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Platelet counts affect the prognostic value of homocysteine in acute ischemic stroke patients

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HIGHLIGHTS

- First study to measure the effect of platelet (PLT) counts on prognostic value of homocysteine (HCY) in ischemic stroke.
- HCY may be a predictor of prognosis in ischemic stroke patients with low PLT.
- Results with important clinical significance.

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ABSTRACT

Background and aims: The association between homocysteine and prognosis of ischemic stroke remains controversial, and the role of platelet count on the effects of homocysteine in the prognosis of ischemic stroke is still not elucidated.

Methods: A total of 3229 acute ischemic stroke patients from the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS) with homocysteine and platelet measurements were included in this analysis. They were prospectively followed up for death, recurrent stroke and vascular events within 1 year after acute ischemic stroke.

Results: There was a significant interaction effect between platelet count and homocysteine level on death (p for interaction < 0.05) within 1 year after ischemic stroke. After multivariate adjustment, high homocysteine level was associated with increased risk of 1-year mortality in patients with low platelet level (hazard ratio, 1.70; 95% confidence interval, 1.01–2.88) but not in those with high platelet level (hazard ratio, 1.08; 95% confidence interval, 0.65–1.75). The addition of homocysteine to a model containing conventional risk factors improved risk prediction of 1-year death (net reclassification index 0.53%, $p < 0.001$; integrated discrimination improvement 0.07%, $p < 0.001$).

Conclusions: High homocysteine may be merely an independent risk factor of death in ischemic stroke patients with low platelet levels. Further prospective studies from other populations and randomized clinical trials are needed to verify our findings and clarify the potential mechanisms.

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1. Introduction

Stroke is the second leading cause of death and main cause of serious long-term disability worldwide [1,2]. The age standardized annual death rate from stroke was two to three times of that from ischemic heart disease in China, whereas ischemic heart disease-related mortality was two to three times greater than the stroke-related mortality in western nations [3,4]. In China, there are 2.5 million new stroke cases annually and 7.5 million stroke survivors, which represent a major public health challenge [5,6]. Thus, an early risk assessment with an estimation of disease severity and identification of modifiable risk factors may lead to more effective stroke prevention and reduced mortality rates [7].

Elevated homocysteine (HCY) has been hypothesized to be associated with risk of developing coronary heart disease and stroke [8,9]. Previous studies also demonstrated that elevated HCY levels are associated with higher mortality rates from stroke and stroke recurrence [10,11]. Platelet (PLT) plays an important role in the pathogenesis of ischemic stroke due to its participation in thromboemboli that may initiate the symptoms of stroke [12]. Several studies also showed that PLT count was associated with increased risk of stroke and stroke mortality [13–15]. Interestingly, PLT has been shown to affect homocysteine on endothelial injury and atherosclerosis, and previous studies have reported that hypertensive adults with low PLT counts and high HCY at baseline have the highest risk of first stroke [16–18]. Therefore, whether the association of HCY with ischemic stroke clinical outcomes is affected by PLT count because of a potential interaction between the two variables needs to be further elucidated.

To test this hypothesis, we conducted a prospective study to investigate the association between HCY and prognosis of ischemic stroke according to the PLT count, based on a large prospective multicenter study of the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS).

2. Study participants and methods

2.1. Study patients

We conducted this study based on the data of CATIS, which was a multicenter, single-blind, blinded end-point randomized clinical trial conducted in 26 hospitals across China to test whether moderate lowering of blood pressure (BP) within the first 48 h after the onset of an acute ischemic stroke would reduce death and major disability at 14 days or hospital discharge. Details of the design, methods and main results of the CATIS test have been previously reported [19]. In briefly, a total of 4071 patients, 22 years or older who had ischemic stroke, confirmed by computed tomography or magnetic resonance imaging of the brain within 48 h of symptom onset, and who had an elevated systolic BP (SBP) between 140 mmHg and less than 220 mmHg, were recruited in this trial. For the present study, 842 participants were further excluded because they did not offer blood samples, or collected samples were hemolyzed in storage or transport, and 3229 patients were finally included in the analysis. There were no significant differences between enrolled and all participants in CATIS (Supplemental Table 1), indicating that those enrolled basically represented the total participants of CATIS.

