

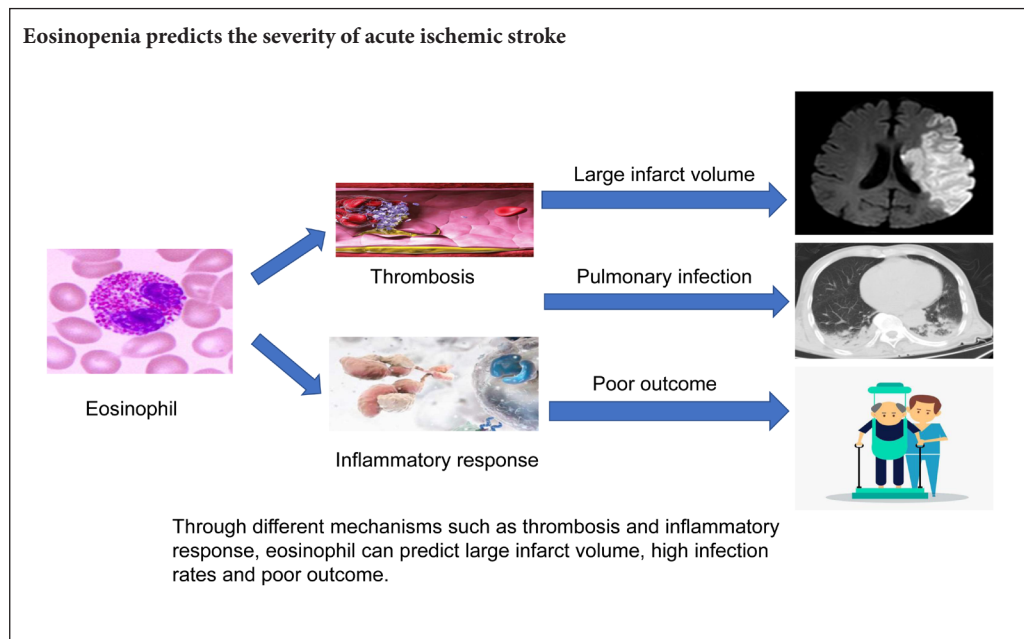
# Eosinopenia is a predictive factor for the severity of acute ischemic stroke

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## Graphical Abstract



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## Abstract

Previous data have revealed an association between eosinopenia and mortality of acute ischemic stroke. However, the relationship of eosinopenia with infarct volume, infection rate, and poor outcome of acute ischemic stroke is still unknown. The retrospective study included 421 patients (273 males, 65%; mean age,  $68.0 \pm 13.0$  years) with first acute ischemic stroke who were hospitalized in the Second Affiliated Hospital of Soochow University, China, from January 2017 to February 2018. Laboratory data, neuroimaging results, and modified Rankin Scale scores were collected. Patients were divided into four groups according to their eosinophil percentage level ( $< 0.4\%$ ,  $0.4\text{--}1.1\%$ ,  $1.1\text{--}2.3\%$ ,  $\geq 2.3\%$ ). Spearman's correlation analysis showed that the percentage of eosinophils was negatively correlated with infarct volume ( $r_s = -0.514$ ,  $P < 0.001$ ). Receiver operating characteristic analysis demonstrated that eosinopenia predicted a large infarct volume more accurately than neutrophilia; the area under curve was 0.906 and 0.876, respectively; a large infarct was considered as that with a diameter larger than 3 cm and involving more than two major arterial blood supply areas. Logistic regression analysis revealed that eosinophil percentage was an independent risk factor for acute ischemic stroke ( $P = 0.002$ ). Moreover, eosinophil percentage was significantly associated with large infarct volume, high infection rate (pulmonary and urinary tract infections), and poor outcome (modified Rankin Scale score  $> 3$ ) after adjusting for potential confounding factors ( $P\text{-trend} < 0.001$ ). These findings suggest that eosinopenia has the potential to predict the severity of acute ischemic stroke. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University, China (approval number: K10) on November 10, 2015.

