JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 71, NO. 19, 2018

PUBLISHED BY ELSEVIER

Platelet Count Affects Efficacy of Folic Acid in Preventing First Stroke

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ABSTRACT

BACKGROUND The role of platelets and important effect modifiers on the risk of first stroke is unknown.

OBJECTIVES This study examined whether low platelet count (PLT) and elevated total homocysteine (tHcy) levels jointly increase the risk of first stroke, and, if so, whether folic acid treatment is particularly effective in stroke prevention in such a setting.

METHODS A total of 10,789 Chinese hypertensive adults (mean age 59.5 years; 38% male, with no history of stroke and myocardial infarction) were analyzed from the China Stroke Primary Prevention Trial, where participants were randomly assigned to daily treatments of 10 mg enalapril and 0.8 mg folic acid (n = 5,408) or 10 mg enalapril alone (n = 5,381). The primary endpoint was first stroke.

RESULTS During 4.2 years of follow-up, a total of 371 first strokes occurred. In the enalapril-alone group, the lowest rate of first stroke (3.3%) was found in patients with high PLT (quartiles 2 to 4) and low tHcy (<15 μmol/l); and the highest rate (5.6%) was in patients with low PLT (quartile 1) and high tHcy (≥15 μmol/l) levels. Following folic acid treatment, the high-risk group had a 73% reduction in stroke (hazard ratio: 0.27; 95% confidence interval: 0.11 to 0.64; p = 0.003), whereas there was no significant effect among the low-risk group.

CONCLUSIONS Among Chinese hypertensive adults, the subgroup with low PLT and high tHcy had the highest risk of first stroke, and this risk was reduced by 73% with folic acid treatment. If confirmed, PLT and tHcy could serve as biomarkers to identify high-risk individuals who would particularly benefit from folic acid treatment. (China Stroke Primary Prevention Trial [CSPPT]; NCT00794885) (J Am Coll Cardiol 2018;71:2136–46) © 2018 by the American College of Cardiology Foundation.

Stroke is the second leading cause of death in the world (1). It is the number 1 cause of death in China, and the number 4 cause of death in the United States (2,3). Globally, there is an urgent need to develop safe, inexpensive, and effective primary prevention strategies for stroke to halt or reverse the rapidly rising trend of stroke in China and many other developing countries (4), given that about 77% of strokes are first events (5,6).

Platelets play an important role in the pathogenesis of vascular disease (7). Previous relevant studies have examined the role of platelets in the acute phase of stroke (8,9). Only 1 prospective study has investigated the relationship between platelets and risk of

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ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2018.02.072

incident stroke (10), and no studies have examined the association of platelet count (PLT) with first stroke.

A recognized mechanism by which platelets contribute to the development of atherosclerosis is by interacting with endothelium or connective tissue secondary to endothelial injury. In the normal physiological state, endothelial cells can inhibit platelet adherence by their capacity to produce antithrombotic substances (e.g., prostacyclin and heparin) (11). It is possible that various forms of endothelial injury may promote platelet adherence and the release of platelet constituents, as in, for example, the presence of elevated total homocysteine (tHcy) (12,13). As such, low PLT could be a marker of endothelial injury and platelet adherence.

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Elevated tHcy is a known risk factor of endothelium injury, atherosclerosis, and cardiovascular disease (13–16). In animal models, experimental homocysteinemia in baboons caused a spectrum of endothelial injury, ranging from alterations in cell-surface constituents to increased turnover (nondenuding injury) or, after extreme injury, loss of endothelial cover (17). The atherogenic mechanism of homocysteinemia has been demonstrated in a primate model by measuring endothelial cell loss and regeneration, platelet consumption, and intimal lesion formation (18). It is conceivable that platelets and tHcy may enhance each other, thereby jointly affecting an individual's risk of stroke. However, to date, there are no adequately powered prospective studies to examine the joint association of PLT and elevated tHcy with the risk of first stroke.

From the standpoint of primary prevention of stroke, there is considerable interest as to whether folic acid (a synthetic form of folate, an essential B vitamin) supplementation can effectively reduce the risk of stroke among those individuals characterized by low PLT and high tHcy levels, particularly among populations residing in regions with low folate intake and without mandatory folic acid fortification of grain products. This interest was heightened by the main finding of the CSPPT (China Stroke Primary Prevention Trial), which showed that folic acid treatment could reduce the risk of first stroke by 21% in hypertensive men and women (19). Given that folic acid can effectively lower tHcy, can improve endothelial function, and has antioxidant properties, it is biologically plausible that folic acid treatment may be particularly effective under the conditions of low PLT and high tHcy (20,21).

ABBREVIATIONS AND ACRONYMS

MPV = mean platelet volume

MTHFR = methylenetetrahydrofolate reductase

PLT = platelet count

tHcy = total homocysteine

Using data from the CSPPT, a high-risk population for low folate, high tHcy, and stroke, the present study sought to address 2 related questions:

1. What is the prospective association between baseline PLT and tHcy levels and first stroke risk in Chinese hypertensive adults? We hypothesized that low PLT and elevated tHcy can jointly increase the risk of first stroke; and
2. Provided our hypothesis is correct, can folic acid treatment reduce the risk of first stroke associated with low PLT and high tHcy levels? We hypothesized that this subgroup would particularly benefit from folic acid treatment.

METHODS

STUDY DESIGN AND SETTING. All participants were part of the CSPPT (NCT00794885). Our paper adheres to the American Heart Association Journal's implementation of the Transparency and Openness Promotion Guidelines. The CSPPT was approved by the ethics committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China (FWA assurance number FWA00001263). All participants provided written informed consent.

