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Original Article

Relationship between acute stroke outcome, aspirin resistance, and humoral factors

Ping-Tsun Lai ^a, Shu-Yi Chen ^{b,c}, Yu-Sun Lee ^c, Yu-Ping Ho ^c,
Yi-Ying Chiang ^{b,c}, Hung-Yi Hsu ^{a,c,d,*}

^a Department of Internal Medicine, Tungs' Taichung Metro Harbor Hospital, Taichung, Taiwan, ROC

^b Department of Internal Neurology, Tungs' Taichung Metro Harbor Hospital, Taichung, Taiwan, ROC

^c Department of Neurology, Taichung Veterans General Hospital, Taichung, Taiwan, ROC

^d Department of Neurology, School of Medicine, Chung Shan Medical University, Taichung, Taiwan, ROC

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Abstract

Background: The relationship between biochemical aspirin resistance (AR) and functional outcome of acute ischemic stroke is uncertain.

Methods: Prospectively, 269 patients with acute ischemic stroke were recruited. Their responsiveness to aspirin was evaluated by platelet function analyzer (PFA-100). All patients received blood tests for fibrinogen, high-sensitivity C-reactive protein (hs-CRP), CD40-ligand, P-selectin, intercellular adhesion molecule -1, von Willebrand factor (vWF), and D-dimer. The patients' National Institutes of Health Stroke Scale and modified Rankin Scale scores were recorded on admission, at 30 days, and at 90 days after stroke.

Results: Closure-time measured by PFA-100 equipped with epinephrine/collagen cartridge (Epi-CT) was <193 seconds (defined as AR) in 83 patients (30.9%). Patients with AR were less likely to have favorable outcome at 30 days (47.0%, $p = 0.047$; odds ratio: 0.69, 0.48–0.99) and 90 days (57.8%, $p = 0.037$; odds ratio: 0.69, 0.47–0.97) after stroke compared with those of patients without AR (60.2% and 71.0%, respectively). The Epi-CT correlated with closure-time measured by adenosine diphosphate/collagen cartridge ($r = 0.241$, $p < 0.001$), blood white cell count ($r = -0.125$, $p = 0.041$), low density lipoprotein cholesterol ($r = 0.120$, $p = 0.050$), hs-CRP ($r = -0.150$, $p = 0.015$), vWF ($r = -0.134$, $p = 0.028$), and body mass index ($r = 0.143$, $p = 0.019$). Multivariate logistic regression analysis showed that higher National Institutes of Health Stroke Scale at admission, atrial fibrillation, increased plasma levels of hs-CRP, and D-dimer were independent predictors for unfavorable stroke outcome at 90 days.

Conclusion: Aspirin resistance evaluated by PFA-100 test was associated with unfavorable 90-day outcome. However, AR determined by PFA-100 dose not predict 90-day functional outcome. The results of PFA-100 testing represented a complex interaction between drug effect, inflammatory reaction, and prothrombotic activity.

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Keywords: acute stroke; aspirin; outcome; platelet aggregation; platelet function tests

1. Introduction

Platelet activation is a crucial mechanism in the pathophysiology of ischemic stroke.^{1–3} Activated platelets release various mediators and chemokines to recruit more activated

platelets and leukocytes, which, in turn, accentuate local thrombogenesis and inflammatory responses.^{1,3} Propagation of thrombosis and inflammation might cause more injury to the ischemic brain tissue and lead to poor outcomes.² Several markers of platelet activation were linked to poor outcomes of acute ischemic stroke.^{2,4,5} Inhibition of platelet function is an important therapeutic strategy in ischemic vascular diseases. Aspirin is an important and effective therapeutic strategy in secondary prevention of atherothrombotic events in acute ischemic stroke.⁶ The bioavailability and responsiveness of

* Corresponding author. Dr. Hung-Yi Hsu, Department of Internal Medicine, Tungs' Taichung Metro Harbor Hospital, 699, Section 1, Zhongci Road, Wuci District, Taichung 435, Taiwan, ROC.