This study was approved by the institutional review boards at Soochow University in China and Tulane University in the USA, as well as ethical committees at the 26 participating hospitals. Written consent was obtained from all study participants or their immediate family members.

2.2. Data collection

Baseline data, including demographic characteristics, clinical characteristics, medical histories, and lifestyles, were collected at the time of enrollment. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS; scores range from 0 to 42, with higher scores indicating a more severe neurologic deficit) by trained neurologists at baseline, as well as one-year follow-up visits [20]. Three BP measurements were obtained at baseline by trained nurses according to a common protocol adapted from procedures recommended by the American Heart Association [21]. BP was measured with the participant in a supine position using a standard mercury sphygmomanometer based on the participant arm circumference. The mean of three BP measurements was used in the analyses.

2.3. Measurements and outcome assessment

Blood samples were collected within 24 h of hospital admission after at least 8 h of fasting. Routine laboratory analyses (blood glucose, blood lipids, blood creatinine, etc.) were performed for all enrolled patients, in each participating hospital at admission. All plasma and serum samples were frozen at −80 °C in the Central Laboratory of School of Public Health in Soochow University, until laboratory testing. Plasma total HCY was determined by enzymatic cycling assay on the Cobas c 501 analyzer (Roche Diagnostics, Indianapolis, IN) and the PLT count on admission was quantified by automated blood cell counters in hospital laboratories. Laboratory technicians who performed these measurements were blind to the clinical characteristics and outcomes of the study participants. The outcomes in this study were defined as death, recurrent stroke and vascular events in the 1-year follow-up period after stroke onset.

2.4. Statistical analysis

PLT count and HCY levels were dichotomized using a median split for the participants. Baseline characteristics were compared between HCY groups in both low and high PLT count subgroups using $\chi 2$ tests, Student-t tests, or Wilcoxon rank-sum tests, as appropriate. Cox proportional hazards regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (95% CIs) of poor prognosis for the high HCY group, with the low HCY group as reference, among the patients stratified by PLT counts. Model 1 was performed to adjust for conventional risk like age, antiplatelet therapy, sex, time from onset to hospitalization, estimated glomerular filtration rate (eGFR), lipid-lowering therapy, antihypertensive treatment, current smoking, C-reaction protein, alcohol consumption, hemoglobin, body mass index, dyslipidemia, heart disease, high-density lipoprotein cholesterol (HDL-C), blood glucose, SBP at baseline, ischemic stroke and subtypes. Model 2 further adjusted for baseline NIHSS score. To examine effect modification by PLT count, we tested the statistical significance of HCY category × PLT count on the outcomes in Cox proportional hazards regression models by the likelihood ratio test.

In addition, C statistics, net reclassification indexes (NRI), and integrated discrimination improvement indexes (IDI) were calculated to evaluate improvement in risk classification by HCY over conventional risk factors [22]. The DeLong non-parametric test was used to compare the two C statistics [23]. NRI is a measure to evaluate the improvement in prediction performance gained by adding a marker to a set of baseline predictors, and IDI is a popular tool to evaluate the capacity of a marker to predict a binary outcome of interest. To test the robustness of our findings, we further categorized patients into four subgroups according to quartiles of HCY concentrations among the patients stratified by PLT counts. Two-tailed $p < 0.05$ was considered to be statistically significant. All statistical analyses were conducted using SAS statistical software (version 9.4, Cary, NC, USA).

Table 1
Baseline characteristics of study participants with different PLT levels according to HCY levels.