The methods and primary results of the CSPPT have been previously reported (19). Briefly, the CSPPT was a multicommunity, randomized, double-blind, controlled trial conducted from May 2008 to August 2013 in 32 communities in China. Eligible participants were men and women age 45 to 75 years who had hypertension, defined as seated resting systolic blood pressure ≥140 mm Hg or diastolic blood

Specialized for "Major New Drugs Innovation and Development" during the 12th Five-Year Plan Period: China Stroke Primary Prevention Trial (grant zx09101105 to Dr. Binyan Wang); the National Key Research and Development Program (2016YFC0903101, 2016YFE0205400 to Dr. Huo); the Projects of the National Natural Science Foundation of China (81402735 to Dr. Qin); the Science and Technology Planning Project of Guangzhou (201707020010 to Dr. Binyan Wang); and the Science, Technology and Innovation Committee of Shenzhen (KQCX20120816105958775, JSGG20160229173428526, JSGG20170412155639040, GJHS20170314114526143, KC2014JSCX0071A to Dr. Binyan Wang). Dr. Qin has received consulting fees from AUSA Research Institute, Shenzhen AUSA. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. This work was presented in part as an abstract by Dr. Xiangyi Kong at the 2017 American College of Cardiology Scientific Session in Washington, DC.

Manuscript received January 22, 2018; revised manuscript received February 23, 2018, accepted February 26, 2018.

pressure ≥90 mm Hg at both the screening and recruitment visit, or who were on antihypertensive medication. The major exclusion criteria included history of physician-diagnosed stroke, myocardial infarction, heart failure, post-coronary revascularization, or congenital heart disease.

Eligible participants were first stratified by methylenetetrahydrofolate reductase (MTHFR) C677T genotypes (CC, CT, or TT). Then, within each genotype group, they were randomly assigned in a 1:1 ratio to receive 1 of 2 treatments: a daily oral dose of 1 tablet containing 10 mg enalapril and 0.8 mg folic acid, or a daily oral dose of 1 tablet containing 10 mg enalapril only. During the trial period, concomitant use of other antihypertensive drugs (mainly calcium-channel blockers or diuretics) was allowed, but not B vitamins. Participants were scheduled for follow-up visits every 3 months.

LABORATORY ASSESSMENT. Overnight fasting venous blood samples were collected at baseline. Laboratory tests were performed at the core laboratory of the National Clinical Research Center for Kidney Disease (Nanfang Hospital, Guangzhou, China) as previously described (19). Baseline complete blood count, including the measurement of PLT, platelet crit, platelet distribution width, and mean platelet volume (MPV), was obtained using a BC-3200 hematology analyzer (Mindray Medical, Shenzhen, China). Fasting glucose, fasting lipids (serum total cholesterol, high-density lipoprotein cholesterol [HDL-C], and triglycerides), serum tHcy, and creatinine were measured using automatic clinical analyzers (Beckman Coulter, Brea, California). An ABI Prism 7900HT sequence detection system (Life Technologies, Carlsbad, California) was used to detect MTHFR gene C677T genotypes. Serum folate and vitamin B12 were measured at baseline by a commercial laboratory using a chemiluminescent immunoassay (New Industrial, Shenzhen, China).

OUTCOMES. As detailed in a previous report (19), the primary outcome was a first nonfatal or fatal stroke (ischemic or hemorrhagic) occurring between baseline and follow-up (a median of 4.2 years), excluding subarachnoid hemorrhage and silent stroke. All stroke cases were adjudicated by experts on the Endpoint Adjudication Committee using pre-defined criteria (19).

COVARIABLES. In the adjusted hazard models, pertinent covariables were included, including age, sex, MTHFR C677T genotypes, systolic and diastolic blood pressure at baseline, mean systolic and diastolic blood pressure over the treatment period, pulse, smoking status, alcohol consumption, body mass index, baseline vitamin B12, folate, glucose, creatinine, total cholesterol, triglycerides, and HDL-C. These covariables were chosen based on our previous CSPPT research findings, and the details regarding covariable definitions have been previously published (19).

STATISTICAL ANALYSES. Data are presented as mean ± SD for continuous variables and as frequency (%) for categorical variables by baseline PLT quartiles. The effects of baseline PLT on first stroke and the effect modification on folic acid efficacy were first evaluated using Kaplan-Meier curves (log-rank test). The hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of first stroke associated with PLT and each subgroup as defined by PLT and tHcy levels were estimated using Cox proportional hazards models with adjustment for pertinent variables. Similarly, the HRs and 95% CIs of first stroke in response to folic acid supplementation across each PLT/tHcy subgroup were estimated and their interactions were tested. A 2-tailed $p < 0.05$ was considered to be statistically significant in all analyses. Data were analyzed using the statistical package R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) and Empower (X&Y Solutions, Inc. Boston, Massachusetts).

RESULTS

The flow chart of the participants is presented in Online Figure 1. Of the 20,702 participants in the CSPPT, our analyses were limited to 10,789 participants from 1 study center (Lianyungang) with baseline measures on PLT and tHcy and with no antiplatelet drug usage. Of those, 5,408 received the enalapril-folic acid treatment, and 5,381 received enalapril alone.

As shown in **Table 1**, participants' baseline characteristics were presented by PLT quartiles (Q): Q1: $<210 \times 10^9$/l; Q2: ≥210 to $<248 \times 10^9$/l; Q3: ≥248 to $<291 \times 10^9$/l; and Q4: $\geq 291 \times 10^9$/l. There were no significant differences in baseline characteristics between each quartile, with the exception of the lowest PLT quartile, which had more men and higher MPV. Within each PLT quartile, the baseline characteristics were comparable between the enalapril-folic acid group and the enalapril group, including age, body mass index, MTHFR genotypes distribution, blood pressure, smoking status, alcohol consumption, self-reported diabetes, fasting lipids, fasting glucose, creatinine, tHcy, vitamin B12, and folic acid.

For those subjects excluded from the analyses due to missing PLT or tHcy values, their baseline characteristics were similar to their counterparts who were included in the analyses (Online Table 1).

