on admission were found to be independently associated with symptomatic intracerebral hemorrhages (sICH) and worse outcome at 3 months in patients with IV r-tPA treatment [22]. Duan and colleague also showed that the baseline

**Table 2** Prospective associations of neutrophil and lymphocyte with parenchymal hemorrhage (PH) and 3-month functional outcomes

Log-transformed cell count	PH OR (95%CI)	<i>P</i>	Poor Outcome OR (95%CI)	<i>P</i>
Cell count on admission				
Neutrophil	0.71(0.29, 1.74)	0.452	0.78(0.39, 1.55)	0.478
Lymphocyte	0.64(0.33, 1.24)	0.188	1.41(0.74, 2.70)	0.301
NLR	1.18(0.70, 1.98)	0.534	0.72(0.44, 1.19)	0.724
Cell count at 24 h after r-tPA				
Neutrophil	2.86(1.11, 7.40)	0.029	2.67(1.28, 5.58)	0.009
Lymphocyte	0.30(0.29, 1.15)	0.659	1.35(0.66, 2.74)	0.41
NLR	2.22(1.11, 4.07)	0.023	1.58(1.02, 2.45)	0.04
Cell count at 7 days				
Neutrophil			3.64(1.68, 7.87)	0.001
Lymphocyte			0.46(0.18, 1.13)	0.088
NLR			2.61(1.49, 4.58)	0.001

NLR neutrophil-to-lymphocyte ratio, OR odds ratio, CI confidence interval

Adjusted for age, sex, hypertension, diabetes, smoke, drink, hyperlipidemia, atrial fibrillation, DNT, NIHSS

NLR predicts 3-month functional outcome and sICH after endovascular treatment for acute anterior circulation large vessel occlusion stroke patients [23]. Nevertheless, the results of the present study suggested that neutrophil count and NLR, measured before r-tPA thrombolysis, are not reliable predictors of PH and prognosis in acute stroke patients undergoing systemic thrombolysis. The conflicting data presented above, which highlight a controversial role of early neutrophil count and NLR on the outcome, may result from the exclusion of patients with infections on admission in the present study. Moreover, infection has been demonstrated to affect stroke outcome in several different mechanisms. For example, infection may accelerate the inflammatory cascade and exceed central nervous system immune response [24]. Moreover, MMP-9 levels were found to be increased by the presence of infection and then enhance the ischemia-induced degradation of collagen IV, which may lead to the disruption of BBB [25]. Additionally, infection has also been observed to increase platelet activation and platelet-leukocyte aggregation [26, 27]. Therefore, the presence of infected patient may contribute to the difference in the predictive values of the neutrophils on admission for poor outcome at 3 months.

Numerous studies have demonstrated that inflammation response plays an important role in the formation and progression of ischemic stroke. When ischemic stroke occurs, a large quantity of leukocytes infiltrate in peripheral blood immediately [28]. Each subtype of leukocytes has a specific inflammatory function and may contribute differentially to the process of ischemic stroke [29]. Among peripheral circulating leukocytes, neutrophils are recruited to the brain ischemic area in the early phase of inflammation [9]. Brain infiltrating neutrophils release free oxygen radicals and inflammatory mediators and cause ischemic tissue damage by cellular necrosis and apoptosis [30]. Moreover, neutrophils release cytokines, chemokines, and adhesion molecules, resulting in BBB disruption and hemorrhagic transformation [31]. So far, many studies suggested that the high neutrophils and NLR are associated with stroke severity, hemorrhagic transformation [22], infarct volume [32], mortality, and clinical function outcome [33]. However, most of these previous studies focused on the neutrophils on admission but not the dynamic change of neutrophils levels. The present study observed that a higher increase of neutrophils within 24 h is independently associated with hemorrhagic transformation in acute ischemic stroke patients after IV r-tPA. The mechanisms underlying by which

**Table 3** Associations of percent increase in neutrophil within 24 h after r-tPA with parenchymal hemorrhage (PH) and 3-month poor outcome after stroke

Neutrophil change	PH		Poor outcome	
	OR	<i>P</i>	OR	<i>P</i>
Decrease or no change	1	Reference	1	Reference
0 < increase ≤ 25%	0.56(0.13, 2.35)	0.425	1.25(0.52, 2.97)	0.621
25% < increase ≤ 50%	2.54(1.36, 10.74)	0.027	3.77(1.31, 10.88)	0.014
Increase > 50%	4.00(1.42, 11.3)	0.009	3.86(1.64, 9.13)	0.002

