Predictive Value of High Residual Platelet Reactivity by Flow Cytometry for Outcomes of Ischemic Stroke Patients on Clopidogrel Therapy

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High residual platelet reactivity (HRPR) assessed by multiple tests has been associated with worse clinical outcomes. However, the clinical impact of HRPR assessed by flow cytometry is unknown. The aim of this study was to validate the predictive value of HRPR measured by flow cytometry for clinical outcomes in ischemic stroke patients during clopidogrel therapy. Overall, 198 consecutive patients with ischemic stroke taking clopidogrel underwent platelet function testing on flow cytometer including adenosine diphosphate (ADP)–induced platelet aggregation (PAg) and platelet activation markers (CD62P, CD63, and PAC-1). Poor outcome was defined as poor prognosis and ischemic events during 12-month follow-up. By receiver operating characteristic curve analysis, residual platelet reactivity assessed by flow cytometry was able to distinguish between patients with and without poor outcomes, when platelet inhibition was evaluated with ADP-PAg (area under the curve [AUC], .77; 95% confidence interval [CI], .69-.84; $P < .001$), CD62P (AUC, .73; 95% CI, .64-.81; $P < .001$), CD63 (AUC, .72; 95% CI, .64-.80; $P < .001$), and PAC-1 (AUC, .70; 95% CI, .62-.78; $P < .001$). The prevalence of HRPR was 25.8% for ADP-PAg, 32.8% for CD62P, 41.4% for CD63, and 56.1% for PAC-1. The multiple logical regression analysis demonstrated that HRPR was an independent predictor of poor outcomes (ADP-PAg: odds ratio [OR] 13.03, 95% CI 5.66-29.98, $P < .001$; CD62P: OR 8.55, 95% CI 3.94-18.57, $P < .001$; CD63: OR 8.74, 95% CI 3.89-19.64, $P < .001$; PAC-1: OR 4.23, 95% CI 1.98-9.08). In conclusion, HRPR, assessed by flow cytometry, is able to detect ischemic stroke patients at increased risk of 12-month poor outcomes on clopidogrel treatment. **Key Words:** Clopidogrel—platelet function tests—stroke—flow cytometry—treatment outcome.

Introduction

Antiplatelet treatment plays a pivotal role in the secondary prevention of acute coronary disease[1] or noncardioembolic ischemic stroke.[2] Thienopyridines such as clopidogrel are alternative oral antiplatelet agents by inhibiting P2Y12 adenosine diphosphate (ADP) receptor. Although clopidogrel was used later than aspirin, the effect of it was not inferior to aspirin on preventing recurrent ischemic events when on monotherapy[3] or dual-antiplatelet therapy with clopidogrel and aspirin.[4]

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Nonetheless, previous studies have well described that platelet reactivity to clopidogrel existed considerable interindividual variability,[5,6] and high residual platelet reactivity (HRPR) or suboptimal response to clopidogrel was associated with an increased risk of ischemic events in acute coronary disease[7,8] and ischemic stroke.[9,10]

Hence, a reliable test of platelet response to clopidogrel therapy is needed to guide to individualizing regimen. To date, however, there is no uniform and standardized method for quantifying clopidogrel-mediated platelet inhibition. Light transmission aggregometry (LTA) has been widely used but weakly standardized because of its certain drawbacks such as automatic activation and poor specificity.[11] Flow cytometric measurements of platelet activation markers (CD62P, CD63, and PAC-1) show more specificity on evaluating clopidogrel response in individual patients.[12-14] Similarly, free from centrifugation and based on platelet identified monoclonal antibody, measurement of platelet aggregation (PAg) induced by ADP on whole-blood flow cytometer could overcome the limitations of conventional optical PAg assays and may be a reliable method for monitoring platelet function.

Therefore, we aimed to validate whether HRPR assessed by ADP-induced PAg and platelet activation markers with the whole-blood flow cytometric method is able to predict clinical outcomes in patients with ischemic stroke patients on clopidogrel therapy.

