A Comparison of INNOVANCE® PFA P2Y and VerifyNow P2Y12 Assay for the Assessment of Clopidogrel Resistance in Patients Undergoing Percutaneous Coronary Intervention

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Introduction: VerifyNow P2Y12 is commonly used to measure responsiveness to clopidogrel. We sought to compare the results obtained from novel INNOVANCE® PFA P2Y and VerifyNow P2Y12 assay to assess the clopidogrel resistance in patients undergoing percutaneous coronary intervention. **Methods:** A total of 255 patients undergoing percutaneous coronary intervention, preliminarily treated with 100 mg/day of aspirin followed by coadministration of clopidogrel (loading dose, 600 mg; maintenance dose, 75 mg/day), were enrolled in this study. Platelet aggregation was measured by INNOVANCE® PFA P2Y and VerifyNow P2Y12. **Results:** INNOVANCE® PFA P2Y and VerifyNow P2Y12 assay showed moderate correlations with INNOVANCE® PFA P2Y vs. VerifyNow%inhibition: $r = 0.412$, $P < 0.0001$; INNOVANCE® PFA P2Y vs. VerifyNow

P2Y12 reaction units (PRU): $r = -0.402$, $P < 0.0001$. The agreement between INNOVANCE® PFA P2Y and VerifyNow%inhibition was 85% and that of INNOVANCE® PFA P2Y and VerifyNow PRU was 79%. The k statistics between INNOVANCE® PFA P2Y and VerifyNow%inhibition and PRU were 0.52 and 0.44, respectively. **Conclusions:** The sensitivity of INNOVANCE® PFA P2Y in detecting clopidogrel resistance is comparable to that of VerifyNow P2Y12 assay. As the PFA-100® system is already widely used, the new test cartridge may be a useful tool for the assessment of clopidogrel effects. Additional clinical correlation studies are required to validate the effectiveness of INNOVANCE® PFA P2Y in predicting long-term clinical outcomes. J. Clin. Lab. Anal. 26:262–266, 2012. © 2012 Wiley Periodicals, Inc.

Key words: clopidogrel; resistance; platelet function test

INTRODUCTION

Changes in platelet activation and aggregation are an important pathophysiologic feature in acute vessel occlusion by atherosclerotic changes. The inhibition of platelet function is a major principle in the treatment of cardiovascular diseases.

Clopidogrel effectively inhibits adenosine diphosphate (ADP)-induced platelet activation and aggregation by selectively and irreversibly blocking the P2Y12 receptor (1).

The addition of clopidogrel to aspirin treatment reduces ischemic events in a wide range of patients with cardiovascular disease. However, ischemic event recurrence during dual antiplatelet therapy, including stent thrombosis, remains a major concern. Interindividual variability in response to clopidogrel and a significant propor-

tion of suboptimal platelet inhibition have been reported (2, 3).

Light transmittance aggregometry (LTA) is still regarded as the method of choice for the assessment of platelet function (4). However, LTA is not standardized or labor intensive. Thus, point-of-care (POC) platelet function assays have been developed for use in routine clinical

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Received 15 August 2011; Accepted 8 March 2012

DOI 10.1002/jcla.21515

Published online in Wiley Online Library (wileyonlinelibrary.com).

practice. A need exists for a validated bedside or POC device, to allow clinicians to assess platelet function and titrate antiplatelet drugs in a more timely fashion.

VerifyNow P2Y12 Assay (Accumetrics, San Diego, CA) has been commonly used worldwide as a POC device for clopidogrel resistance. VerifyNow assay correlates best with the maximal plasma level of the active metabolite of clopidogrel and is good for predicting early clinical outcomes in patients undergoing percutaneous coronary intervention (5, 6).

The PFA-100[®] System (Siemens, Marburg, Germany) provides a test method that is rapid and easy to perform. The manufacturer currently provides a new cartridge, the INNOVANCE[®] PFA P2Y, which uses prostaglandin E1 (PGE1) as the adenylate cyclase activator.

In this study, we sought to compare the results obtained from novel INNOVANCE[®] PFA P2Y and VerifyNow P2Y12 assay for the assessment of clopidogrel resistance in patients undergoing percutaneous coronary intervention.