**Key Words:** nerve regeneration; eosinopenia; eosinophil; ischemia; stroke; infarct volume; infection; clinical outcome; neutrophilia; risk factors; predictive factor; neural regeneration

**Chinese Library Classification No.** R446; R741

## Introduction

Many studies have demonstrated an association of a poor stroke prognosis with neutrophilia (Wang et al., 2016), monocytosis, lymphopenia (Zierath et al., 2018), an increased neutrophil lymphocyte ratio (Tokgoz et al., 2013; Wang et al., 2016; Zhao et al., 2016; Qun et al., 2017), and C-reactive protein level (Wassertheil-Smoller et al., 2014; Lind et al., 2015). However, only a few studies have studied the severity of stroke in relation to eosinophils, which lead to endothelial dysfunction, thrombosis, and worsen inflammation (Ten et al., 1989; Slungaard et al., 1993; Wang et al., 2006; Moosbauer et al., 2007; Marzano et al., 2009; Kecoglu et al., 2014). Some researchers have reported that higher eosinophil levels are associated with stent thrombosis and stent restenosis (Niccoli et al., 2009). Reduced eosinophil count ( $< 40$  cells/mm<sup>3</sup>) was initially found in patients with febrile diseases, cachectic, and the moribund state (Kirkeby, 1956; Abidi et al., 2011), and more recently found in those with acute coronary syndrome (Jiang et al., 2015) and heart failure (Shah et al., 2016). Recent studies have shown that eosinopenia is strongly associated with mortality and high infection rate in patients with stroke (Hori et al., 2016; Bolayir et al., 2017; Wang et al., 2017; Fan et al., 2018). Therefore, we investigated the association between eosinopenia and acute ischemic stroke (AIS), as well as the ability for eosinopenia to predict AIS severity.

## Participants and Methods

### Participants

A total of 421 patients with acute ischemic stroke (AIS) admitted to the Second Affiliated Hospital of Soochow University, China, from January 2017 to February 2018 after symptom onset were enrolled in this retrospective analysis. A control group of 104 age- and sex-matched healthy individuals were recruited from the medical examination center of our hospital.

The inclusion criterion was a first onset of AIS within 72 hours. According to World Health Organization-defined criteria, the diagnosis of ischemic stroke was based on patient history, clinical data, computed tomography (CT) and/or magnetic resonance imaging (MRI) (Sacco et al., 2013).

Patients with infection, asthma, eosinophilic esophagitis, and/or hypereosinophilic syndrome were excluded from this study.

This study was approved by Ethics Committee of the Second Affiliated Hospital of Soochow University (approval number: K10, **Additional file 1**) on November 10, 2015. Patients or their family members provided written informed consent.

### Laboratory measurements and CT/MRI scan

Blood samples were collected from patients the day after admission and analyzed using XN-10 (Sysmex, Kobe, Japan), which was calibrated daily. Laboratory variables, including neutrophil and eosinophil counts, eosinophil percentage, C-reactive protein, fasting plasma glucose, low density lipoprotein-cholesterol, and homocysteine, were assayed at local laboratories. Eosinopenia was defined as an eosinophil percentage less than 0.4% (Krause and Boggs, 1987).

CT and MRI images were acquired by a multislice CT-scanner (Hispeed NX, GE, USA) and a 1.5 or 3.0 Tesla Philips Ingenia scanner, respectively. All patients received repeated CT or MRI within 24 to 96 hours after admission ( $n = 72$  for CT,  $n = 349$  for MRI). CT or diffusion-weighted MR imaging volume was measured using the ABC/2 formula (ellipsoid). Large infarct volume was defined as a large hemisphere infarction with a diameter more than 3 cm and that involved the blood supply area of two large, main arteries (Adams et al., 1993). Infection was defined as fever with clinical manifestations (such as urinary tract symptoms, productive cough, and pleuritic pain), laboratory inspection (such as leukopenia [4/L] or leukocytosis [12/L]), and imaging examination (such as chest CT penetration).

