Spontaneous Micro-Aggregation of Platelets Predicts Clinical Outcome in Acute Ischemic Stroke

Risa Kudoh,* Kasumi Mikami, RN, PhD,* Maiko Kitajima, RN, PhD,* Keiko Aizu, RN, PhD,* Yui Kitajima, RN, PhD,* Joji Hagii, MD,† Hirofumi Metoki, MD,† Satoshi Seino, MD,† Yoshiko Baba, MD,† Minoru Yasujima, MD,† and Tomohiro Osanai, MD*

Backgrounds: Spontaneous micro-aggregation of platelets (SMAP) is frequently observed in stroke patients and is a trigger for the additional development of larger thrombi. We tested the hypothesis that SMAP may predict clinical outcome in acute ischemic stroke patients. *Methods and Results:* Consecutive acute ischemic stroke patients (n = 358) who were transferred to our hospital within 24 hours after its onset were enrolled. Peripheral venous blood was sampled to measure various parameters when they arrived. SMAP was correlated with plasma brain natriuretic peptide and diastolic blood pressure positively, and with serum albumin and body weight negatively. Multivariable Cox regression analysis showed that only serum albumin was an independent predictor of the SMAP ($P = .0023$). The proportion of patients who were functionally independent (score 0-2 on the modified Rankin Scales) at discharge was lower in the third tertile of SMAP (higher level) as compared with the first and the second tertiles in ischemic stroke (odds ratio [OR], 5.76; 95 % confidence interval [CI], 3.31-10.05; $P < .0001$) and atherothrombotic stroke ($P = .02$ by chi-square test). The lower proportion of patients achieving independence was found in the first tertile of serum albumin (lower level) as compared with the second and third tertiles in ischemic (OR, 4.60; 95% CI, 2.66-7.95; $P < .0001$), atherothrombotic, and cardioembolic stroke ($P = .004$ and $P < .0001$ by chi-square test). On logistic regression analysis, SMAP and serum albumin remained independent predictors of poor outcome in ischemic stroke. *Conclusions:* SMAP within 24 hours after stroke onset is a novel independent predictor of clinical outcome in acute ischemic stroke patients. **Key Words:** Spontaneous micro-aggregation of platelets—stroke—prognosis—albumin—modified Rankin Scale.

Introduction

An important role of platelet micro-aggregation in systemic circulation and its involvement in pathogenesis were demonstrated in patients with stroke and diabetes after the creation of a laser LS system for micro-aggregates.[1-3] The spontaneous micro-aggregation of platelets (SMAP) formed under no stimulation with exogenous agonists is frequently observed in stroke and diabetic patients.[4-6] The SMAP is a trigger for the additional development of larger thrombi, leading to vascular occlusions, but its role in a predictor of clinical outcome in ischemic stroke remained to be determined.

From the *Department of Nursing Science, Hirosaki University Graduate School of Health Science, Hirosaki, Japan; and †Hirosaki Stroke and Rehabilitation Center, Hirosaki, Japan.

Received January 30, 2018; revision received February 24, 2018; accepted March 8, 2018.

Address correspondence to Tomohiro Osanai, MD, Department of Nursing Science, Hirosaki University Graduate School of Health Science, 66-1 Hon-cho, Hirosaki, 036-8564, Japan. E-mail: osanait@hirosaki-u.ac.jp.

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.03.004

Stroke is the fourth leading cause of death in Japan, the second leading cause of death globally, and a major cause of disability.[7] By 2030, stroke-related disability is estimated to become the fourth most important cause of disability-adjusted life years in developed countries.[8] One of the specific treatments shown to improve outcome in patients with acute ischemic stroke is the intravenous tissue plasminogen activator, but the therapeutic benefit of this treatment declines in the first few hours after stroke onset.[9] Aspirin has been used as a primary and a secondary strategy to prevent cardiovascular events including ischemic stroke in diabetic and nondiabetic individuals. The meta-analysis of large-scale collaborative trials suggests that a low dose of aspirin should be used as a primary prevention strategy with diabetes that is at high risk of cardiovascular diseases.[10] However, a considerable number of patients receiving aspirin still display SMAP formation.[5]