Characteristics	PLT < median			PLT ≥ median		
	HCY ≥ median	HCY < median	*p* value	HCY ≥ median	HCY < median	*p* value
Number of subjects	848	747		786	848	
Demographics						
Age, years	64.50 ± 11.35	62.39 ± 10.49	0.012	61.79 ± 11.23	60.88 ± 10.05	< 0.001
Male	690 (81.37)	446 (59.71)	< 0.001	237 (30.15)	461 (65.11)	< 0.001
Current cigarette smoking	380 (44.81)	245 (32.80)	< 0.001	331 (42.11)	251 (29.60)	< 0.001
Current alcohol drinking	316 (37.26)	222 (29.72)	0.002	254 (32.32)	218 (25.72)	0.004
Medical history						
History of hypertension	663 (78.18)	587 (78.58)	0.855	623 (79.26)	664 (78.30)	0.672
History of hyperlipidemia	52 (6.13)	62 (8.30)	0.098	47 (5.98)	72 (8.49)	0.057
History of diabetes mellitus	119 (14.03)	173 (23.16)	< 0.001	106 (13.49)	169 (19.93)	< 0.001
Clinical features						
NIHSS score	5 (3–8)	4 (2–7)	0.012	4 (2–7)	4 (2–7)	0.925
SBP, mmHg	167.56 (17.30)	165.70 (17.33)	0.959	166.44 (16.69)	165.61 (16.91)	0.723
DBP, mmHg	97.30 (11.30)	95.83 (11.24)	0.716	97.08 (11.25)	96.01 (10.53)	0.107
BMI, kg/m^2	24.78 (2.93)	24.93 (3.20)	0.043	24.91 (2.94)	25.08 (3.18)	0.074
Blood glucose, mmol/L	5.70 (5.00–7.00)	5.80 (5.15–7.54)	0.001	5.76 (5.00–6.99)	5.85 (5.10–7.52)	0.004
TG, mmol/L	1.39 (0.95–1.98)	1.48 (1.01–2.20)	0.021	1.41 (1.04–2.07)	1.61 (1.09–2.29)	< 0.001
TC, mmol/L	4.72 (4.10–5.47)	4.93 (4.26–5.62)	0.002	5.10 (4.39–5.78)	5.24 (4.46–5.95)	0.016
LDL-C, mmol/L	2.70 (2.19–3.36)	2.81 (2.28–3.41)	0.057	2.96 (2.38–3.55)	3.03 (2.35–3.67)	0.335
HDL-C, mmol/L	1.18 (1.00–1.42)	1.22 (1.03–1.50)	0.020	1.23 (1.02–1.48)	1.27 (1.07–1.27)	0.014
HCY, μmol/L	29.77 (19.94)	11.29 (2.22)	< 0.001	30.19 (19.80)	11.26 (2.23)	< 0.001
PLT, 10^9/L	169 (145–189)	172 (149–190)	0.212	248 (227–279)	249 (227–280)	0.506
Hematocrit, %	42 (39–45)	42 (39–45)	0.789	43 (40–46)	41 (39–44)	0.652
Ischemic stroke subtype						
Thrombotic	666 (78.54)	553 (74.03)	0.039	602 (76.59)	642 (75.71)	0.685
Embolic	46 (5.42)	38 (5.09)	0.823	42 (5.34)	40 (4.72)	0.573
Lacunar	136 (16.04)	156 (20.88)	0.062	142 (18.07)	166 (19.57)	0.150

PLT: platelet; HCY: homocysteine; NIHSS, National Institute of Health Stroke Scale; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; TG: triglycerides; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.
Median (interquartile range) for PLT: 208 (171–249) × 10^9/L; median (interquartile range) for HCY: 14.9(11.5–22.2) μmol/L.

3. Results

3.1. Baseline characteristics

Among 3229 individuals in the present analysis, 2064 were male and the average age was 61.81 ± 10.89 years. Among the patients with low PLT count, those with high HCY levels had lower HDL-C and history of diabetes mellitus and higher baseline NIHSS score, BMI, blood glucose, triglycerides (TG), total cholesterol (TC), and prevalence of smoking, drinking, thrombotic subtype, and tended to be male. In patients with high PLT count, those with high HCY had lower HDL-C and prevalence of history of diabetes compared with those with low HCY level (Table 1).