E-mail address: hungyihsu@gmail.com (H.-Y. Hsu).

aspirin is variable among different individuals. Previous studies showed that 30%–40% of patients who experienced ischemic stroke were taking aspirin at the time of their events.⁷ The so-called aspirin resistance (AR) or aspirin unresponsiveness is widely discussed in the literature, though few studies focused on acute ischemic stroke patients.^{7–10} Effective antithrombotic management of acute ischemic stroke depends on a better understanding of AR and its potential influences on stroke outcomes.

The usefulness of *in vitro* tests for AR remains undetermined.¹¹ Among various platelet function tests, closure-time (CT) measured by platelet function analyzer-100 (PFA-100, Dade Behring, Marburg, Germany) equipped with collagen/epinephrine cartridge is able to evaluate the response to aspirin and is a point-of-care test among different tests of platelet function.^{12,13} Aspirin resistance determined by PFA-100 has shown that AR was associated with a higher risk of future cardiovascular events.^{14,15} Aspirin resistance assessed by PFA-100 occurred in approximately 15%–60% of patients with ischemic stroke, depending on the age, the history of diabetes and hypertension, and the acuteness of the ischemic event.^{7,16,17} Recent studies demonstrate that CT measured by PFA-100, as an indicator of platelet functions, might help in guiding antiplatelet therapy.¹⁸ The role of PFA in assessing platelet activation during the acute stage of ischemic stroke has only been evaluated in a few studies.^{7,9,10} The prognostic significance of AR in acute stroke patients is also rarely reported.¹⁰ Whether AR assessed by PFA-100 or humoral markers of platelet activation during the acute stroke period could predict patient outcome, or the likelihood of recurrent ischemic events is of interest. We conducted this study to evaluate the relationship between AR, plasma markers associated with platelet activation, and outcomes in patients with acute ischemic stroke.

2. Methods

2.1. Participants

From Feb 1, 2005, to July 31, 2006, a total of 347 consecutive patients who were admitted to this hospital due to ischemic stroke within 7 days after onset were prospectively screened by experienced neurologists. All stroke patients fulfilled the World Health Organization criteria of stroke and had no evidence of hemorrhage on their cranial computed tomography or magnetic resonance imaging. Patients were excluded due to: (1) having used anticoagulants for secondary prevention (29 patients), (2) having contraindication for aspirin use (21 patients), (3) other known causes of ischemic stroke (arterial dissection in four patients, protein C deficiency in two patients, anticardiolipin syndrome in two patients, hypercoagulation state due to malignancy in one patient), (4) severe renal or hepatic diseases (10 patients), (5) hematocrit less than 30% (11 patients), (6) platelet count less than 100,000/cumm limbs (five patients), and (7) allergy to aspirin (three patients). After screening was completed, 269 patients were enrolled into this study. The study was approved by the

institutional review committee of this hospital and all enrolled participants gave informed consent before entering this study. We recorded patient medical histories, cardiovascular risk factors, and currently used medications. Ancillary tests (chest radiography, electrocardiography, complete blood cell count, prothrombin time, activated partial thromboplastin time, fibrinogen, fasting plasma sugar, and routine biochemical tests) were further performed on all studied patients. In addition, all recruited patients received duplex sonography of cervical arteries and transcranial color-coded sonography. Echocardiography was performed if there was an abnormal electrocardiographic finding or finding on physical examination suggesting organic cardiac disease. Twenty-four-hour Holter electrocardiography was performed if paroxysmal arrhythmia was clinically suspected. All stroke patients were classified into different stroke subgroups based on results of the aforementioned investigations and criteria adapted from the Trial of Org 10172 in Acute Stroke Treatment (TOAST) study.¹⁹ National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) scores were recorded on admission, 30 days, and 90 days after the index stroke for all recruited patients. The stroke outcome was dichotomized by mRS for statistic analysis. A mRS ≤ 2 was defined as favorable outcome, and a mRS > 2 was defined as unfavorable outcome.

All patients received 100 mg aspirin (standard form or enteric-coated form) per day for at least 5 days before being tested for platelet function and plasma markers. Venous blood was obtained by venipuncture at the forearm by 21-gauge needle for all participants after an overnight fast. Whole blood samples for platelet function study were anticoagulated with 3.8% sodium citrate and were analyzed immediately. After centrifugation, the citrated plasma samples were then stored at -70°C until further measurements were necessary.