At 90 days after stroke onset, patient disability was evaluated using the modified Rankin Scale (Harrison et al., 2013; Pan et al., 2017; Zhou et al., 2019). Disability is rated on a scale from 0 to 6, as follows: 0: no symptoms; 1: no significant disability, able to carry out all usual activities despite the presence of some symptoms; 2: slight disability, able to look after own affairs without assistance, but unable to carry out all previous activities; 3: moderate disability, requires some help, but able to walk unassisted; 4: moderately severe disability, unable to attend to own bodily needs without assistance and unable to walk unassisted; 5: severe disability, requires constant nursing care and attention, bedridden, incontinent; 6: dead. A poor outcome was defined as a modified Rankin Scale score  $> 3$ .

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### Outcomes

The severity of AIS was determined by infarct volume, infection, and poor outcome.

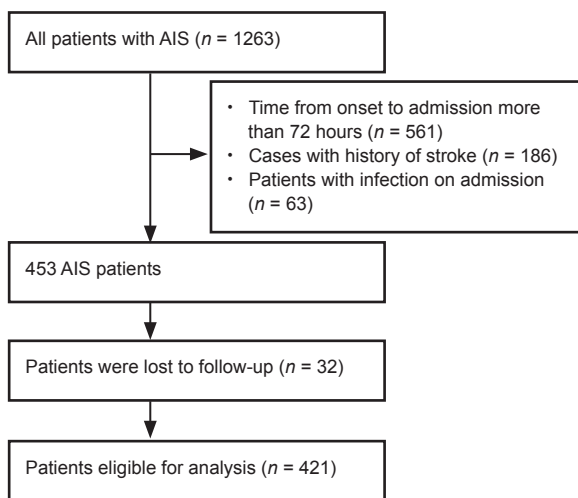
### Statistical analysis

SPSS statistical package 21.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Eosinophil percentage at admission was collapsed into quartiles. Normal distribution variables are presented as the mean  $\pm$  SD and were compared using Student's *t* tests. Non-normally distributed variables are presented as the interquartile range and were compared using Mann-Whitney *U* test or the Kruskal-Wallis rank sum test. Pearson's chi-square test was used to compare the differences in categorical variables, including gender, hypertension, diabetes mellitus, coronary artery disease, current smoking, atrial fibrillation, thrombolytic therapy, infection, 30-day mortality, and stroke subtype. Spearman's correlation test was used to explore correlations between variables. To predict the large infarct volume, the eosinophil percentage's cut-off values were evaluated using receiver operating characteristic analyses. Youden's index was used to test the eosinophil cut-off value. Backward stepwise logistic regression was applied to analyze predictor variables for large infarct volume, infection, and poor outcome. A value of  $P < 0.05$  was considered statistically significant.

## Results

### Baseline characteristics of patients

**Table 1** shows the baseline characteristics of participants. There were 273 men (65%) and 148 women (35%) and the average age was  $68.0 \pm 13.0$  years old. Compared with the control group, patients with AIS had lower eosinophil counts in peripheral blood. Blood samples were collected at a mean  $\pm$  SD of  $39.1 \pm 33.5$  hours after AIS onset. The average counts of total peripheral eosinophil and neutrophil were  $0.1 \pm 0.1 \times 10^9$  and  $5.4 \pm 2.7 \times 10^9$  cells/L, respectively. A flow chart of the study procedure is shown in **Figure 1**.



**Figure 1** Flow chart of the study procedure. AIS: Acute ischemic stroke.

### Eosinopenia percentage is an independent risk factor for AIS

Compared with control group, the AIS group had higher levels of systolic blood pressure, fasting plasma glucose, homocysteine, C-reactive protein, low density lipoprotein-cholesterol, and neutrophil counts, and had a lower eosinophil count and eosinophil percentage (all  $P < 0.05$ ). Meanwhile, the AIS group had higher rate of smoking and history of atrial fibrillation compared with the control group (both  $P < 0.05$ ; **Table 1**). Furthermore, the binary logistic regression analysis revealed that, compared with the control group, the AIS group had significantly increased odds of having a current smoking status ( $OR = 3.504$ , 95%  $CI$ : 1.301–7.167,  $P = 0.010$ ), atrial fibrillation ( $OR = 8.456$ , 95%  $CI$ : 2.327–30.726,  $P = 0.001$ ), higher neutrophil levels ( $OR = 1.311$ , 95%  $CI$ : 1.082–1.580,  $P = 0.006$ ), low-density lipoprotein cholesterol levels ( $OR = 1.982$ , 95%  $CI$ : 1.428–2.749,  $P < 0.001$ ), and homocysteine levels ( $OR = 1.058$ , 95%  $CI$ : 1.002–1.118,  $P = 0.044$ ), and lower levels of eosinophil percentage ( $OR = 0.711$ , 95%  $CI$ : 0.572–0.884,  $P = 0.002$ ; **Table 2**).