3.2. Association between HCY and stroke prognosis

There were 175 (5.42%), 143 (4.43%) and 182 (5.64%) patients who experienced death, recurrent stroke and vascular events within 1 year after stroke, respectively. The association between HCY and the risk of 1-year death was appreciably modified by PLT counts (p-$_{interaction}$ < 0.05). After adjustment for conventional risk factors and baseline NIHSS score, HRs (95% CIs) associated with high HCY levels were 1.70 (1.01–2.88) and 1.08 (0.65–1.75) for 1-year death among patients with low and high PLT count, respectively (Table 2, multivariable model 2). There were no significant associations between high HCY levels and recurrent stroke and vascular events in both low and high PLT count subgroups.

Adding HCY to a model containing conventional risk factors did not significantly improve the discriminatory power but did significantly improve risk reclassification for 1-year death among patients with low PLT counts (category-free NRI = 0.53, 95% CI = 0.31–0.75, p < 0.001; IDI = 0.07, 95% CI = 0.03–0.09, p < 0.001) (Table 3). As shown in Fig. 1, to examine the robustness of the effects of PLT counts and HCY levels on stroke prognosis, we further divided the patients into four groups according to HCY quartiles, and similar detrimental effects of high HCY levels on 1-year death after ischemic stroke were evident in patients with low PLT counts (see Table 4).

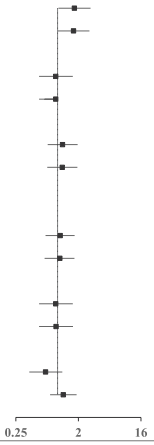
4. Discussion

In this large prospective multicenter study from CATIS, we found a significant association of elevated HCY levels with risk of death in acute ischemic stroke patients with low PLT counts, independent of established risk factors for stroke prognosis. The present study highlights the modification role of PLT count on the effect of HCY levels on prognosis after ischemic stroke.

Previous studies reported that elevated HCY levels were associated with cardiovascular morbidity and mortality in the general population and patients with cardiovascular diseases [24–26]. Recently, homocysteine-lowering vitamin therapy was independently associated with lower all-cause mortality in patients with coronary artery disease and elevated HCY levels [26]. In terms of ischemic stroke, several studies have also reported a detrimental effect of elevated HCY on clinical outcomes. In an analysis based on 3799 ischemic stroke patients, elevated HCY levels in the acute phase were associated with mortality after a follow-up of 48 months [11]. Li et al. also found that elevated HCY levels at admission were significantly associated with 3-month depression after stroke [27]. In contrast, a study based on 845 stroke cases and 421 health subjects found that elevated HCY levels in the acute phase of stroke patients were not associated with stroke severity and outcome measured by the Barthel Index at 6 or 12 months, and the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial revealed that moderate reduction of total HCY levels after non-disabling cerebral infarction had no effect on vascular outcomes during a follow-up of 2 years [28,29]. It has to be mentioned that all previous studies did not consider the effect of PLT count and did not

Table 2
Hazard ratio and 95% confidence interval of outcomes according to HCY levels at baseline in patients with different PLT levels.

Characteristics	HCY≥ median	HCY< median	HR (95% CI)	*p value*
PLT< median				
Death, n (%)	54(6.37)	29(3.88)		
Model 1			1.75(1.03-2.99)	0.040
Model 2			1.70(1.01-2.88)	0.044
Recurrent stroke, n (%)	34(4.01)	30(4.02)		
Model 1			0.94(0.54-1.66)	0.840
Model 2			0.94(0.54-1.65)	0.837
Vascular events, n (%)	49(5.78)	35(4.69)		
Model 1			1.18(0.72-1.95)	0.510
Model 2			1.17(0.71-1.93)	0.522
PLT≥ Median				
Death ,n (%)	49(6.23)	43(5.07)		
Model 1			1.09(0.67-1.76)	0.730
Model 2			1.08(0.65-1.75)	0.727
Recurrent stroke, n (%)	39(4.96)	40(4.72)		
Model 1			0.94(0.54-1.66)	0.840
Model 2			0.95(0.54-1.67)	0.855
Vascular events, n (%)	52(6.62)	46(5.42)		
Model 1			0.67(0.39-1.17)	0.566
Model 2			1.21(0.78-1.88)	0.386



HR, hazard ratio; 95%CI, 95% confidence interval; eGFR, estimated glomerular filtration rate
Model 1: adjusted for age, antiplatelet therapy, sex, time from onset to hospitalization, eGFR, lipid-lowering therapy, antihypertensive treatment, current smoking, C-reaction protein, alcohol consumption, hemoglobin, body mass index, dyslipidemia, heart disease, HDL-C, blood glucose, SBP at baseline and ischemic stroke subtypes.
Model 2: further adjusted for baseline NIHSS score

adjust it in a multivariable model as an important confounder.