MATERIALS AND METHODS

Patients

A total of 255 patients undergoing percutaneous coronary intervention in the cardiology clinic of Seoul St. Mary's Hospital, South Korea, from January 2011 to June 2011, were enrolled in this study. All patients were preliminarily treated with 100 mg/day of aspirin followed coadministration of clopidogrel (loading dose, 600 mg; maintenance dose, 75 mg/day). Blood samples were collected from the antecubital vein at 48 h after clopidogrel loading dose. Exclusion criteria were platelet count outside the $100\text{--}450 \times 10^9/\text{L}$ range; hematocrit $<25\%$ or hemoglobin $<8 \text{ g/dl}$; and chronic renal failure requiring dialysis. All blood samples were processed within 2 h of collection. This study was approved by the institutional review board of Seoul St. Mary's Hospital (Approval number: KC10DISI0554).

Platelet Function Tests

(1) INNOVANCE[®] PFA P2Y

The PFA-100[®] system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) is a system for the assessment of high shear stress dependent platelet function by a procedure simulating the complex process of primary hemostasis in vitro. The device aspirates 800 μl of whole blood, anticoagulated with 0.129 M (3.2%) trisodium citrate, at high shear rates ($5,000\text{--}6,000 \text{ s}^{-1}$), first through a stainless steel capillary and then through an aperture in a membrane coated with agonists. The agonists at the

membrane trigger platelet adhesion, activation, and aggregate formation at the aperture.

The membrane of INNOVANCE[®] PFA P2Y is coated with 20- μg ADP, 5-ng PGE1, and 125- μg calcium (as calcium chloride). The time required to occlude the central aperture (diameter, 100 μm) resulting in the cessation of blood flow is defined as the closure time (CT); the maximum value for CT is determined by the maximum measurement time of 300 s (7).

(2) VerifyNow assay

The VerifyNow P2Y12 assay (Accumetrics, San Diego, CA) is based on the optical detection of platelet aggregation in the whole blood (8). The assay contains 20 $\mu\text{mol l}^{-1}$ ADP to induce platelet aggregation and 22 nmol l^{-1} PGE1 to suppress the undesirable contribution from ADP-agonism of P2Y1 receptors to platelet aggregation.

The magnitude of ADP-induced platelet activation is expressed as P2Y12 reaction units (PRU). In addition, the device calculates the percentage of P2Y12-inhibition, based on thrombin receptor activating peptide (TRAP)-induced platelet aggregation (BASE value) and PRU.

Characterization of Clopidogrel Resistance

The cutoff levels were derived from medical literature for each platelet-function test or manufacturer's recommendation. The clopidogrel resistances determined by using the INNOVANCE P2Y were defined as a $\text{CT} < 106 \text{ s}$. The clopidogrel resistances determined by using the VerifyNow assay were defined as $\text{PRU} \geq 240$ and $\text{P2Y12\%inhibition} < 20\%$ (9, 10).

Statistical Methods

Continuous variables are presented as the mean $\pm$ standard deviation and the categorical variables are presented as frequencies and percentages. Correlations between the results obtained with the various assays were established using Spearman's correlation coefficient. The agreement between the clopidogrel resistance statuses assessed by two platelet function tests was evaluated with the use of the k statistic. A two-sided P -value of <0.05 was considered significant. Analyses were performed with SPSS 18.0 for Windows (SPSS Chicago, IL).

RESULTS

INNOVANCE[®] PFA P2Y and VerifyNow P2Y12 assay were simultaneously performed in 255 patients undergoing percutaneous coronary intervention. Baseline characteristics of patients investigated are shown in Table 1.

TABLE 1. Baseline characteristics (*N* = 255)

Variables	No. of patients (<i>N</i> = 255)
Sex (female/male)	84/171
Age	64.5 ± 10.9
Risk factors	
Current smoker	56 (22%)
Diabetes mellitus	86 (33.7%)
Hypercholesterolemia	117 (45.8%)
Hypertension	151 (59.2%)
Laboratory parameters	
Platelet count (×10 ³ /mm ³)	203 ± 56.9
Hematocrit (%)	37.3 ± 5.05
Total cholesterol (mg/dl)	161 ± 42.5
LDL-cholesterol (mg/dl)	95.1 ± 34.3
Triglyceride(mg/dl)	130 ± 80.6
hs CRP(mg/dl)	1.00 ± 3.16

Continuous variables are presented as mean ± SD, and categorical variables as counts (%).