Adjusted for age, sex, hypertension, diabetes, smoke, drink, hyperlipidemia, atrial fibrillation, DNT, NIHSS

increased neutrophils within 24 h compromises outcomes remain to be fully elucidated. Higher level of neutrophils within 24 h may indicate more active inflammation status. In animal experiment, MMP-9-positive neutrophil infiltration has been shown to contribute to an increased risk of hemorrhage after r-tPA treatment in middle cerebral artery occlusion (MCAO) rat models [34]. McColl and colleagues found the beneficial effect of treatments of preventing neutrophil infiltration on reducing MMP-9 released in the brain [35]. Additionally, the infarct volume of transient brain ischemia is found to be reduced when the infiltration of neutrophil is inhibited [36]. Moreover, inhibition of neutrophils also could reduce the BBB disruption and the rate of HT in ischemic stroke [34, 37]. In humans, MMP-9-driven neutrophil infiltration has been found to contribute to the BBB breakdown, basal lamina type IV collagen degradation, and HT [11]. Neutrophils are regarded as one of the main sources of matrix metalloproteinase-9. When neutrophils enter into brain, it can release MMP-9 which may act on tight junction proteins and then disrupt the BBB from the luminal side of the vessel [38]. Therefore, the inflammation process may contribute to the PH after ischemic stroke.

The present study also demonstrated that high neutrophils at 24 h are associated with 3-month poor outcome. Compared with favorable clinical outcome group, neutrophil count in the poor outcome group was significantly higher. The mechanisms underlying the relationship between high neutrophil and poor functional outcomes remain to be elucidated. Neutrophil has been demonstrated to release chemokines and cytokines and other molecules in inflammation process. Neutrophil can also disrupt the BBB by releasing MMP-9 [39] and increase reactive oxygen and nitrogen species [40]. Moreover, previous studies showed that BBB disruption, cerebral edema, and hemorrhagic transformation after ischemic stroke are highly associated with poor outcome and contribute to the morbidity and mortality of ischemic stroke. Patients with a neutrophil increase  $> 50\%$  had numerically higher OR compared with patients with a  $25\% < \text{neutrophil increase} \leq 50\%$  in our study, which indicate the greater the neutrophil elevation within 24 h, the higher the possibility of hemorrhagic transformation and poor prognosis. Based on these findings, inhibition of neutrophil or neutrophil infiltration may be a reasonable peripheral target for clinical treatment of stroke.

There is accumulating evidence supporting that NLR, which reflects systemic inflammation and the balance between neutrophils and lymphocytes, was regarded as a reliable biomarker in acute ischemic stroke [16]. Consistent with previous studies, the present study demonstrated that higher level of NLR may predict 3-month poor functional outcome in AIS patients. For example, NLR on admission and within 24 h was a predictor of 3-month outcome in patients with first-ever AIS [17, 18]. The present study also examined the predictive ability of lymphocyte on PH and outcome in AIS patients with the

r-tPA treatment. Nevertheless, the present study failed to observe any significant links to lymphocyte and 3-month functional outcome. Therefore, more work will be required to identify the reasons. It is well-established that inflammation response in the process of ischemic stroke is complicated. In addition to neutrophils and lymphocytes, other indicators of inflammation such as leukocyte and C-reactive protein (CRP) are also dynamically altered in stroke patients after thrombolysis [41]. CRP level measured within 24 h after stroke has been reported to predict 1-year functional outcome [42]. An increase in CRP during the first 24 h was also strongly associated with lack of clinical improvement and poor outcome in patients treated with r-tPA [43]. Another study showed that changes in leukocytes levels 24 h after intravenous thrombolysis could predict the short-term functional outcome of AIS patients.

It needs to be mentioned that there are still some limitations. In the current study, we finally recruited only 208 patients, and further study such as the association between neutrophil with PH-1 and PH-2 could not be addressed, and we would carry on including the patients. Additionally, we only examined neutrophil at three time points, and more time points of the examination would help to understand more precise dynamic change of neutrophil following IV r-tPA. Moreover, more work is required to explore the association between neutrophil with infarct volume. Furthermore, more studies are needed to characterize the underlying molecular mechanisms by which the neutrophil and NLR predict the PH and outcome in AIS patients with r-tPA treatment.

## Conclusion

In conclusion, our results suggest that the neutrophil is a dynamic variable following the r-tPA treatment of AIS. Dynamic increase in neutrophil after stroke significantly and reliably predicts PH and 3-month poor outcome in acute ischemic stroke patients receiving r-tPA treatment. In light of our findings, neutrophil level after r-tPA treatment may be regarded as an activity marker for PH and unfavorable prognosis in AIS patients with intravenous thrombolysis. Therefore, inhibition of neutrophil may serve as a reasonable peripheral target for clinical treatment of stroke.