Recently, a study found that PLT count, especially low PLT count, was associated with cardiovascular disease mortality and ischemic stroke prognosis [30]. Moreover, some studies reported that PLT count was inversely correlated with HCY level in women ($R = -0.88$, $p < 0.001$), and antiplatelet therapy modified the potential benefits of lowering HCY with B-vitamin supplementation in the secondary prevention of major vascular events in patients with stroke or transient ischemic attack [31,32]. Thus, the prognostic value of HCY in ischemic stroke may be modified by PLT count. In the present study, we found a significant interaction between HCY and PLT on 1-year death after ischemic stroke, and the results indicated that elevated HCY might be an independent risk indicator for mortality in patients with low PLT count compared to those with high PLT count. Moreover, a previous clinical trial reported that hypertensive adults with low PLT count and high HCY level had the highest risk of first stroke, and this risk was reduced by 73% with folic acid treatment, which suggested that there was a joint effect between low PLT and elevated HCY [18].

If our findings are valid, the mechanisms by which elevated HCY level increase risk of stroke mortality in patients with low PLT count remain to be elucidated. Laboratory investigations suggest several potential mechanisms of elevated HCY on the prognosis in the ischemic stroke patients with low PLT count. HCY may result in endothelial injury [33,34], and can stimulate aggregation and adherence of platelets

Table 3
Reclassification and discrimination statistics for clinical outcomes by serum HCY among patients with different PLT levels.

Characteristics	C statistic		NRI (categorical)		IDI	
	Estimate (95% CI)	*p* value	Estimate (95% CI)	*p* value	Estimate (95% CI)	*p* value
PLT < median						
Death						
Conventional model	0.754 (0.731–0.755)		Reference		Reference	
Conventional model + HCY	0.755 (0.733–0.777)	0.563	0.53 (0.31–0.75)	< 0.001	0.07 (0.04–0.09)	< 0.001
Recurrent stroke						
Conventional model	0.690 (0.665–0.713)		Reference		Reference	
Conventional model + HCY	0.692 (0.668–0.715)	0.673	−0.02 (-0.23-0.20)	0.884	0.02 (-0.01-0.03)	0.442
Vascular events						
Conventional model	0.689 (0.665–0.712)		Reference		Reference	
Conventional model + HCY	0.691 (0.667–0.714)	0.719	0.01 (-0.02-0.20)	0.985	0.01 (-0.01-0.02)	0.830
PLT ≥ median						
Death						
Conventional model	0.874 (0.856–0.891)		Reference		Reference	
Conventional model + HCY	0.875 (0.858–0.892)	0.658	−0.02 (-0.23-0.19)	0.845	0.02 (-0.02-0.04)	0.932
Recurrent stroke						
Conventional model	0.777 (0.755–0.798)		Reference		Reference	
Conventional model + HCY	0.785 (0.763–0.805)	0.071	0.04 (-0.23-0.24)	0.974	0.01 (-0.02-0.03)	0.599
Vascular events						
Conventional model	0.690 (0.665–0.713)		Reference		Reference	
Conventional model + HCY	0.691 (0.667–0.715)	0.861	0.06 (-0.16-0.27)	0.591	0.03 (-0.01-0.05)	0.861

Conventional model included age, antiplatelet therapy, sex, time from onset to hospitalization, eGFR, lipid-lowering therapy, antihypertensive treatment, current smoking, C-reaction protein, alcohol consumption, hemoglobin, body mass index, dyslipidemia, heart disease, HDL-C, blood glucose, SBP at baseline, ischemic stroke subtypes and NIHSS score.

Table 4
Hazard ratio and 95% confidence interval of outcomes for quartiles of HCY at baseline in patients with different PLT levels.