### Association between eosinopenia and infarct volume

Demographic and clinical features of the four groups of patients based on blood eosinophil percentage quartiles are shown in **Table 3**. There were no significant differences in

**Table 1** Features of enrolled patients on admission

	Controls (n = 104)	Stroke patients (n = 421)	P-value
Median age (years)	69±10	68±13	0.065
Male sex	47(47)	273(65)	0.001*
Hypertension	73(70)	305(72)	0.782
Diabetes	28(27)	108(26)	0.889
Coronary artery disease	8(8)	16(4)	0.15
Atrial fibrillation	3(3)	88(21)	< 0.001*
Current smoking	15(14)	130(31)	0.001*
Systolic blood pressure (mmHg)	134±22	152±22	< 0.001*
Fasting plasma glucose (mM)	5.20(4.79–5.95)	5.66(5.01–6.99)	< 0.001*
Neutrophils ( $\times 10^9/L$ )	3.40(2.73–4.40)	4.50(3.50–6.70)	< 0.001*
Eosinophils ( $\times 10^9/L$ )	0.11(0.08–0.17)	0.07(0.03–0.15)	< 0.001*
Eosinophil percentage (%)	1.80(1.30–2.88)	1.20(0.40–2.35)	< 0.001*
LDL-c (mM)	2.43(1.83–3.15)	2.89(2.25–3.50)	< 0.001*
Homocysteine (mM)	11.65(9.28–14.30)	13.20(10.90–17.45)	< 0.001*
C-reactive protein (mg/L)	5.30(5.20–5.50)	5.40(5.01–6.99)	0.001*

Data are expressed as the mean  $\pm$  SD, median (interquartile range) or  $n(\%)$ . \*Indicates significance, chi-square test, Mann-Whitney  $U$  test, or Student's  $t$  test. LDL-c: Low density lipoprotein-cholesterol.

**Table 2** Variables related to acute ischemic stroke analyzed by binary logistic regression

Category	OR	95% CI	P-value
Current smoking	3.504	1.301–7.167	0.01
Atrial fibrillation	8.456	2.327–30.726	0.001
Neutrophils	1.311	1.082–1.580	0.006
Eosinophil percentage	0.711	0.572–0.884	0.002
LDL-c	1.982	1.428–2.749	< 0.001
Homocysteine	1.058	1.002–1.118	0.044

Model adjusted for sex, age, hypertension, diabetes mellitus, coronary artery disease, current smoking, atrial fibrillation, fasting plasma glucose, neutrophil count, LDL-c, homocysteine, and eosinophil percentage (backward stepwise (wald)). OR: Odds ratio; CI: confidence interval; LDL-c: low density lipoprotein-cholesterol.

admission blood pressure, fasting plasma glucose, or the premorbid risk factor profile expect atrial fibrillation across eosinophil percentage quartiles. Bivariate regression analysis revealed that patients with the lowest eosinophil percentage quartile ( $< 0.4\%$ ) had significantly larger lesion volumes than patients with the other three higher quartiles ( $P$ -trend  $< 0.001$ ) (**Figure 2** and **Table 4**). However, no significant differences were observed between the other three groups with higher quartiles. Spearman's correlation analysis showed that infarct volume was negatively correlated with eosinophil percentage ( $r_s = -0.514$ ,  $P < 0.001$ ). Finally, receiver operating characteristic curves were used to investigate the diagnostic value of eosinophils in predicting large infarct volume. An eosinophil percentage of 0.35% independently predicted a large infarct volume in AIS patients, with a sensi-