Antiplatelet therapy with cilostazol attenuates SMAP formation in type 2 diabetic patients who had an insufficient platelet response to aspirin. SMAP formation induced by shear stress is mediated through the P2Y12 receptor,[4] and the interaction of the von Willebrand factor, platelet receptor protein glycoprotein Ib/V/IX, and glycoprotein IIb/IIIa is attributable to the onset of platelet thrombosis at sites exposed to shear stress.[11,12] As platelets obtained from stroke patients are activated by low shear stress, micro-thrombi formed from platelets could be present in the systemic circulation of stroke patients. Thus, it is likely that platelet-derived micro-thrombi contribute to determining the severity and prognosis of ischemic stroke patients. We tested the hypothesis that the SMAP within 24 hours after stroke onset is an independent predictor of clinical outcome in acute ischemic stroke.

Methods

Study Patients

The protocol of the present study was approved by the ethics committee of the Hirosaki Stroke and Rehabilitation Center, and informed consent was obtained from all patients or families. A total of 358 consecutive acute ischemic stroke patients from April 2014 through March 2015 who were transferred to the emergency room of the center within 24 hours after stroke onset were enrolled. The reason for the limitation of a 24-hour window after stroke was to collect patients before receiving initial medical treatment to acute ischemic stroke. Indeed, no patients had undergone acute therapy until their arrival to our center, because they were directly transferred to our center after the confirmation of stroke attack. They underwent head computed tomography scan and magnetic resonance imaging, and the peripheral venous blood was sampled for the measurement of biochemical parameters before any treatments at arrival to the emergency room. The National Institutes of Health Stroke Scale (NIHSS) was assessed at admission and 1 week later to estimate stroke severity, and modified Rankin Scales (mRSs) before stroke onset, at admission, and at discharge were scored.

Table 1 shows the clinical profiles of the stroke patients. The types of 358 ischemic strokes were atherothrombotic (n = 160), lacunar (n = 30), cardioembolic (n = 108), and unclassified type of ischemic stroke (n = 60). Age, gender, and incidence of risk factors such as hypertension, dyslipidemia, and diabetes did not differ among the types of stroke. At admission, some stroke patients had been already administered aspirin (100 mg/d, n = 36), thienopyridine (clopidogrel at 75-150 mg/d or ticlopidine at 100-200 mg/d, n = 29), cilostazol (100-200 mg/d, n = 10), and combined medicines (n = 10) to prevent cardiovascular diseases.

At admission, 31 patients (atherothrombotic = 7, lacunar = 0, cardioembolic = 21, and unclassified type of ischemic stroke = 3) received an intravenous administration of the tissue plasminogen activator in our hospital, and most of the patients received conventional therapies such as antithrombotic and antiradical injections. The atherothrombotic or lacunar stroke patients were administered antiplatelet medicines, whereas the cardioembolic stroke patients received direct oral anticoagulants during the course of the observation.

Detection of Platelet Microaggregation

Light intensities of small- (9-25 μn), medium- (25-50 μm), and large-sized (50-70 μm) platelet aggregates were measured by the laser light scattering aggregometer (model PA-200C; Kowa, Tokyo, Japan) immediately after sampling the peripheral venous blood. Briefly, platelet-rich plasma (PRP) was obtained from blood collected into sodium citrate (14 μmol/L) by centrifugation at 155*g* for 12 minutes at room temperature, and the density of platelets in PRP was adjusted to 300,000 of platelets per microliter. We usually selected 1 reading of light scattering in PRP with 1 test, but in some cases, double sampling of 1 venous blood sample was performed in order to detect variability of the measurement on repetition. Platelet micro-aggregation was determined by measuring the light scattering intensity on a PA-200 aggregometer. The SMAP was observed under low shear stress conditions at a stirring speed of 1000 rpm (26 dyn/cm^2) without stimulation by an exogenous agonist. This degree of low shear stress is considered to be the same degree of shear stress occurring in the arterial stream. The data were recorded as a 2-dimensional graph showing the change in total light intensity over time, expressed as a cumulative summation at 10-second intervals of scattered light intensity and the number of particles corresponding to that intensity in terms of particle size (volts × counts per second). Particles with an intensity of 25-400 mV represented small aggregates (9-25 μm). The degree of the SMAP was described by the area under