Variable	PLT < median					PLT ≥ median				
	Q1	Q2	Q3	Q4	*p* value	Q1	Q2	Q3	Q4	*p* value
Death, n (%)	11 (2.94)	18 (4.83)	26 (6.21)	28 (6.53)		22 (5.14)	21 (5.00)	25 (6.33)	24 (6.14)	
Model 1	1.00	1.70 (0.71–4.08)	2.14 (0.94–4.91)	2.61 (1.16–5.86)	0.016	1.00	0.72 (0.37–1.40)	0.79 (0.42–1.54)	1.14 (0.59–2.19)	0.612
Model 2	1.00	1.55 (0.65–3.69)	1.83 (0.80–4.20)	2.25 (1.01–5.01)	0.042	1.00	0.83 (0.42–1.62)	0.94 (0.48–1.84)	1.03 (0.53–2.01)	0.815
Recurrent stroke, n (%)	15 (4.01)	15 (4.020	19 (4.53)	15 (3.50)		21 (4.91)	19 (4.52)	17 (4.30)	22 (5.63)	
Model 1	1.00	1.29 (0.59–2.84)	0.95 (0.42–2.16)	1.22 (0.55–2.68)	0.734	1.00	0.82 (0.43–1.57)	1.06 (0.56–2.01)	0.87 (0.44–1.72)	0.875
Model 2	1.00	1.29 (0.59–2.84)	0.95 (0.42–2.15)	1.23 (0.56–2.70)	0.786	1.00	0.83 (0.43–1.59)	1.09 (0.57–2.09)	0.86 (0.43–1.71)	0.856
Vascular events, n (%)	17 (4.55)	18 (4.830	26 (6.21)	23 (5.36)		25 (5.84)	21 (5.00)	22 (5.57)	30 (7.67)	
Model 1	1.00	1.21 (0.58–2.51)	1.28 (0.63–2.60)	1.37 (0.68–2.77)	0.383	1.00	0.82 (0.44–1.51)	1.27 (0.71–2.30)	0.95 (0.51–1.77)	0.787
Model 2	1.00	1.27 (0.61–2.64)	1.25 (0.61–2.54)	1.36 (0.68–2.75)	0.431	1.00	0.82 (0.44–1.51)	1.27 (0.71–2.30)	0.95 (0.51–1.77)	0.836

Model 1: adjusted for age, antiplatelet therapy, sex, time from onset to hospitalization, eGFR, lipid-lowering therapy, antihypertensive treatment, current smoking, C-reaction protein, alcohol consumption, hemoglobin, body mass index, dyslipidemia, heart disease, HDL-C, blood glucose, SBP at baseline, ischemic stroke and subtypes. Model 2: further adjusted for baseline NIHSS score.

[35].The endothelial injury resulting from HCY promotes platelet consumption and adherence as well as activation, which consequently stimulate the proliferation of vascular smooth muscle cells by releasing mitogen factors [17]. Furthermore, HCY could also induce the proliferation of vascular smooth muscle cells [36], probably through interaction with platelets, suggested by an HCY-dependent expression of the platelet derived growth factor [37], thereby contributing to atherosclerosis and arterial stiffness [38,39]. Moreover, joint low PLT and high tHcy may be a marker for endothelial injury, platelet adherence, and consumption, which is in line with the findings in animal models [40].

Several strengths of our study deserve to be mentioned. First, this is the first study to investigate the association between HCY and prognosis of acute ischemic stroke according to admission PLT count. Second, this is a large prospective study based on data of CATIS, a randomized clinical trial with strict quality controls in data collection and outcome assessment.