Platelet function was studied by using the PFA-100 device. The PFA-100 device measured the time necessary to form a hemostatic platelet plug and to occlude the central hole of an epinephrine/collagen- or adenosine diphosphate/collagen-coated membrane, which is called “closure time” and is related to platelet function.¹² These results were recorded as epinephrine closure-time (Epi-CT) and adenosine diphosphate closure-time (ADP-CT). Patients who had an Epi-CT < 193 seconds were classified as AR.²⁰

Commercial enzyme-linked immunoassay test kits were used to measure plasma concentrations of high sensitive C-reactive protein (hs-CRP), soluble P-selectin, soluble CD40-ligand (sCD40L), soluble intercellular adhesion molecule-1 (ICAM-1), von Willebrand factor (vWF), and D-dimer. Test kits from R&D Systems, Inc. (Minneapolis, MN, USA) were used for measuring P-selectin, sCD40L, hs-CRP, and ICAM-1, and test kits from American Diagnostica, Inc. (Stamford, CT, USA) were used for measuring vWF and D-dimer.

2.2. Statistical analysis

Continuous variables are expressed as mean \pm standard deviation. Nonparametric Mann-Whitney test was used to

compare variables between patients with and without AR, and between patients with favorable outcomes and unfavorable outcomes. Chi-square test was used for evaluating categorical variables. The Spearman rank correlation coefficient was used to evaluate the relationship between Epi-CT and other studied parameters. Univariate and multivariate logistic stepwise regression analysis were used to evaluate the relationship between stroke outcome and studied parameters. Age, sex, and AR were forced into the multivariate regression model in order to assess their influence. All statistical calculations were performed using a commercially available statistical package (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL, USA). A *p* value less than 0.05 was considered statistically significant.

3. Results

The study population consisted of 70 women (26%) and 199 men, and they had a mean age of 68.7 ± 12.2 years (range: 21–91 years). Hypertension was observed in 210 patients (78.1%), diabetes in 99 patients (36.8%), hyperlipidemia in 71 patients (26.4%), history of previous transient ischemic attack or stroke in 141 patients (52.4%), coronary heart disease in 81 patients (30.1%), and current smoking status in 106 patients (39.4%); AR was observed in 83 patients (30.9%). The frequencies of AR in TOAST stroke subtypes

were 33.0% (31 of 93) for atherothrombotic stroke, 42% (eight of 19) for cardioembolic stroke, 27.0% (26 of 95) for small vessel disease, and 29% (18 of 44) for undetermined cause (overall $p < 0.001$). Enteric-coated aspirin was not associated with AR. The demographic characteristics, National Institutes of Health Stroke Scale (NIHSS) at admission, and plasma levels of the previously mentioned factors were not different between patients with and without AR (Table 1). The outcome data at 90 days were not available in three patients without AR. Patients with AR were less likely to have favorable outcome at 30 days (47.0%, $p = 0.047$, odds ratio: 0.69, 0.48–0.99) and 90 days (57.8%, $p = 0.037$, odds ratio: 0.69, 0.47–0.97) after stroke compared with those of patients without AR (60.2% and 71.0%, respectively). Recurrent ischemic stroke occurred in three patients (3.6%) with AR and seven patients (3.8%) without AR ($p = 1.000$) during a 90-day follow-up. The Epi-CT significantly correlated with ADP-CT ($r = 0.241$, $p < 0.001$), white cell count ($r = -0.125$, $p = 0.041$), low density lipoprotein cholesterol ($r = 0.120$, $p = 0.050$), hs-CRP ($r = -0.150$, $p = 0.014$), vWF ($r = -0.134$, $p = 0.028$), and body mass index (BMI; $r = 0.143$, $p = 0.019$).

The clinical and laboratory profiles of patients with favorable outcome and unfavorable outcome are shown in Table 2. Unfavorable stroke outcomes were associated with older age,

Table 1
Comparison of clinical profiles and laboratory data between patients with and without aspirin resistance.