Subjects and Methods

Study Population

Consecutive patients with acute ischemic stroke admitted to our institution within a week after symptom onset were eligible for this prospective study if they were given clopidogrel (75 mg per day) treatment after admission. The exclusion criteria were the presence of cranial bleeding; a history of atrial fibrillation or percutaneous coronary intervention; previous or current intake of antiplatelet agents, anticoagulants, cilostazol, and glycoprotein IIb/IIIa inhibitors within 2 weeks or consideration for thrombotic therapy; and the presence of severe hepatic or renal dysfunction, tumors, or infectious conditions at study entry.

Clinical data including demographic characteristics, stroke risk factors, National Institutes of Health Stroke Scale (NIHSS) scores, and auxiliary examination were collected from all patients on admission. All patients underwent platelet function testing before and after clopidogrel treatment for 7 days. Patients were encouraged to adhere to clopidogrel treatment for at least 1 month after discharge.

The study was approved by the Ethics Committee at the Second Hospital of Tianjin Medical University. All participants gave their written informed consent before enrollment.

Blood Sampling and Platelet Function Testing

Venipuncture of forearm veins was performed under minimal stasis with the first 2 mL blood discarded. Blood was anticoagulated with 3.8% sodium citrate .129 M/L in the dilution 1:10. Platelets were stimulated in whole blood with 20 μmol/L ADP (Sigma Biosciences, St. Louis, MO), and aggregation was assessed by FCL500MPL (Beckman Coulter, Pasadena, CA). The peridinin chlorophyll protein–conjugated anti-CD61 (Becton-Dickinson Biosciences) binding to the fibrinogen receptor glycoprotein (GP) IIb/IIIa. of platelet was used to identify the platelets. After platelet in whole blood was stimulated with ADP for 5 minutes, the blood mixture corresponded with saturating concentrations of CD61 and incubated at room temperature in the dark for 15 minutes. Then, 1% paraformaldehyde in phosphate buffered saline was added for fixation. The result was expressed as the percentage of aggregates in the CD61-identified platelets.

Platelet activation was determined by quantifying platelet surface CD62P, CD63, and PAC-1 (Becton-Dickinson Biosciences) within 2 hours of blood sampling. Similarly, the CD61 was used to identify the platelets. While Arg-Gly-Asp-Ser (RGDS) tetrapeptide (Sigma Biosciences) and mouse-IgG1 (Becton-Dickinson Biosciences) were used as isotype-negative controls to define nonspecific bindings. After immunolabeling and fixation, the samples were analyzed on flow cytometer to measure fluorescent light scatter as previously described.[15,16] Platelets were identified by side light scatter and expression of CD61, and 5000 platelets were gated. The results were expressed as a percentage of antibody-positive platelets exceeding 99% of the control platelets.

Clinical Outcomes and Definition

Poor outcome was defined as poor prognosis and ischemic events. Others were considered as good outcome. Poor prognosis was defined as a score of more than 2 on modified Rankin Scale. The ischemic events were defined as a composite of nonfatal ischemic stroke/transient ischemic attack (TIA), nonfatal myocardial infarction, and vascular death. Ischemic stroke recurrence was diagnosed as a focal neurologic deficit lasting more than 24 hours, complicating ischemic cerebral lesions confirmed by computed tomography/magnetic resonance imaging. TIA was defined as a focal neurologic deficit lasting within 24 hours. Myocardial infarction was diagnosed when 2 of the following 3 criteria were met: typical symptoms, increased cardiac enzyme levels, and diagnostic electrocardiographic changes. Vascular death was defined as deaths occurring within 24 hours after stroke, myocardial infarction, and other unexpected symptoms onset without other causes evidence.[17]

All clinical outcomes were judged by independent investigators who were unaware of the clinical information. All patients were followed via telephone contact or