TABLE 2. Agreement of two platelet function tests of clopidogrel resistance

Platelet function assays	<i>k</i> statistic	Concordance (%)
INNOVANCE® PFA P2Y vs. VerifyNow (%inhibition)	0.52	85
INNOVANCE® PFA P2Y vs. VerifyNow (PRU)	0.44	79

It is generally accepted that a *k* statistic between 0 and 0.2 translates into slight agreement, 0.2 to 0.4 into fair agreement, 0.4 to 0.6 into moderate agreement, 0.6 to 0.8 into substantial agreement, and 0.8 to 1 into almost perfect agreement.

Among the 255 patients, 84 were women and 171 were men (1:2.03). The mean age was 64.5 ± 10.9 years (range from 37 to 87 years). Comparisons between the results of two platelet function tests are shown in Table 2. This was supported by the Spearman's correlation coefficients and the *k* statistics that were calculated to assess the correlation and the agreement between the various platelet function tests.

INNOVANCE® PFA P2Y and VerifyNow P2Y12 assay showed moderate correlations with INNOVANCE® PFA P2Y vs. VerifyNow[®]inhibition: *r* = 0.412, *P* < 0.0001; INNOVANCE® PFA P2Y vs. VerifyNow PRU: *r* = - 0.402, *P* < 0.0001. The agreement between INNOVANCE® PFA P2Y and VerifyNow[®]inhibition was 217/255 (85%) and that of INNOVANCE® PFA P2Y and VerifyNow PRU was 201/255 (79%): The *k* statistics between INNOVANCE® PFA P2Y and VerifyNow[®]inhibition and PRU were 0.52 and 0.44, respectively.

TABLE 3. Prevalence of clopidogrel resistance determined using two platelet function tests

Platelet function assays	Prevalence (%)
INNOVANCE® PFA P2Y	37 (14.5%)
VerifyNow (%inhibition)	59 (23.1%)
VerifyNow (PRU)	83 (32.5%)

The prevalence of clopidogrel resistance by using INNOVANCE® PFA P2Y, VerifyNow[®]inhibition, and VerifyNow PRU was 14.5%, 23.1%, and 32.5%, respectively (Table 3).

DISCUSSION

Clopidogrel is an effective inhibitor of platelet activation and aggregation due to its selective and irreversible blocking of the P2Y₁₂ receptor. Combination antiplatelet therapy with clopidogrel and aspirin is an important strategy for patients with acute coronary syndromes and those undergoing percutaneous interventions. Despite significant benefits demonstrated with combination antiplatelet treatment in large clinical trials, the occurrence of adverse ischemic events, including stent thrombosis, remains a serious clinical problem (11). LTA had been the standard test, but it is time intensive, labor intensive, and therefore not practical.

A simple, reliable whole blood test system for the assessment of clopidogrel resistance would be of great value in clinical practices. So POC tests, including VerifyNow, multiplate analyzer assays and the novel INNOVANCE® PFA P2Y cartilage of the PFA-100® system, have been developed.

Yong-Guk ko et al. showed that VerifyNow assay was an efficient POC platelet test to identify patients with hyporesponsiveness to clopidogrel who were at increased risk for periprocedural MI and early adverse cardiovascular events (5).

VerifyNow assay was significantly correlated with LTA-based high posttreatment platelet reactivity, and the result of VerifyNow assay for the clopidogrel resistance has shown a good correlation with that of ADP-induced LTA (6).

VerifyNow assay has been commonly used as a POC device for the assessment of clopidogrel resistance. In this study, we evaluated the correlation and agreement between INNOVANCE® PFA P2Y and VerifyNow assays.

The PRU and P2Y₁₂inhibition results obtained from the VerifyNow assay and CT of INNOVANCE® PFA P2Y assay were moderately correlated.

The agreement between INNOVANCE® PFA P2Y and VerifyNow[®]inhibition was 217/255 (85%) and that of INNOVANCE® PFA P2Y and VerifyNow PRU was 201/255 (79%).