	Aspirin resistance (<i>n</i> = 83)	Aspirin responder (<i>n</i> = 186)	<i>p</i> value
Age (yr)	69.6 ± 11.1	68.3 ± 12.8	0.447
Sex (male/female)	62/21	137/49	0.882
Coronary heart diseases (%)	25 (30.1%)	56 (30.1%)	1.000
Previous stroke or transient ischemic attack (%)	42 (51.9%)	99 (55.9%)	0.591
Atrial fibrillation (%)	10 (12.0%)	17 (9.1%)	0.512
Hypertension (%)	61 (73.5%)	149 (80.1%)	0.264
Diabetes mellitus (%)	27 (32.5%)	72 (38.7%)	0.342
Hyperlipidemia (%)	23 (27.7%)	48 (25.9%)	0.766
Smoking (%)	38 (45.8%)	68 (36.6%)	0.177
NIHSS at admission	7.7 ± 6.0	6.7 ± 4.7	0.453
Body mass index	24.0 ± 3.2	24.8 ± 2.9	0.084
Pre-ASA (%)	49 (26.3%)	22 (26.5%)	1.000
Fibrinogen (g/L)	4.09 ± 0.51	4.20 ± 0.99	0.309
hs-CRP (mg/L)	1.4 ± 2.5	1.0 ± 1.7	0.133
D-dimer (mg/L)	1.09 ± 1.49	1.00 ± 1.70	0.678
vWF (mU/mL)	1,930 ± 891.8	1,746 ± 836.5	0.105
ICAM (ng/mL)	293.7 ± 176.4	332.4 ± 659.3	0.599
CD40 ligand (ng/mL)	4.64 ± 3.16	4.40 ± 2.99	0.544
P-selectin (ng/mL)	99.7 ± 28.8	101.3 ± 44.6	0.805
Stroke outcome			
At 7 days			
mRS ≤ 2	27 (32.5%)	71 (38.2%)	$p = 0.412$; OR = 1.08, (0.57–1.24)
mRS > 2	56 (67.5%)	115 (61.8%)	
At 30 days			
mRS ≤ 2	39 (47.0%)	112 (60.2%)	$p = 0.047$; OR = 1.183, (1.00–1.40)
mRS > 2	44 (53.0%)	74 (39.8%)	
At 90 days			
mRS ≤ 2	48 (57.8%)	132 (71.0%)	$p = 0.037$; OR = 1.21, (1.01–1.46)
mRS > 2	35 (42.2%)	54 (29.0%)	

hs-CRP = high-sensitivity C-reactive protein; ICAM = intercellular adhesion molecule; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale; OR = odd ratios for aspirin non-responder to have mRS ≤ 2; pre-ASA = aspirin use prior to the index stroke; vWF = von Willebrand factor.

Table 2
Comparison of clinical profiles between patients with favorable (mRS ≤ 2) and unfavorable 90-day outcome (mRS > 2).

	Favorable outcome (n = 180)	Unfavorable outcome (n = 89)	p value
Age (yr)	66.6 \pm 12.7	73.0 \pm 10.0	<0.001
Sex (female/male)	38/142	32/57	0.012
Coronary heart disease (%)	43 (23.9%)	38 (42.7%)	0.002
Previous stroke or transient ischemic attack (%)	90 (50%)	51 (57.3%)	0.143
Atrial fibrillation (%)	8 (4.4%)	19 (21.3%)	<0.001
Hypertension (%)	137 (76.1%)	73 (82.0%)	0.347
Diabetes mellitus (%)	66 (36.7%)	33 (37.1%)	1.000
Hyperlipidemia (%)	54 (30%)	17 (19.1%)	0.057
Smoking (%)	72 (40%)	34 (38.2%)	0.792
NIHSS at admission	5.4 \pm 4.0	11.4 \pm 6.2	<0.001
Body mass index	25.0 \pm 3.0	23.7 \pm 2.9	0.002
Pre-ASA (%)	53 (29.4%)	18 (20.2%)	0.141
Aspirin resistance (%)	48 (27.7%)	35 (39.3%)	0.035
Fibrinogen (g/L)	4.12 \pm 0.81	4.25 \pm 0.97	0.263
D-dimer (mg/L)	0.73 \pm 0.95	1.64 \pm 2.40	<0.001
hs-CRP (mg/L)	0.8 \pm 1.4	1.8 \pm 2.7	0.001
vWF (mU/mL)	1,708 \pm 839.6	1,996.1 \pm 862.5	0.009
ICAM-1 (ng/mL)	273.5 \pm 110.6	415.4 \pm 951.4	0.049
CD40-ligand (ng/mL)	4.50 \pm 3.07	4.43 \pm 2.97	0.853
P-selectin (ng/mL)	101.0 \pm 45.0	100.2 \pm 29.0	0.876