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## Compliance with ethical standards

**Conflict of interest** None.

**Ethical approval** None.

## References

- Sacco RL, Kasner SE, Broderick JP et al (2013) An updated definition of stroke for the 21st century a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke -Dallas* 44(7):2064–2089
- Meschia JF, Bushnell C, Bodenbala B et al (2014) Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 45(12):3754–3832
- Group IC, Sandercock P, Wardlaw JM et al (2012) The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet*. 379(9834):2352–2363
- Hacke W, Kaste M, Bluhmki E et al (2008) Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*
- Jickling GC, Liu D, Stamova B, Ander BP, Zhan X, Lu A, Sharp FR (2014) Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab* 34(2):185–199
- Zhu W, Guo Z, Yu S, Leys D, Bordet R (2015) Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology* 85(16):1408–1416
- Guo Z, Yu S, Xiao L, Chen X, Ye R, Zheng P, Dai Q, Sun W, Zhou C, Wang S, Zhu W, Liu X (2016) Dynamic change of neutrophil to lymphocyte ratio and hemorrhagic transformation after thrombolysis in stroke. *J Neuroinflammation* 13(1):199
- Kim JY, Park J, Ji YC, Kim SH, Lee JE. Inflammation after ischemic stroke: the role of leukocytes and glial cells. *Exp Neurobiol*. 2016. 25(5): 241-
- Herz J, Sabellek P, Lane TE, Gunzer M, Hermann DM, Doeppner TR (2015) Role of neutrophils in exacerbation of brain injury after focal cerebral ischemia in hyperlipidemic mice. *Stroke* 46(10):2916–2925
- Perez-De-Puig I, Miró-Mur F, Ferrer-Ferrer M et al (2015) Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathol* 129(2):239–257
- Tokgoz S, Keskin S, Kayrak M, Seyithanoglu A, Ogmegul A (2014) Is neutrophil/lymphocyte ratio predict to short-term mortality in acute cerebral infarct independently from infarct volume. *J Stroke Cerebrovasc Dis* 23(8):2163–2168
- Schwartz M, Moalem G (2001) Beneficial immune activity after CNS injury: prospects for vaccination. *J Neuroimmunol* 113(2):185–192
- Fang YN, Tong MS, Sung PH et al (2017) Higher neutrophil counts and neutrophil-to-lymphocyte ratio predict prognostic outcomes in patients after non-atrial fibrillation-caused ischemic stroke. *Biom J* 40(3):154–162
- Rosell A, Cuadrado E, Ortegaaznar A, Hernándezguillamon M, Lo EH, Montaner J (2008) MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. *Stroke*. 39(4):1121–1126
- Mao Leilei, Li Peiying, Zhu Wen et al. Regulatory T cells ameliorate tissue plasminogen activator-induced brain haemorrhage after stroke.[J]. *Brain*, 2017, 140: 1914-1931
- Xue J, Huang W, Chen X, Li Q, Cai Z, Yu T, Shao B (2017) Neutrophil-to-lymphocyte ratio is a prognostic marker in acute ischemic stroke. *J Stroke Cerebrovasc Dis* 26(3):650–657
- Qun S, Tang Y, Sun J et al (2017) Neutrophil-to-lymphocyte ratio predicts 3-month outcome of acute ischemic stroke. *Neurotox Res* 31(3):1–9
- Pektezel M Yasir, Yilmaz Ezgi, Arsava E Murat et al. Neutrophil-to-lymphocyte ratio and response to intravenous thrombolysis in patients with acute ischemic stroke.[J]. *J Stroke Cerebrovasc Dis*, 2019, 28: 1853–1859
- Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T (1980) Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ* 58(1):113–130
- Jauch EC, Saver JL, Adams HP et al (2013) Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Circulation*. 8(3):870–947
- Churilov L, Arnup S, Johns H et al (2015) An improved method for simple, assumption-free ordinal analysis of the modified Rankin scale using generalized odds ratios. *Int J Stroke* 9(8):999–1005
- Maestrini I, Strbian D, Gautier S, Haapaniemi E, Moulin S, Sairanen T, Dequatre-Ponchelle N, Sibolt G, Cordonnier C, Melkas S, Leys D, Tatlisumak T, Bordet R (2015) Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology*. 85(16):1408–1416
- Duan Z, Wang H, Wang Z, Hao Y, Zi W, Yang D, Zhou Z, Liu W, Lin M, Shi Z, Lv P, Wan Y, Xu G, Xiong Y, Zhu W, Liu X, ACTUAL Investigators (2018) Neutrophil-lymphocyte ratio predicts functional and safety outcomes after endovascular treatment for acute ischemic stroke. *Cerebrovasc Dis* 45(5–6):221–227
- Raymond S, Connie W (2016) Ischemia, immunosuppression and infection—tackling the predicaments of post-stroke complications. *Int J Mol Sci* 17(1):551–556
- Muhammad S, Haasbach E, Kotchourko M et al (2011) Influenza virus infection aggravates stroke outcome. *Stroke* 42(3):783
- Mccoll BW, Allan SM, Rothwell NJ (2009) Systemic infection, inflammation and acute ischemic stroke. *Neuroscience*. 158(3): 1049–1061
- Zeller JA, Lenz A, Eschenfelder CC et al (2005) Platelet-leukocyte interaction and platelet activation in acute stroke with and without preceding infection. *Arter Thromb Vas Biol* 25(7):1519
- Barone FC, Hillegass LM, Price WJ, White RF, Lee EV, Feuerstein GZ, Sarau HM, Clark RK, Griswold DE (1991) Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. *J Neurosci Res* 29(3):336–345
- Kim J, Song T, Park JH, Lee HS, Nam CM, Nam HS, Kim YD, Heo JH Different prognostic value of white blood cell subtypes in patients with acute cerebral infarction. *Atherosclerosis*. 2012. 222(2): 464–467
- Matsuo Y, Kihara T, Ikeda M, Ninomiya M, Onodera H, Kogure K (1995) Role of neutrophils in radical production during ischemia and reperfusion of the rat brain: effect of neutrophil depletion on extracellular ascorbyl radical formation. *J Cereb Blood Flow Metab* 15(6):941–947
- Dirnagl U (1999) Pathobiology of ischaemic stroke : an integrated view. *Trends Neurosci* 22(9):391–397
- Buck BH, Liebeskind DS, Saver JL, Bang OY, Yun SW, Starkman S, Ali LK, Kim D, Villablanca JP, Salamon N, Razinia T, Ovbiagele B (2008) Early Neutrophilia is associated with volume of ischemic tissue in acute stroke. *Stroke*. 39(2):355–360
- Shi J, Peng H, You S, Liu Y, Liu CF (2018) Increase in neutrophil after r-tPA thrombolysis predicts poor functional outcome of ischemic stroke: a longitudinal study. *Eur J Neurol* 25(4):687–e645
- Gautier S, Ouk T, Tagzirt M et al (2014) Impact of the neutrophil response to granulocyte colony-stimulating factor on the risk of hemorrhage when used in combination with tissue plasminogen activator during the acute phase of experimental stroke. *J Neuroinflammation* 11(1):96
- Mccoll BW, Rothwell NJ, Allan SM (2007) Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via