**Table 3 Demographic and clinical features of the four patient groups based on blood eosinophil percentage quartiles**

Characteristic	Blood eosinophils percentage quartiles				P-value
	1 <sup>st</sup> , < 0.4% (n = 104)	2 <sup>nd</sup> , 0.4–1.1% (n = 105)	3 <sup>rd</sup> , 1.1–2.3% (n = 107)	4 <sup>th</sup> , ≥ 2.3% (n = 105)	
<b>Demographics</b>					
Median age (years)	69±15	65±12	66±14	67±12	0.296
Male sex	6(59)	68(65)	69(65)	75(71)	0.29
<b>History</b>					
Hypertension	75(72)	77(73)	79(74)	75(71)	0.784
Diabetes	18(17)	29(28)	32(30)	29(28)	0.155
Coronary artery disease	9(9)	1(1)	3(3)	3(3)	0.023
Atrial fibrillation	44(42)	19(18)	11(10)	14(13)	< 0.001*
Current smoking	23(22)	27(26)	36(34)	44(42)	0.01
<b>Admission</b>					
Systolic blood pressure (mmHg)	150±24	150±20	156±22	152±21	0.819
Diastolic blood pressure (mmHg)	86±15	85±13	84±15	84±14	0.639
Fasting plasma glucose (mM)	6.01(5.06–7.09)	5.44(4.76–6.58)	5.60(4.94–7.33)	5.34(4.95–6.61)	0.049
Admission NIHSS	15(6–18)	4(2–7)	3(2–5)	3(2–4)	< 0.001*
Discharge NIHSS	12(4–17)	3(1–6)	2(1–5)	2(1–3)	< 0.001*
Infarct volume (cm <sup>3</sup> )	28.95(4.20–87.5)	0.90(0.25–4.75)	0.50(0.23–1.40)	0.45(0.18–1.23)	< 0.001*
<b>Thrombolytic therapy</b>					
Infection	27(26)	19(18)	13(12)	7(7)	0.001*
30-day mortality	47(45)	9(9)	5(5)	3(3)	< 0.001*
Modified Rankin Scale Stroke subtype	23(30)	2(2)	0	0	< 0.001*
<b>Modified Rankin Scale Stroke subtype</b>					
Large vessel atherosclerosis	3(0–5)	1(0–2)	1(0–1)	0(0–1)	< 0.001*
Cardioembolic	34(44)	36(47)	39(52)	30(42)	0.62
Small vessel disease	29(38)	14(18)	6(8)	9(12)	< 0.001*
Other	9(12)	23(30)	26(35)	32(44)	< 0.001*
Unknown	3(4)	0	1(1)	0	0.119
Unknown	2(2)	3(4)	3(4)	1(1)	0.940

Data are expressed as the mean ± SD, median (interquartile range), or n(%). The stroke subtypes included dissecting aneurysm, vascular malformations, hypercoagulable states, hematologic disorders, fibromuscular dystrophy, and vasculitis. \*Indicates significance, chi-square test, Kruskal-Wallis rank sum test. NIHSS: National Institutes of Health Scale Score.

**Table 4 Eosinopenia predicts large infarct volume**

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-trend	OR (95% CI)	P-trend
Eosinophil (%)		< 0.001		< 0.001
≥ 2.3	1.00(Ref.)		1.00(Ref.)	
2.3–1.1	4.039 (0.444–36.750)		2.416 (0.543–10.749)	
1.1–0.4	9.750 (1.213–78.391)		4.988 (1.227–20.274)	
< 0.4	88.324 (25.237–605.333)		50.791 (12.751–426.635)	

Model adjusted for sex, age, hypertension, diabetes mellitus, coronary artery disease, current smoking, fasting plasma glucose, neutrophil count, stroke subtype, and thrombolytic therapy [multivariate logistic regression, backward stepwise (wald)]. Large infarct volume was defined as a large hemisphere infarction: the diameter of infarct was more than 3 cm and involved more than two large arteries in the main supply area. OR: Odds ratio; CI: confidence interval; Ref.: referent quartile.