Aspirin resistance = a epinephrine/collagen closure-time > 193 seconds; hs-CRP = high-sensitivity C-reactive protein; ICAM = intercellular adhesion molecule; NIHSS = National Institutes of Health Stroke Scale; pre-ASA = aspirin use prior to the index stroke; vWF = von Willebrand factor.

female sex, history of coronary arterial diseases, atrial fibrillation, lower BMI, higher NIHSS score at admission, AR, and higher plasma concentrations of hs-CRP, D-dimer, vWF, and ICAM-1 (Table 2). Multivariate logistic stepwise regression analysis showed that NIHSS score at admission, atrial fibrillation, increased plasma levels of hs-CRP, and D-dimer were independent predictors for unfavorable 90-day outcome. The predictive power of AR became insignificant when hs-CRP was put into the regression model as a predictor for 90-day outcome. AR remained an insignificant factor in the final regression analysis after adjusting for age and sex (Table 3).

4. Discussion

Our study demonstrated that biochemical AR, as evaluated by Epi-CT on PFA-100, is a common condition in acute ischemic stroke patients. The prevalence of AR in our patients

was lower than those observed in other studies using Epi-CT in acute ischemic stroke patients.^{9,18} A low noncompliance rate and ethnic difference in responsiveness and bioavailability to aspirin may contribute to the lower prevalence of AR in our patients. Enteric-coated aspirin was not associated with higher frequency of AR in our study in contrast to previous study.¹⁸ The lipid profile and vascular risk factors were different between patients with and without AR.

AR was associated with unfavorable 30-day and 90-day outcome in our patients. More interestingly, the predictors for 90-day outcome in multivariate regression analysis included NIHSS score, atrial fibrillation, plasma levels of hs-CRP, and D-dimer, but not AR. The Epi-CT significantly correlated with hs-CRP. Although AR was associated with unfavorable outcome in univariate analysis, the predictive power of AR (as defined by Epi-CT) was overridden by the power of hs-CRP in the regression model.

CRP is an inflammatory marker, and it is associated with various cardiovascular diseases. Infection may precipitate an ischemic stroke attack.³ On the other hand, infectious complications during acute ischemic stroke period have an adverse effect on stroke recovery.²¹ Only a few patients (two patients with AR and one patient with non-AR) in our study had fever and CRP higher than 4.0 mg/dL, which suggested substantial infection at the time of blood test. We did not have enough data to comment on the relationship between infection and stroke outcome. The higher plasma concentration of CRP was associated with larger brain infarcts and a worse neurologic outcome.²² Some published data linked inflammation with AR.^{22–24} Inflammation, as represented by elevated CRP level, could accentuate platelet activation, and cytokines released by activated platelets could induce inflammatory responses. Both inflammation and platelet activation play

Table 3
Independent predictors for unfavorable outcome by multivariate logistic regression analysis.