TABLE 1 Baseline Characteristics of Study Participants by Platelet Count Quartiles (N = 10,789)

	Platelet Count Quartiles							
	Q1		Q2		Q3		Q4	
	<210 × 10^9/l		≥210–<248 × 10^9/l		≥248–<291 × 10^9/l		≥291 × 10^9/l	
	Enalapril Group (n = 1,354)	Enalapril-Folic Acid Group (n = 1,293)	Enalapril Group (n = 1,334)	Enalapril-Folic Acid Group (n = 1,379)	Enalapril Group (n = 1,317)	Enalapril-Folic Acid Group (n = 1,389)	Enalapril Group (n = 1,376)	Enalapril-Folic Acid Group (n = 1,347)
Male	662 (48.9)	605 (46.8)	564 (42.3)	576 (41.8)	476 (36.1)	498 (35.9)	377 (27.4)	389 (28.9)
Age, yrs	59.9 ± 7.6	59.9 ± 7.5	59.5 ± 7.6	59.1 ± 7.4	59.3 ± 7.8	59.4 ± 7.5	59.2 ± 7.5	59.4 ± 7.7
Body mass index, kg/m^2	25.4 (3.5)	25.4 ± 3.5	25.5 ± 3.6	25.7 ± 3.6	25.7 ± 3.6	25.6 ± 3.6	25.7 ± 3.7	25.8 ± 3.7
MTHFR C677T genotypes								
CC	325 (24.0)	294 (22.7)	306 (22.9)	327 (23.7)	300 (22.8)	343 (24.7)	323 (23.5)	291 (21.6)
CT	664 (49.0)	654 (50.6)	678 (50.8)	682 (49.5)	655 (49.7)	672 (48.4)	677 (49.2)	691 (51.3)
TT	365 (27.0)	345 (26.7)	350 (26.2)	370 (26.8)	362 (27.5)	374 (26.9)	376 (27.3)	365 (27.1)
Mean SBP, mm Hg	140.2 ± 11.9	139.5 ± 11.0	139.7 ± 11.0	139.8 ± 11.6	139.9 ± 11.1	139.3 ± 10.4	140.0 ± 10.9	140.1 ± 11.2
Mean DBP, mm Hg	83.9 ± 7.7	83.6 ± 7.5	84.1 ± 7.5	84.3 ± 7.4	83.8 ± 7.5	83.6 ± 7.0	83.4 ± 7.2	83.5 ± 7.1
Baseline SBP, mm Hg	167.8 ± 21.6	168.2 ± 21.8	168.5 ± 20.6	167.0 ± 20.3	168.1 ± 21.3	167.9 ± 20.3	167.9 ± 20.4	168.0 ± 20.9
Baseline DBP, mm Hg	94.7 ± 12.3	94.6 ± 12.3	95.4 ± 12.2	95.4 ± 11.7	95.4 ± 11.9	95.0 ± 11.4	94.2 ± 11.6	94.9 ± 11.3
Cardiovascular risk factors								
Smoking status								
Never	871 (64.3)	864 (66.8)	911 (68.3)	953 (69.1)	943 (71.6)	990 (71.3)	1,051 (76.4)	1,022 (75.9)
Former	151 (11.2)	119 (9.2)	109 (8.2)	114 (8.3)	93 (7.1)	79 (5.7)	73 (5.3)	82 (6.1)
Current	332 (24.5)	310 (24.0)	314 (23.5)	312 (22.6)	281 (21.3)	320 (23.0)	251 (18.3)	242 (18.6)
Alcohol consumption								
Never	900 (66.5)	898 (69.5)	904 (67.8)	945 (68.5)	962 (73.0)	1,015 (73.1)	1,056 (76.7)	1,023 (76.0)
Former	106 (7.8)	99 (7.7)	101 (7.6)	94 (6.8)	86 (6.5)	82 (5.9)	69 (5.0)	72 (5.3)
Current	347 (25.6)	295 (22.8)	329 (24.7)	340 (24.7)	269 (20.4)	292 (21.0)	251 (18.2)	251 (18.6)
Self-reported diabetes	53 (3.9)	40 (3.1)	57 (4.3)	54 (3.9)	42 (3.2)	43 (3.1)	48 (3.5)	52 (3.9)
Laboratory results								
PLT, × 10^9/l	177.9 ± 27.6	178.0 ± 27.7	228.5 ± 11.0	228.7 ± 10.5	267.9 ± 12.2	267.2 ± 12.1	351.8 ± 139.3	351.4 ± 108.9
PCT	0.1 (0.0)	0.1 (0.0)	0.2 (0.0)	0.2 (0.0)	0.2 (0.0)	0.2 (0.0)	0.3 (0.1)	0.3 (0.1)
MPV, fl	7.9 ± 0.7	7.9 ± 0.7	7.6 ± 0.6	7.7 ± 0.6	7.5 ± 1.7	7.5 ± 0.6	7.4 ± 0.6	7.5 ± 0.7
PDW, fl	15.7 ± 0.3	15.7 ± 0.3	15.5 ± 0.5	15.6 ± 0.3	15.5 ± 1.1	15.5 ± 0.3	15.4 ± 0.4	15.5 ± 0.4
Total cholesterol, mmol/l	5.4 ± 1.1	5.4 ± 1.1	5.6 ± 1.1	5.7 ± 1.2	5.8 ± 1.2	5.8 ± 1.2	5.8 ± 1.2	5.8 ± 1.2
Triglycerides, mmol/l	1.6 ± 0.9	1.6 ± 1.0	1.7 ± 0.9	1.7 ± 1.1	1.8 ± 1.1	1.8 ± 2.7	1.8 ± 1.0	1.8 ± 1.0
HDL-C, mmol/l	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4
Fasting glucose, mmol/l	6.1 ± 1.8	5.9 ± 1.6	6.1 ± 1.8	6.1 ± 1.9	6.1 ± 1.8	6.1 ± 1.9	6.0 ± 1.7	6.1 ± 1.8
Creatinine, μmol/l	67.5 ± 18.9	67.3 ± 20.6	64.9 ± 14.6	66.6 ± 20.0	63.8 ± 15.3	64.1 ± 25.8	61.2 ± 13.4	63.0 ± 19.1
tHcy, μmol/l	14.8 ± 8.6	14.9 ± 8.8	14.9 ± 9.6	14.8 ± 9.2	14.5 ± 9.0	14.6 ± 8.5	14.2 ± 8.2	14.7 ± 9.1
Vitamin B_{12}, pg/ml	400.2 ± 173.5	392.3 ± 163.2	397.6 ± 157.4	407.1 ± 162.8	405.8 ± 157.7	394.1 ± 136.6	416.2 ± 209.2	400.8 ± 162.6
Folic acid, ng/ml	7.9 ± 3.4	7.7 ± 3.2	7.8 ± 3.0	7.9 ± 3.1	7.9 ± 3.3	7.8 ± 3.2	7.8 ± 3.6	7.8 ± 3.2

Values are n (%) or mean ± SD.