- interleukin-1- and neutrophil-dependent mechanisms. *J Neurosci* 27(16):4403–4412
36. Garau A, Bertini R, Colotta F, Casilli F, Bigini P, Cagnotto A, Mennini T, Ghezzi P, Villa P (2005) Neuroprotection with the CXCL8 inhibitor repertaxin in transient brain ischemia. *Cytokine* 30(3):125–131
  37. Li Z, Zheng GZ, Rui LZ, Mei L, Chopp M (2003) Effects of a selective CD11b/CD18 antagonist and recombinant human tissue plasminogen activator treatment alone and in combination in a rat embolic model of stroke. *Stroke* 34(7):1790–1795
  38. Harris AK, Ergul A, Kozak A, Machado LS, Johnson MH, Fagan SC (2005) Effect of neutrophil depletion on gelatinase expression, edema formation and hemorrhagic transformation after focal ischemic stroke. *BMC Neurosci* 6(1):49
  39. Ludewig P, Sedlacik J, Gelderblom M, Bernreuther C, Korkusuz Y, Wagener C, Gerloff C, Fiehler J, Magnus T, Horst AK (2013) Carcinoembryonic antigen-related cell adhesion molecule 1 inhibits MMP-9-mediated blood-brain-barrier breakdown in a mouse model for ischemic stroke. *Circ Res* 113(8):1013–1022
  40. Dziedzic T (2015) Systemic inflammation as a therapeutic target in acute ischemic stroke. *Expert Rev Neurother* 15(5):523–531
  41. Tiainen M, Meretoja A, Strbian D, Suvanto J, Curtze S, Lindsberg PJ, Soenne L, Tatlisumak T, Helsinki Stroke Thrombolysis Registry Group (2013) Body temperature, blood infection parameters, and outcome of thrombolysis-treated ischemic stroke patients. *Int J Stroke* 8(8):632–638
  42. Napoli MD, Papa F, Boccola V (2001) Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. *Stroke* 32(1):133–138
  43. Gill D, Sivakumaran P, Wilding P, Love M, Veltkamp R, Kar A (2016) Trends in C-reactive protein levels are associated with neurological change twenty-four hours after thrombolysis for acute ischemic stroke. *J Stroke Cerebrovasc Dis* 25(8):1966–1969

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