	B	SE	p value	Odds ratio (95% CI)
Age (yr)	0.028	0.020	0.154	1.029 (0.989–1.070)
Sex (female as baseline)	−0.797	0.436	0.067	0.451 (0.192–1.059)
NIHSS at admission	0.221	0.038	<0.001	1.245 (1.148–1.345)
hs-CRP (mg/dL)	0.233	0.107	0.039	1.262 (1.023–1.556)
Atrial fibrillation	1.379	0.633	0.029	3.973 (1.148–13.745)
D-dimer	0.232	0.133	0.042	1.346 (1.122–1.639)
Aspirin resistance	−0.198	0.452	0.662	0.820 (0.338–1.991)
Constant	−0.071	2.740	0.979	0.931

B = B coefficient; CI = confidence interval; hs-CRP = high-sensitivity C reactive protein; NIHSS = National Institutes of Health Stroke Scale; SE = standard error.

important roles in acute ischemic vascular events. Inflammation and AR are not mutually exclusive, but rather two complementing elements of ischemic brain damage.²⁴ CRP and D-dimer were independent predictors of poor stroke outcome in other studies.^{25,26} However, the usefulness of D-dimer in predicting stroke outcome needs more study.²⁷

The ADP-CT measured by PFA-100 is not affected by the use of aspirin.²⁸ The significant correlation between ADP-CT and Epi-CT suggested that AR in our patients might result from abnormal platelet activation through noncyclooxygenase-mediated mechanisms. The PFA-100 test may unveil other biological factors that contribute to shortening Epi-CT, not necessarily resistant to aspirin. The results of the PFA-100 test represent the global status of platelet activation via various mechanisms including inadequate suppression effect of drug, systemic inflammation, and prothrombotic activity.

Meta-analysis studies showed that patients who had laboratory AR are more likely to have clinical ischemic events.^{14,15,29} On the contrary, AR measured by PFA-100 did not predict new thrombotic event in patients with stable cardiovascular diseases in other studies.^{30–32} The frequency of previous history of stroke or transient ischemic attack was not different between our patients with and without AR. The discrepancies between studies could result from the fact that some studies recruited patients with chronic stable stroke and some studies recruited patients with acute ischemic stroke.¹⁷ Cohort study also demonstrated that the prevalence of AR decreased during follow-up tests.^{9,33} The recurrent ischemic vascular events during a 90-day follow-up in our study were too few to draw any conclusions.

The relationship between different stroke subtypes and AR was unclear.^{10,22,34,35} Previous study showed that AR occurred more frequently in lacunar stroke.²² Our patients with cardioembolic stroke had a higher percentage of AR (seven of the eight patients with AR had atrial fibrillation), and increased platelet activation in patients with atrial fibrillation had also been reported.^{4,36} Atrial fibrillation is associated with inflammation and coagulation cascade activation.³⁷ Inflammatory cytokines and activated coagulation factors such as thrombin could subsequently activate more platelets. Patients with atrial fibrillation could have high residual platelet aggregability, which was mediated through a non-cyclooxygenase pathway and could not be suppressed by aspirin. Clinically, an oral anticoagulant is more effective than aspirin in preventing recurrent ischemic stroke for patients with atrial fibrillation.

The PFA-100 results are strongly correlated with vWF in our study and previous reports.³⁸ vWF is also a marker of cardiovascular risk and an independent risk factor for recurrent myocardial infarctions and death.³⁹ Plasma vWF level was significantly higher in patients with unfavorable outcome in our study. Plasma levels of P-selectin, ICAM-1, and CD40 ligand were not associated with stroke outcome in our study. The application of these parameters in daily clinical practice is limited, partly because of insufficient reproducibility and the expense of time and required equipment. More studies are necessary to prove whether these markers could predict

functional outcomes after stroke and could be helpful to guide therapeutic decisions.

There are some limitations of this study. The study evaluated the short-term outcome only during a 90-day follow-up. The relationship between long-term outcome and AR was not studied. Furthermore, higher dose of aspirin was not allowed in this study. Another methodical problem is artifactual in vitro activation of platelets during blood sampling and processing.

In conclusion, AR evaluated by PFA-100 was associated with unfavorable 30- and 90-day stroke outcome, but it was not predictive of 90-day functional outcome in patients with acute ischemic stroke. NIHSS score at admission, atrial fibrillation, and plasma levels of hs-CRP and D-dimer were predictors of 90-day outcomes. The results of PFA-100 test represented an overall effect of platelet inhibition by drug, systemic inflammation, and prothrombotic activity.

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