DBP = diastolic blood pressure; HDL-C = high-density lipid cholesterol; MPV = mean platelet volume; MTHFR = methylenetetrahydrofolate reductase; PCT = platelet crit; PDW = platelet distribution width; PLT = platelet count; SBP = systolic blood pressure; tHcy = total homocysteine.

PLATELET COUNT AND EFFICACY OF FOLIC ACID IN PREVENTION OF FIRST STROKE. Among the 10,789 participants included in the analyses, a total of 371 first strokes occurred over the median treatment duration of 4.2 years, including 210 in the enalapril group and 161 in the enalapril-folic acid group. For those who did not receive folic acid treatment (the enalapril group), participants with low PLT (Q1) showed the highest risk of first stroke (4.6%) compared to those in the higher PLT quartiles (3.7%) (Table 2).

Online Figure 2 shows Kaplan-Meier curves illustrating the cumulative event rate of first stroke by the 2 treatment groups, stratified by PLT quartiles. In the lowest quartile of PLT (Q1), the risk of stroke incidence was substantially reduced in the enalapril-folic acid group compared with the enalapril group. For the remaining 3 quartiles of PLT (Q2 to Q4), there was no significant difference in the risk of stroke incidence between the 2 treatment groups.

Table 2 further quantifies the effect modification of PLT on efficacy of folic acid treatment in stroke

TABLE 2 Effect Modification of PLT on Folic Acid Supplementation in Prevention of First Stroke

	Enalapril Group		Enalapril-Folic Acid Group		Crude		p Value* for Interaction	Adjusted†		p Value* for Interaction
	Total	Events (%)	Total	Events (%)	HR (95% CI)	p Value		HR (95% CI)	p Value	
Total stroke										
PLT Q1 (<210 × 10^9/l)	1,354	62 (4.6)	1,293	24 (1.9)	0.40 (0.25–0.64)	<0.001	Ref	0.42 (0.26–0.68)	<0.001	Ref
PLT Q2–Q4	4,027	148 (3.7)	4,115	137 (3.3)	0.91 (0.72–1.14)	0.410	0.002	0.90 (0.71–1.14)	0.382	0.004
PLT Q2 (≥210-<248 × 10^9/l)	1,334	44 (3.3)	1,379	43 (3.1)	0.95 (0.62–1.44)	0.794		0.97 (0.63–1.49)	0.884	
PLT Q3 (≥248-<291 × 10^9/l)	1,317	48 (3.6)	1,389	44 (3.2)	0.87 (0.58–1.31)	0.508		0.92 (0.61–1.40)	0.694	
PLT Q4 (≥291 × 10^9/l)	1,376	56 (4.1)	1,347	50 (3.7)	0.91 (0.62–1.34)	0.645		0.86 (0.57–1.28)	0.460	
Ischemic stroke										
PLT Q1	1,354	55 (4.1)	1,293	21 (1.6)	0.39 (0.24–0.65)	<0.001	Ref	0.43 (0.26–0.72)	0.001	Ref
PLT Q2–Q4	4,027	126 (3.1)	4,115	120 (2.9)	0.93 (0.73–1.20)	0.591	0.002	0.94 (0.73–1.21)	0.637	0.007
PLT Q2	1,334	38 (2.8)	1,379	38 (2.8)	0.97 (0.62–1.52)	0.891		0.98 (0.61–1.55)	0.918	
PLT Q3	1,317	42 (3.2)	1,389	39 (2.8)	0.88 (0.57–1.36)	0.573		0.93 (0.60–1.45)	0.746	
PLT Q4	1,376	46 (3.3)	1,347	43 (3.2)	0.96 (0.63–1.45)	0.842		0.95 (0.61–1.47)	0.806	
Hemorrhagic stroke										
PLT Q1	1,354	7 (0.5)	1,293	3 (0.2)	0.45 (0.12–1.73)	0.243	Ref	0.32 (0.06–1.61)	0.166	Ref
PLT Q2–Q4	4,027	22 (0.5)	4,115	16 (0.4)	0.71 (0.37–1.36)	0.302	0.531	0.67 (0.35–1.30)	0.239	0.367
PLT Q2	1,334	6 (0.4)	1,379	5 (0.4)	0.81 (0.25–2.65)	0.724		0.82 (0.24–2.82)	0.750	
PLT Q3	1,317	6 (0.5)	1,389	5 (0.4)	0.79 (0.24–2.58)	0.694		0.85 (0.25–2.86)	0.790	
PLT Q4	1,376	10 (0.7)	1,347	6 (0.4)	0.61 (0.22–1.69)	0.343		0.46 (0.15–1.37)	0.164	

*p value for interaction test: 2-way interaction of PLT (Q1 vs. Q2–Q4) and treatment groups (enalapril vs. enalapril + folic acid) on first stroke. †Adjusted for age, sex, MTHFR C677T genotypes, systolic and diastolic blood pressure at baseline, mean systolic and diastolic blood pressure over the treatment period, pulse, smoking status, alcohol consumption, body mass index, baseline vitamin B_{12}, folate, total homocysteine, glucose, creatinine, total cholesterol, triglycerides, and HDL-C. The enalapril group is the reference group.

CI = confidence interval; HR = hazard ratio; PLT = platelet count; Q = quartile.

prevention, accounting for important covariates. For those with folic acid treatment (compared with the enalapril group), the stroke incidence was reduced from 4.6% to 1.9% in the low PLT quartile (Q1), representing an absolute risk reduction of 2.7% and a relative risk reduction of 58% in the adjusted model (HR: 0.42; 95% CI: 0.26 to 0.68; $p < 0.001$). In contrast, the stroke risk reduction in the high PLT quartiles (Q2 to Q4) was modest (HR: 0.90; 95% CI: 0.71 to 1.14; $p = 0.382$). A test of interaction between PLT and folic acid treatment on first stroke was statistically significant ($p = 0.004$).