The study has some limitations. First, some serious patients or those treated with intravenous thrombolytic therapy at admission were excluded because of CATIS design requirements. Therefore, a selection bias might be unavoidable. However, the proportion of patients treated with intravenous thrombolytic therapy is low in China and baseline characteristics of participants in this study were similar to those from the China National Stroke Registry [41]. Second, PLT and HCY levels were only measured at the acute stage of stroke in patients. Thus, without serial measurements, we have no data to examine the association between PLT and HCY change and stroke prognosis. Third, this study included some patients who had been hospitalized for more than 24 h from onset to admission; thus, admission PLT and HCY levels of these patients might not very accurately reflect the levels at stroke onset. However, previous studies had reported that PLT count and plasma HCY level were not elevated immediately after atherothrombotic stroke [42,43]. Fourth, a previous study indicated that aspirin therapy may downregulate homocysteine formation [44]. However, single antiplatelet agents or dual antiplatelet therapy was an important treatment for ischemic stroke secondary prevention, and we adjusted antiplatelet therapy in a multivariable model as an important confounder. Thus, the effect of aspirin therapy to the credibility of the present results is minimal. Fifth, a relatively small number of stroke recurrence and vascular events were observed within 1-year follow-up, which might limit our power to detect significant associations between HCY and stroke recurrence and vascular events. Finally, the information about homocysteine-lowering vitamin therapy was not collected in this study, which might influence the association between HCY and prognosis of acute ischemic stroke according to PLT counts.

In conclusion, the present study suggests that elevated HCY is associated with 1-year mortality in ischemic stroke patients with low PLT count compared to those with high PLT count. Further prospective studies from other populations and randomized clinical trials are needed to verify our findings and clarify the potential mechanisms.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

Tan Xu, Jing Chen, Yanbo Peng, Jing Chen, Qunwei Li, Zhong Ju, Deqin Geng, Yonghong Zhang, and Jiang He conceived and designed the study. Tan Xu, Yanbo Peng, Qunwei Li, and Yonghong Zhang coordinated the study. Xiaowei Zheng, Daoxia Guo, Hao Peng, MD, Chongke Zhong, Xiaoqing Bu, Tan Xu, Zhengbao Zhu, Aili Wang, Jing Chen, Tian Xu, Yanbo Peng, Qunwei Li, Zhong Ju, Deqin Geng, Jiang He, and Yonghong Zhang oversaw subjects' recruitment and monitored gathering of clinical data. Xiaowei Zheng and Daoxia Guo conducted

Characteristics	PLT< Median		PLT≥ Median	
	HR(95%CI)	p_{trend}	HR(95%CI)	p_{trend}
HCY quartiles	Death		Death	
Q1(Reference)	1.00	0.042	1.00	0.815
Q2	1.55(0.65-3.69)		0.83(0.42-1.62)	
Q3	1.83(0.80-4.20)		0.94(0.48-1.84)	
Q4	2.25(1.01-5.01)		1.03(0.53-2.01)	
	Recurrent stroke		Recurrent stroke	
Q1(Reference)	1.00	0.786	1.00	0.856
Q2	1.29(0.59-2.84)		0.83(0.43-1.59)	
Q3	0.95(0.42-2.15)		1.09(0.57-2.09)	
Q4	1.23(0.56-2.70)		0.86(0.43-1.71)	
	Vascular events		Vascular events	
Q1(Reference)	1.00	0.431	1.00	0.836
Q2	1.27(0.61-2.64)		0.82(0.44-1.51)	
Q3	1.25(0.61-2.54)		1.27(0.71-2.30)	
Q4	1.36(0.68-2.75)		0.95(0.51-1.77)	

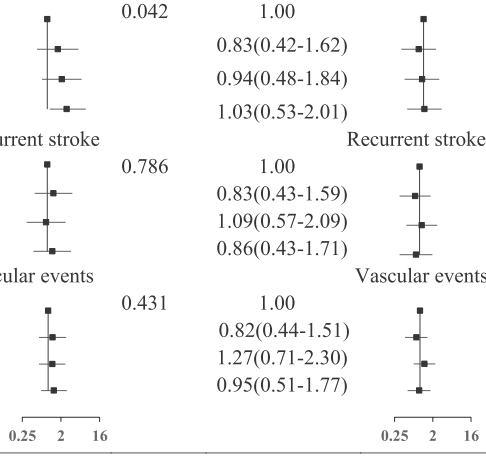


Fig. 1. Hazard ratio and 95% confidence interval of outcomes for quartiles of HCY at baseline in patients with different PLT levels.

the statistical analysis and prepared the paper. Yonghong Zhang and Jiang He revised the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.04.203.

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