tivity of 82.7% and a specificity of 89.1% (area under curve = 0.906; 95% CI: 0.870–0.943). Furthermore, by comparing the area under curve, we found that eosinopenia identified large

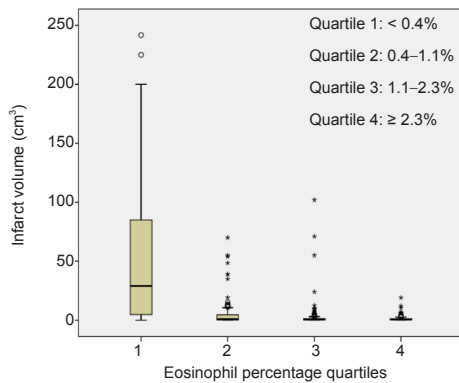
infarct volume more accurately than neutrophilia (Figure 3 and Table 5).

#### Eosinopenia is associated with high infection rate and poor outcome

As shown in Table 3, patients with eosinopenia showed a worse clinical outcome (modified Rankin Scale scores > 3) and higher infection rate than patients without eosinopenia ( $P < 0.001$ ). After adjusting for potential confounders, the odds of infection and poor outcome were significantly higher in participants with the lowest eosinophil quartile compared with those in the highest quartile ( $OR = 7.275$ ; 95%CI: 1.881–28.133;  $P$ -trend < 0.001;  $OR = 8.855$ ; 95% CI: 2.130–36.815;  $P$ -trend < 0.001) (Tables 6 and 7).

#### Association between eosinophil percentage quartiles and stroke subtype

As illustrated in Table 3, the Trial of Org 10172 in Acute Stroke Treatment (TOAST) stroke subtypes exhibited significantly different eosinophil percentages ( $P < 0.001$ ). In the lowest eosinophil percentage quartile, 38% of stroke cases were classified as the cardioembolic subtype, with a greater proportion than the other three higher eosinophil percent-



**Figure 2** Infarct volume distribution in patients with AIS according to eosinophil percentage quartiles. Box plot showing the median volumes (solid bar), minimum/maximum values (whiskers), and interquartile range (bar width) across eosinophil quartiles.

**Table 5** Area under curve, sensitivity, and specificity of the different predictors

Variable	Area under curve		Sensitivity	Specificity	Cut-off value
	95% CI	P-value			
Eosinophil (%)	0.906 (0.870–0.943)	< 0.001	0.827	0.891	0.35
Eosinophil ( $\times 10^9/L$ )	0.884 (0.838–0.929)	< 0.001	0.79	0.897	0.025
Neutrophil ( $\times 10^9/L$ )	0.876 (0.835–0.917)	< 0.001	0.877	0.726	5.45

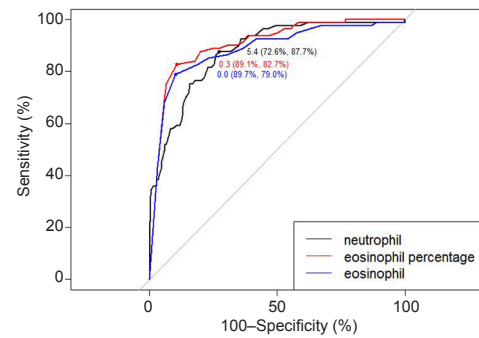
CI: Confidence interval.

**Table 7** Eosinopenia predicts poor outcomes (modified Rankin Scale scores > 3)

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-trend	OR (95% CI)	P-trend
Eosinophil (%)		< 0.001		< 0.001
≥ 2.3	1.00(Ref.)		1.00(Ref.)	
2.3–1.1	2.747 (0.707–10.656)		2.416 (0.543–10.749)	
1.1–0.4	4.804 (1.327–17.394)		4.988 (1.227–20.274)	
< 0.4	30.291 (9.024–101.676)		8.855 (2.130–36.815)	