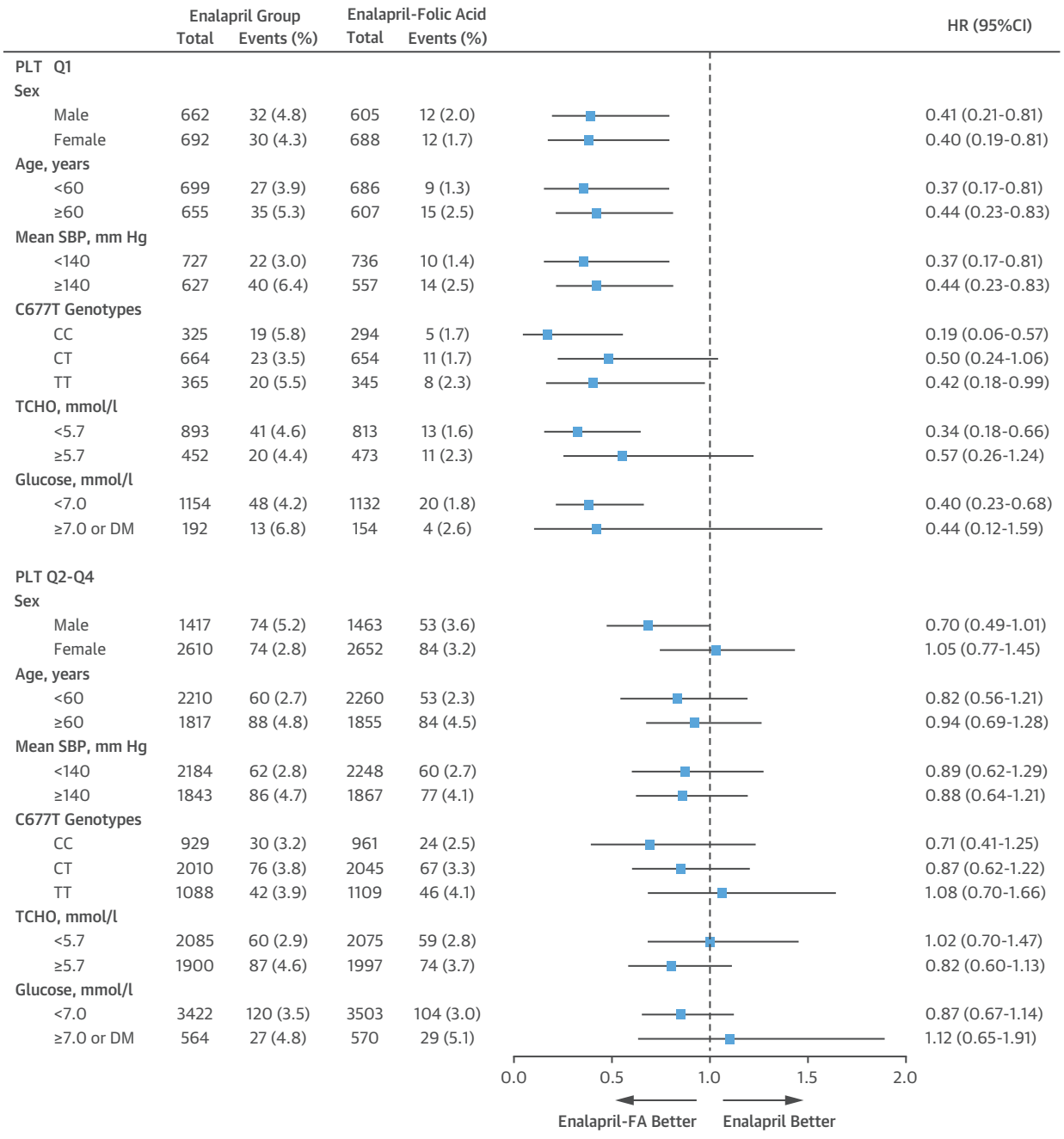
We performed similar analyses for 2 subtypes of stroke: ischemic and hemorrhagic stroke. For ischemic stroke, the adjusted HR for PLT (Q1) was 0.43 (95% CI: 0.26 to 0.72; $p = 0.001$). Again, a significant interaction between PLT and folic acid treatment on ischemic stroke was observed ($p = 0.007$). However, for hemorrhagic stroke, the sample size was too small to perform a meaningful analysis.

Given the above results showing distinct patterns between PLT Q1 and PLT Q2 to Q4 in terms of the association with stroke and effect modification on folic acid efficacy, in the subsequent analyses, PLT was dichotomized into low (Q1) and high (Q2 to Q4) (Online Table 2).

STRATIFIED ANALYSES BY IMPORTANT COVARIABLES. To further confirm that the findings observed in Table 2 are robust to potential confounders, we performed stratified analyses by subgroups defined by major covariables known to affect stroke risk, including sex, age, mean systolic blood pressure over the treatment period, MTHFR C677T genotypes, baseline fasting total cholesterol, and glucose. All analyses adjusted for age, sex, MTHFR C677T genotypes, systolic and diastolic blood pressure at baseline, mean systolic and diastolic blood pressure over the treatment period, pulse, smoking status, alcohol consumption, body mass index, baseline vitamin B12, folate, tHcy, glucose, creatinine, total cholesterol, triglycerides, and HDL-C, except for the variable that was stratified. Figure 1 reveals a highly consistent pattern: among patients with low PLT (Q1), regardless of subgroup, folic acid treatment resulted in a significant reduction in first stroke risk, with HRs ranging from 0.19 to 0.57. In contrast, among patients with higher PLT (Q2 to Q4), the efficacy of folic acid was greatly attenuated, with HRs ranging from 0.70 to 1.12.

