# Comparison of Aspirin Response Measured by Urinary 11-Dehydrothromboxane B2 and VerifyNow Aspirin Assay in Patients with Ischemic Stroke

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*Background:* We looked for the prevalence of aspirin nonresponders, compared the results of 2 tests assessing aspirin responses—measurement of urinary 11-dehydrothromboxane B2 (dTXB2) and VerifyNow Aspirin assay—in patients with ischemic stroke, and examined the relationship of aspirin nonresponse and the outcomes of the patients. *Methods:* One hundred one patients with ischemic stroke were prospectively included. Aspirin response was assessed by urinary dTXB2 measurement and VerifyNow Aspirin assay. The Spearman correlation coefficients and kappa statistics were calculated to assess correlation and agreement between the 2 tests. The measured outcome was the occurrence of cardiovascular events and death. *Results:* Prevalence of aspirin nonresponders was 40% and 6%, if they were measured by urinary dTXB2 and VerifyNow Aspirin assay, respectively. Poor correlation in the results between the 2 tests was found ($r = .135$, $P = .190$). The degree of agreement between the 2 tests in relation to resistance status was weak (kappa = .032, $P = .590$). With a mean follow-up time of 17 months, the outcomes occurred significantly higher in aspirin nonresponders who were diagnosed by urinary dTXB2 measurement as compared with patients with aspirin response (18% versus 2%, odds ratio 8.8, 95% confidence interval 1.18-65.4, $P = .037$). *Conclusions:* Our research confirmed poor correlation and lack of agreement between the 2 tests. Only aspirin nonresponders who were diagnosed by dTXB2 measurement were related to having cardiovascular events and death. Further research is still needed to identify the best method of diagnosis of aspirin nonresponders. **Key Words:** Stroke—aspirin—aspirin nonresponder—VerifyNow—11-dehydrothromboxane B2—Asia.


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Aspirin therapy for secondary stroke prevention reduces risk of recurrent stroke by 15%.[1] American Stroke Association recommends the use of aspirin 50-325 mg/d in patients with noncardioembolic stroke or transient ischemic attack.[2] However, approximately a third to half of patients have recurrent strokes while on antiplatelet therapy.[3] "Aspirin resistance" has been defined as the inability of aspirin to protect individuals from thrombotic complications or to produce an anticipated effect on one or more in vitro tests of platelet function.[4,5] However, different terms—"aspirin nonresponders" or "low response" or "high residual platelet reactivity"—have been used to describe patients who had low or no anticipated response after antiplatelet treatment by different laboratory methods/criteria. Systemic reviews

and meta-analysis showed that patients who were aspirin nonresponders had greater risk of clinically cardiovascular events than *aspirin-sensitive* patients.[6,7]

There are many laboratory methods used to measure aspirin responses. However, poor correlation among methods is usually found. It was proposed that methods that directly measure the capacity of platelets to synthesize thromboxane A2 (TXA2) may be preferable.[8] Thromboxane B2 is a stable metabolite of TXA2. The results from measurement of urinary 11-dehydrothromboxane B2 (dTXB2) have been shown to correlate with future cardiovascular events in patients at high vascular risk who were treated with aspirin.[9] VerifyNow is a fully automated point-of-care platelet aggregometer to measure antiplatelet therapy. VerifyNow Aspirin assay has US Food and Drug Administration approval for monitoring aspirin therapy.[10] There is less data about aspirin nonresponders in patients with ischemic stroke as compared with those with coronary artery diseases, especially in Asian patients. The purpose of this research is to look for the prevalence of aspirin nonresponders, compare the results of the tests assessing aspirin responses between urinary dTXB2 measurement and VerifyNow Aspirin assay in patients with ischemic stroke, and compare those results with the clinical outcome of the patients.

## Methods

Patients with ischemic stroke who were treated with aspirin at Thammasat University Hospital during April 2011 to August 2011 were prospectively included. Patients who were on other antiplatelets, besides aspirin, having contraindications to aspirin, noncompliance or poor compliance, allergy to aspirin, renal failure (required dialysis), or who declined to participate in the study were excluded. Noncompliance or poor compliance was defined by inability to take aspirin every day for at least 7 days before the enrollment in patients with stable ischemic stroke. For the included patients with acute ischemic stroke, uncoated aspirin 325 mg was prescribed at the stroke unit and aspirin 81 or 325 mg was used to treat patients with stable ischemic stroke at the outpatient clinic. All patients were followed up as outpatients at the clinic or by telephone calls or mail in case of patients who were unable to come to the hospital. Information about baseline characteristics, stroke subtypes, stroke severity, compliance of medications, doses of aspirin, side effects of aspirin, and concomitant medications, especially NSAIDS, were studied. The outcomes of the study were cardiovascular events, including recurrent ischemic stroke, transient ischemic attack, myocardial infarction, unstable angina, cardiac interventions, cardiovascular death, and all-cause mortality.

Urine samples and blood samples of the patients were collected at the same time, 2-6 hours after taking aspirin. A study revealed that there was no significant difference in urine dTXB2 level whether measured on day 3 or day 7 after daily aspirin administration.[11] Thus, in patients with acute ischemic stroke, urine and blood samples were collected on day 3 or after 3 doses of 325 mg aspirin. All urine samples were kept at −80° C all the time until analysis. Urine samples were assayed for dTXB2 levels with a commercially available enzyme immunoassay (Cayman Chemical). Urinary dTXB2 was normalized to urine creatinine. Subjects presenting urinary dTXB2 levels of 67.9 ng/mmol of creatinine or more were considered "aspirin nonresponders."[9]

VerifyNow Aspirin assay is a test to detect platelet dysfunction because of aspirin ingestion in whole blood for the point-of-care laboratory setting. The assay incorporates the agonist arachidonic acid to activate platelets, and it measures platelet function based on the ability of activated platelets to bind to fibrinogen. Besides a platelet activator, the cartridges contain fibrinogen-coated beads. Blood sample tubes are mixed before insertion onto the cartridge that has been premounted onto the instrument. Aggregation in response to the agonist is monitored by light transmission through chambers in each cartridge. The assay reports the extent of platelet aggregation as aspirin reaction units (ARUs). ARU values of 550 ARUs or more are considered inadequate antiplatelet effect.[10]

Continuous variables were presented as mean ± standard deviation, and categorical variables were presented as frequencies and percentages. Correlation between results from the 2 tests was analyzed using Spearman correlation coefficient. The agreement between the aspirin resistance status assessed by the 2 platelet function tests was evaluated with kappa statistics. The demographics and vascular risk factors were compared between patients with and without aspirin nonresponse using Student *t* test (for the continuous variables) and the chi-square test (for the proportions). The study was approved by the Faculty of Medicine, Thammasat University's ethical review committee. All patients read an information sheet and gave their consent to participate in the study.

## Results

There were 101 patients with ischemic stroke who had both of the antiplatelet function tests during the study period. Baseline characteristics of the patients are presented in Table 1. One hundred one urine and blood samples were studied. Prevalence of aspirin nonresponders was 40% and 6%, if they were measured by urinary dTXB2 and VerifyNow Aspirin assay, respectively. The Spearman correlation coefficients and kappa statistics were calculated to assess correlation and agreement between the 2 tests. Poor correlation in results between the 2 test was found ($r = .135$, $P = .190$). The degree of agreement between the 2 tests in relation to resistance status was weak (kappa = .032, $P = .590$) (Fig 1).