Model adjusted for sex, age, hypertension, diabetes mellitus, coronary artery disease, current smoking, fasting plasma glucose, neutrophil count, stroke subtype, and thrombolytic therapy [multivariate logistic regression, backward stepwise (wald)]. Poor outcome was defined as having a modified Rankin Scale score > 3. OR: Odds ratio; CI: confidence interval; Ref.: referent quartile.

age quartiles (18%, 8%, and 12%, respectively;  $P < 0.001$ ). Moreover, there was larger proportion of brain small vessel occlusions in the highest quartile than in the lowest quartile (44% vs. 12%,  $P < 0.001$ ). Among eosinophil percentage quartiles, there was no significant difference of the proportions of brain large-vessel stroke.



**Figure 3** Different types of white blood cells in predicting large infarct volume.

Receiver-operating characteristic analysis for neutrophil, eosinophil, and eosinophil percentage in predicting large infarct volume.

**Table 6** Eosinopenia indicates infection

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-trend	OR (95% CI)	P-trend
Eosinophil (%)		< 0.001		< 0.001
≥ 2.3	1.00(Ref.)		1.00(Ref.)	
2.3–1.1	1.667 (0.388–7.158)		1.504 (0.330–6.859)	
1.1–0.4	3.187 (0.838–12.125)		2.465 (0.618–9.831)	
< 0.4	28.035 (8.349–94.139)		7.275 (1.881–28.133)	

Model adjusted for Sex, age, hypertension, diabetes mellitus, coronary artery disease, current smoking, fasting plasma glucose, neutrophil count, stroke subtype, and thrombolytic therapy [multivariate logistic regression, backward stepwise (wald)]. OR: Odds ratio; CI: confidence interval; Ref: referent quartile.

## Discussion

The present study shows that patients with the lowest eosinophil percentage quartile (< 0.4%) had remarkably larger infarct volume, a higher rate of infection, and poorer outcomes relative to the other three higher quartiles. Our results reveal that eosinopenia can predict AIS severity after adjustment for potential confounders.

Our results were consistent with previous studies that have demonstrated the prognostic significance of eosinopenia and mortality in patients with AIS (Hori et al., 2016; Wang et al., 2017; Fan et al., 2018). Some researchers have identified a positive relationship between neutrophilia and large infarct volume (Buck et al., 2008; Pagram et al., 2016). In our study, we found that eosinopenia might identify the large infarct volume more accurately than neutrophilia by comparing the area under curve. Thus, eosinophil percentage might have the potential to serve as a biomarker to predict the severity of cerebral infarction. Even though the proportion of eosinophils in peripheral blood is low, eosinopenia can occur in very rare cases. A previous study reported that only 24 patients out of 24,300 with a normal leukocyte count had

eosinopenia (Krause and Boggs, 1987).