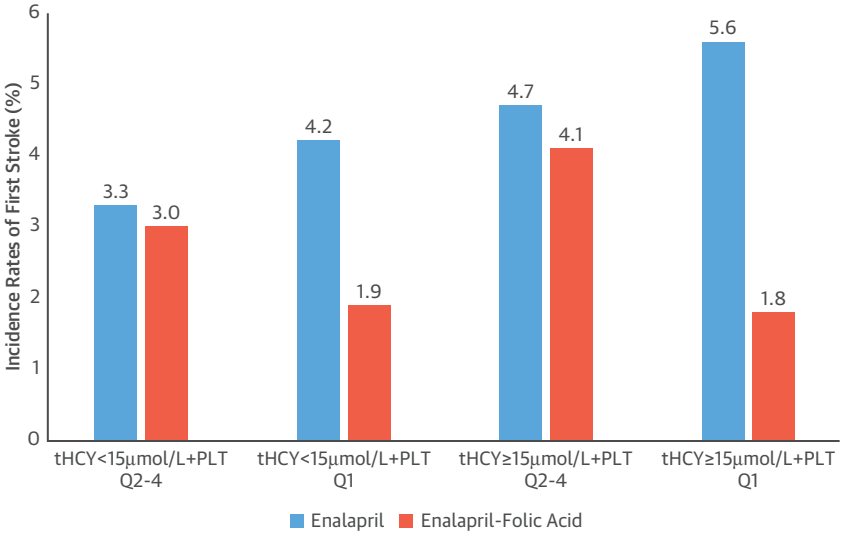
JOINT EFFECT OF PLT AND HOMOCYSTEINE LEVELS. Figure 2 shows that the incidence of first stroke varied significantly among subgroups defined by PLT, tHcy level, and folic acid treatment group. In the enalapril group, the lowest incident rate of first stroke (3.3%) was found in patients with high PLT (Q2 to Q4) and low tHcy (<15 μmol/l), whereas the highest rate (5.6%) was found in those with low PLT (Q1) and

FIGURE 1 **Subgroup Analyses on Effect Modification of PLT on Folic Acid Supplementation in Prevention of First Stroke**



The multivariate model adjusted for age, sex, MTHFR C677T genotypes, systolic and diastolic blood pressure, blood pressure at baseline, mean systolic and diastolic blood pressure over the treatment period, pulse, smoking status, alcohol consumption, body mass index, baseline vitamin B12, folate, total homocysteine, glucose, creatinine, total cholesterol, triglycerides, and HDL-C, except for the variable that is stratified. None of the stratified variables, including sex, age, mean SBP, MTHFR C677T genotype, total cholesterol and glucose, significantly modified the effect of folic acid treatment on first stroke; the p values of all interaction test were >0.1. DBP = diastolic blood pressure; DM = diabetes mellitus; PLT = platelet count; SBP = systolic blood pressure; TCHO = total cholesterol.

FIGURE 2 Incidence Rates of First Stroke in Subgroups Defined By PLT, tHcy Level, and Folic Acid Treatment Group



PLT = platelet count; tHcy = total homocysteine.

high tHcy (≥15 μmol/l). Following folic acid treatment, the risk of first stroke was reduced from 5.6% to 1.8% among patients with low PLT and high tHcy. In contrast, folic acid treatment had no effect on incident stroke among those with high PLT (Q2 to Q4) and low tHcy.

As shown in **Table 3**, to examine the robustness of the joint effect of tHcy and PLT on first stroke, we used 3 different cutoffs for elevated tHcy: ≥10, ≥12, and ≥15 μmol/l, corresponding to various cutoffs used in the published data; the corresponding percentage of the participants who met the cutoffs were 78%, 55%, and 29%, respectively. Regardless of the tHcy cutoffs, the overall pattern remained the same. Using the tHcy >15 μmol/l cutoff as an example, compared with the enalapril group, folic acid treatment was most effective and reduced the risk of first stroke by 73% (HR: 0.27; 95% CI: 0.11 to 0.64; p = 0.003) among patients with high tHcy and low PLT. In contrast, the risk reduction was modest or nonsignificant among other subgroups. A test of interaction between this PLT/tHcy subgroup and folic acid treatment on first stroke was statistically significant (p = 0.009).

DISCUSSION

To our knowledge, this is by far the largest study to demonstrate the prospective association between baseline PLT and tHcy levels and future risk of first stroke. We observed remarkable differences in the risk of first stroke among subgroups as defined by PLT and tHcy levels. Within the enalapril group (without intervention), the lowest rate of first stroke (3.3%) was observed in the high PLT (Q2 to Q4) and low tHcy (<15 μmol/l) subgroup, whereas the highest rate (5.6%) was observed in the low PLT (Q1) and high tHcy subgroup. We also found a dramatic variability in the efficacy of folic acid treatment (0.8 mg daily) across subgroups. The greatest risk reduction in first stroke for those with folic acid treatment was observed in the low PLT and high tHcy subgroup (from 5.6% to 1.8%), a risk reduction of 73% (HR: 0.27; 95% CI: 0.11 to 0.64; p = 0.003). In contrast, folic acid treatment had no effect on first stroke in the high PLT and low tHcy subgroup. A similar association was found for ischemic stroke, which was the majority of stroke cases in the CSPPT. Taken together, our data indicate that among Chinese hypertensive adults, the subgroup with low PLT and high tHcy had the highest risk of first stroke, and this risk was substantially reduced by folic acid treatment. These findings, if confirmed, would help identify those individuals who are at high risk of first stroke and who would particularly benefit from folic acid treatment.

COMPARISONS WITH PREVIOUS STUDIES. Our study differed from previous studies in several important aspects. First, in terms of study design, our study utilized data from the CSPPT, the largest folic acid intervention trial on the primary prevention of stroke (19). Not only were we able to assess the prospective association between baseline PLT and first stroke risk,

TABLE 3 Effect Modification of PLT on Folic Acid Supplementation in Prevention of First Stroke, Stratified By tHcy Levels

	Enalapril Group		Enalapril-Folic Acid Group		Adjusted Model 1*		p Value† for interaction	Adjusted Model 2*		p Value† for interaction
	Total	Events (%)	Total	Events (%)	HR (95% CI)	p Value		HR (95% CI)	p Value	
tHcy low (<10 μmol/l)										
PLT Q1	250	11 (4.4)	244	5 (2.0)	0.54 (0.15–1.93)	0.339		0.54 (0.15–1.93)	0.339	
PLT Q2–Q4	911	25 (2.7)	925	26 (2.8)	1.04 (0.58–1.85)	0.899	0.341	1.04 (0.58–1.85)	0.900	0.342
tHcy high (≥10 μmol/l)										
PLT Q1	1,104	51 (4.6)	1,049	19 (1.8)	0.39 (0.22–0.67)	<0.001		0.39 (0.22–0.67)	<0.001	
PLT Q2–Q4	3,116	123 (3.9)	3,190	111 (3.5)	0.88 (0.68–1.14)	0.334	0.006	0.88 (0.68–1.14)	0.333	0.006
tHcy low (<12 μmol/l)										
PLT Q1	584	20 (3.4)	543	9 (1.7)	0.57 (0.25–1.30)	0.181		0.57 (0.25–1.32)	0.193	
PLT Q2–Q4	1,873	48 (2.6)	1,839	50 (2.7)	1.07 (0.71–1.61)	0.742	0.175	1.08 (0.71–1.62)	0.729	0.183
tHcy high (≥12 μmol/l)										
PLT Q1	770	42 (5.5)	750	15 (2.0)	0.36 (0.19–0.66)	0.001		0.36 (0.19–0.66)	0.001	
PLT Q2–Q4	2,154	100 (4.6)	2,276	87 (3.8)	0.84 (0.62–1.12)	0.238	0.011	0.84 (0.62–1.12)	0.238	0.011
tHcy low (<15 μmol/l)										
PLT Q1	963	40 (4.2)	907	17 (1.9)	0.49 (0.27–0.89)	0.019		0.49 (0.27–0.89)	0.019	
PLT Q2–Q4	2,909	95 (3.3)	2,917	88 (3.0)	0.90 (0.67–1.21)	0.483	0.071	0.90 (0.67–1.21)	0.485	0.070
tHcy high (≥15 μmol/l)										
PLT Q1	391	22 (5.6)	386	7 (1.8)	0.28 (0.12–0.67)	0.004		0.27 (0.11–0.64)	0.003	
PLT Q2–Q4	1,118	53 (4.7)	1,198	49 (4.1)	0.90 (0.61–1.34)	0.614	0.012	0.90 (0.60–1.34)	0.611	0.009

*Model 1 was adjusted for age, sex, MTHFR C677T genotypes, systolic and diastolic blood pressure at baseline, mean systolic and diastolic blood pressure over the treatment period, pulse, smoking status, alcohol consumption, body mass index, baseline vitamin B12, folate, glucose, creatinine, total cholesterol, triglycerides, and HDL-C. Enalapril group is the reference group. Model 2 was adjusted for all covariables in model 1 plus MPV. Enalapril group is the reference group. †p value for interaction test: 2-way interaction of PLT (Q1 vs. Q2–Q4) and treatment groups (enalapril vs. enalapril-folic acid) on first stroke.

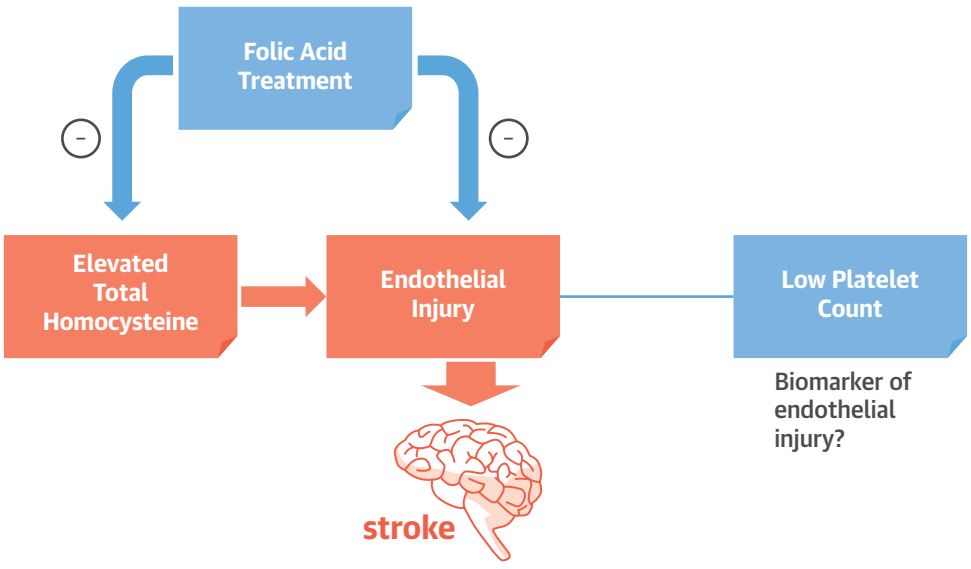
tHcy = total homocysteine; other abbreviations as in Tables 1 and 2.

but we were also able to evaluate whether PLT can modify the efficacy of folic acid treatment. Second, the characteristics of the study population were unique, in that the CSPPT consisted of Chinese hypertensive adults, a population with high prevalence of elevated tHcy compared with western populations. High tHcy levels in this population are due to a combination of reasons including: no mandatory folic acid fortification of grain products in China; insufficient consumption of folate-containing foods; a low rate of folic acid supplementation; and a high prevalence of the MTHFR C677T gene mutation (25% in China vs. 10% to 12% in the general U.S. population) (22,23). As such, our study offers a unique setting to examine the effect of low PLT and elevated tHcy (a high-risk condition leading to endothelial injury, platelet adherence and consumption, and atherosclerosis) on stroke risk. Third, our study, by focusing on first stroke, was less likely confounded by preexisting cardiovascular conditions and medication usage, due to the exclusion of those participants with prior stroke and major cardiovascular diseases, and very low antiplatelet and cholesterol-lowering drug usage among the CSPPT participants. Last, this is by far the first and largest study of its kind focusing on first stroke; such data are critically needed for developing primary prevention strategies for stroke.