The exact mechanisms underlying the relationship between eosinopenia and AIS severity remain unclear. There are several potential explanations for the sharp reduction in the eosinophil count in critical AIS. First, there is an excessive loss of eosinophils through apoptosis and degranulation. Some studies have suggested that eosinopenia can be caused by acute stress (Bass et al., 1980; Pazdrak et al., 2016). An acute stress response may occur in patients with large infarct volume that can stimulate the release of adrenal glucocorticosteroids and epinephrine, leading to eosinopenia by apoptosis (Cayrol and Girard, 2018). However, a decrease in circulating eosinophils does not appear to be a result of adrenal mediation alone, since because this was also noted in adrenalectomized rabbits (Bass et al., 1980). Moreover, acute stress responses involving the hypothalamic-pituitary axis and autonomic nervous system can lead to stroke-induced immunological suppression, and thus increase the risk of infection (Dirnagl et al., 2007). Next, the infarct volume is a major determinant of susceptibility to infection after stroke (Hug et al., 2009). Post-ischemic inflammation is a universally accepted pathogenesis of AIS. A rise in total peripheral leukocyte number is associated with large infarct volume and increased AIS severity (Pozzilli et al., 1985; Audebert et al., 2004). Some cytokines, such as N-formyl-methionyl-leucyl-phenylalanine (Weller and Spencer, 2017) and high-concentration galectin-1 (O'Sullivan and Bochner, 2018), have been found to induce eosinophil apoptosis. Other work has found that the degranulation of eosinophils caused cytotoxic protein-mediated thrombosis and endothelial injury to promote brain infarctions (Wang et al., 2017). Another explanation for the reduction in eosinophil counts in peripheral blood is that eosinophil migrates to tissues. One study has suggested that eosinophils increased in the thrombus and decreased in peripheral blood of patients with acute coronary syndrome (Jiang et al., 2015). However, some researchers have reported that the large thrombus has high eosinophil counts both in thrombi and peripheral blood in patients with acute coronary syndrome (Sakai et al., 2009; Sundstrom et al., 2017). These different results may be attributed to differences in the patient population studied and the timing of blood sample collection. Furthermore, thrombi were noticeably large in patients with great eosinophil infiltration (Sakai et al., 2009). Consistently, our results showed that, in the lowest eosinophil percentage quartile, 38% of stroke cases were classified as the cardioembolic subtype. This may demonstrate that eosinophils promote thrombus formation and growth. Migration of eosinophils to tissues is mediated by numerous inflammatory mediators, including cytokines and activating factors (Koenderman, 2013). Bass (1977) showed that eosinopenia was caused by inflammatory factors in acute infection. Ravin et al. (2016) reported that eosinophils can engulf bacterial organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* via phagocytosis (Weller and Goetzl, 1980; Lehrer et al., 1989). Eosinophil granule proteins, including eosinophil cationic protein, eosinophil peroxidase,

and major basic protein (Wardlaw, 1994), have an anti-bacterial and antiviral effect (Persson et al., 2001; Bystrom et al., 2011; Ohno, 2012). Eosinophil cationic protein also influences the proliferation of T and B lymphocytes (Rosenberg, 1998; Liao et al., 2016; Ravin and Loy, 2016). The number of antigen-specific plasma B cells is reportedly decreased in eosinophil-deficient mice (Pagram et al., 2016). In other work, pneumonia events were negatively correlated with blood eosinophil counts in patients with chronic obstructive pulmonary disease (Pavord et al., 2016).

The above findings indicate that the eosinophils respond to immune cells, infections, and inflammation to protect against pathogenic microorganism. This could explain why patients with eosinopenia had an increased infection rate. Several studies and reviews have emphasized the role of infection as a risk factor for mortality and poor outcome following stroke (Meisel et al., 2005). Recent studies have shown that eosinopenia is strongly associated with mortality and higher infection rate in patients with stroke (Hori et al., 2016; Bolayir et al., 2017; Wang et al., 2017; Fan et al., 2018). In our study, we also found that patients with eosinopenia have poor outcomes.

By exploring how eosinophil count is associated with infarct volume, infection rate, and poor outcome, this is the first report to identify eosinopenia as a predictive factor for AIS severity.

This study has some limitations that should be noted. First, this was a retrospective study, blood samples and CT/MRI collected in different time that may influence the eosinophil counts and infarction volumes. Second, the blood samples were collected from patients the day after admission, and so the eosinophil count might have been influenced by medication. Third, thrombus samples from the infarction area of AIS patients could not be directly obtained so we could not have concluded that eosinophil promotes thrombus formation and growth. These limitations lead to a lack of the direct evidence on association between eosinophils and thrombosis.

In summary, eosinopenia may result in high infection rates, large cerebral infarction volumes, and poor outcomes in patients with AIS.

To conclude, patients with eosinopenia may have high infection rates, large volume of cerebral infarction and poor outcome in AIS.

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**Declaration of participants consent:** The authors certify that they have obtained all appropriate participants consent forms. In the forms, the participants or their family members have given their consent for the participant's images and other clinical information to be reported in the journal. The participants or their family members understand that the

participants' names and initials will not be published and due efforts will be made to conceal the participants' identity.

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**Data sharing statement:** Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices) will be in particular shared. Study protocol form will be available. The data will be available immediately following publication without end date. Anonymized trial data will be available indefinitely at [www.figshare.com](http://www.figshare.com).

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