In the platelet cohort study conducted by van der Bom et al. (10), the investigators observed 5,766 elderly patients over a 12- to 15-year period. PLT was categorized into quintiles (48 [lowest observed value] to 99 $\times$ 10^9,100 to 199 $\times$ 10^9, 200 to 299 $\times$ 10^9, 300 to 399 $\times$ 10^9, and 400 to 1,223 $\times$ 10^9 [highest observed value]) (10). The authors reported that PLT had no association with either ischemic or hemorrhagic stroke. However, they did not examine this association in the context of tHcy levels or other high-risk conditions predisposing to endothelial injury. As demonstrated by our study, in the presence of elevated tHcy, low PLT becomes an important marker of increased risk of first stroke and serves as an indicator of greater efficacy of folic acid treatment.

POTENTIAL MECHANISMS. We do not know the exact mechanisms by which low PLT and elevated tHcy may jointly increase first stroke risk, nor do we fully understand why folic acid treatment may be effective in reducing stroke in this subgroup. However, our findings appear to be consistent with previous experimental evidence in animal models. More than 30 years ago, the studies by Harker et al. (17) demonstrated the important role of platelets, endothelium, and homocysteine in the pathogenesis of atherosclerosis. It appears that atherosclerosis may be a result of concerted actions of multiple factors when platelets interact with damaged endothelium

CENTRAL ILLUSTRATION **Platelet Count and Stroke in the Context of Elevated Homocysteine and Folic Acid Treatment Among Hypertensive Patients**



Kong, X. et al. J Am Coll Cardiol. 2018;71(19):2136–46.

Elevated total homocysteine (tHcy) is a known risk factor of endothelium injury, atherosclerosis, and cardiovascular disease. Low platelet count could be a marker of endothelial injury due to platelet adherence and consumption. Folic acid can effectively lower tHcy, and also has antioxidant and anti-inflammatory properties, thus improving endothelial function. It is biologically plausible that folic acid treatment may be particularly effective in stroke prevention among hypertensive patients with elevated tHcy and low platelet count.

or connective tissue. The atherogenic mechanism of homocysteinemia has been illustrated by its effect on endothelial injury (as measured by endothelial cell loss and regeneration), platelet consumption, and intimal lesion formation in a primate model (18).

As depicted in the **Central Illustration**, although human experimental data are lacking, our findings on the joint effect of low PLT and high tHcy on increased stroke risk is in line with the findings in animal models and suggests the possibility that a combination of low PLT and high tHcy may be a marker for endothelial injury, platelet adherence, and consumption. This speculation is further supported by the elevated MPV found in those in the low PLT group in our study (Online Figure 3). MPV is a physiological variable of hemostatic importance (17). Large platelets are more reactive, produce more prothrombotic factors, and aggregate more easily (8,9,18).

It is well-known that folic acid supplementation can effectively lower tHcy levels (24). The CSPPT was the first randomized clinical trial to reveal that among hypertensive populations without folic acid fortification, folic acid supplementation can reduce stroke risk by 21% on average (19). Our present study further showed that folic acid supplementation can reduce first stroke risk by 73% among individuals with low PLT and high tHcy. Such a remarkable stroke risk reduction in this subgroup further supports our hypotheses and strengthens the biological plausibility of our findings.

In addition to its tHcy-lowering effect, FA may have other beneficial effects due to its multiple mechanisms of action. It plays an important role in deoxyribonucleic acid synthesis, repair, and methylation (25). 5-methyltetrahydrofolate could increase the bioavailability of tetrahydrobiopterin, helping to maintain endothelial nitric oxide synthase in its coupled state to favor the generation of NO (26). Moreover, folic acid can exert antioxidative and anti-inflammatory effects (27,28).

STUDY STRENGTHS AND LIMITATIONS. Despite many strengths, our study has the following limitations. We only examined baseline PLT. In future studies, platelet parameters during the follow-up period should also be examined. We did not have data on flow-mediated dilation (a noninvasive assessment of vascular endothelial function in humans) (29). However, findings from previous

meta-analyses of related randomized trials suggest that folic acid supplementation significantly improves endothelial dysfunction as assessed by flow-mediated dilation (30,31).

The CSPPT chose a fixed dosage of 0.8 mg of FA. Thus, we were unable to evaluate whether higher dosages of FA have additional beneficial effects. In addition, in the CSPPT, folic acid (a synthetic form of folate) was used. In contrast, 5-methyltetrafolate, which has a closer resemblance to naturally occurring folate, has been shown to be effective in tHcy-lowering, but without the potential disadvantage of masking anemia due to vitamin B12 deficiency or increasing the levels of unmetabolized folic acid (32). Whether 5-methyltetrafolate is more effective than folic acid in stroke prevention, particularly in participants with the MTHFR 677 TT genotype, remains to be investigated in future trials.

As this is a post hoc analysis, residual confounding cannot be completely eliminated. This study focused on hypertensive adults in a low folate setting, and the generalizability of our results to nonhypertensive adults in folic acid fortified regions remains to be determined. Finally, we would like to underscore that our findings serve as hypothesis generating. In fact, they raise important questions that may prompt additional clinical and mechanistic investigations to help determine such factors as the optimal folate dosage (given our study only used a fixed dose of 0.8 mg folic acid daily) and the biological mechanisms underlying our observations.

CONCLUSIONS

Among Chinese hypertensive adults from the CSPPT, we observed that the subgroup with low PLT and high tHcy at baseline had the highest risk of first stroke over the 4.2 years of follow-up, and this risk was reduced by 73% with folic acid treatment.

Our findings, if confirmed, have enormous clinical and public health implications, given the rising incident rate of stroke in developing countries, including China. Our data suggest that identifying those patients with a combination of low PLT and high tHcy (both biomarkers are easy to obtain) could help detect those individuals who are at high risk of stroke and who would particularly benefit from folic acid supplementation, a treatment that is simple, safe, and inexpensive.

ACKNOWLEDGMENTS The authors thank the investigators and participants of the CSPPT, the parent study, who made this report possible.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Chinese patients with hypertension, low blood PLTs, and elevated total serum homocysteine levels face an increased risk of first stroke, and folic acid supplementation can substantially reduce this risk.

TRANSLATIONAL OUTLOOK: Additional clinical trials are needed to assess the generalizability of the benefit of folic acid supplementation for stroke prevention to other patient populations.

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KEY WORDS FA supplementation, homocysteine, hypertensive adults, platelet, stroke

APPENDIX For supplemental tables and figures, please see the online version of this paper.