The disagreement between INNOVANCE[®] PFA P2Y and VerifyNow[®] inhibition was 38/255 (14.9%). The cases with resistance for INNOVANCE[®] PFA P2Y and no resistance for VerifyNow[®] inhibition were eight, and the cases with no resistance for INNOVANCE[®] PFA P2Y and resistance for VerifyNow[®] inhibition were 30. Cases with discrepancy were confirmed with LTA. The LTA assay was performed on ChronoLog 700 platelet aggregometer (Chronolog Series 700; Probe and Co., Endingen, Germany). The LTA testing was performed using 20 $\mu\text{mol L}^{-1}$ ADP as the agonist. The clopidogrel resistance determined by performing LTA analysis was defined as 20 $\mu\text{mol L}^{-1}$ ADP-induced platelet aggregation (%) more than 65% (28).

One case (12.5%) among eight cases with resistance for INNOVANCE[®] PFA P2Y and no resistance for VerifyNow[®] inhibition showed resistance for LTA. Three cases (10%) among 30 cases with no resistance for INNOVANCE[®] PFA P2Y and resistance for VerifyNow[®] inhibition showed resistance for LTA.

The disagreement between INNOVANCE[®] PFA P2Y and VerifyNow PRU was 54/255 (21.1%). The cases with resistance for INNOVANCE[®] PFA P2Y and no resistance for VerifyNow PRU were four, and the cases with no resistance for INNOVANCE[®] PFA P2Y and resistance for VerifyNow PRU were 50. One case (25%) among four cases with resistance for INNOVANCE[®] PFA P2Y and no resistance for VerifyNow PRU showed resistance for LTA. Three cases (6%) among 50 cases with no resistance for INNOVANCE[®] PFA P2Y and resistance for VerifyNow[®] inhibition showed resistance for LTA.

The resistance rates in LTA with resistance for INNOVANCE[®] PFA P2Y and no resistance for VerifyNow assay were higher than that with no resistance for INNOVANCE[®] PFA P2Y and resistance for VerifyNow. Linnemann et al. reported that the sensitivity of INNOVANCE[®] PFA P2Y in detecting resistance to clopidogrel was comparable to that of ADP-induced LTA (7).

The two methods measure different aspects of platelet function. Although VerifyNow measures the change in light transmission because of platelet aggregation in a liquid phase, the PFA-100[®] system is a flow-based system with high shear stress. And the ADP/PGE1 ratio contained in each method is different. Also, the VerifyNow system is significantly influenced by hemoglobin/hematocrit levels and INNOVANCE[®] PFA P2Y is significantly influenced by vWF-antigen and vWF-activity (12). These influences and different working mechanisms possibly contribute to the discrepancies of results.

The degree of agreement between two platelet function tests was expressed using k statistic analysis and showed moderate agreement (from 0.44 to 0.52). The degree of

agreement between INNOVANCE[®] PFA P2Y and VerifyNow[®] inhibition ($k = 0.52$) was slightly higher than that between INNOVANCE[®] PFA P2Y and VerifyNow PRU ($k = 0.44$).

The prevalence values of clopidogrel resistance obtained by using INNOVANCE[®] PFA P2Y, VerifyNow[®] inhibition, and VerifyNow PRU was 14.5%, 23.1%, and 32.5%, respectively.

The prevalence of clopidogrel resistance in INNOVANCE[®] PFA P2Y assay was 14.5%, which was comparable to that of clopidogrel resistance in LTA (9.1%) reported by Jin-Yeong Han et al. (13)

The prevalence of clopidogrel nonresponsiveness has been reported to be in a range from 5 to 44%. This wide variation in prevalence is primarily due to dosing and is less related to various definitions, laboratory methods, and the time at which blood samples were evaluated for responsiveness (14–22).

Elsenberg et al. reported that high clopidogrel platelet reactivity was associated with an increased risk of atherothrombotic events and was highly dependent on the type of platelet function assay (12). Each platelet function assay was significantly influenced by the distinct clinical and laboratory variables. Several studies suggested that low responsiveness to antiplatelet therapy, as measured in various platelet-function tests, correlated with high incidence rates of ischemic events (23–28).

The novel INNOVANCE[®] PFA P2Y assay among the various platelet function tests showed moderate correlation with VerifyNow and 85% agreement with VerifyNow[®] inhibition. The resistance rates in LTA with resistance for INNOVANCE[®] PFA P2Y and no resistance for VerifyNow assay were higher than that with no resistance for INNOVANCE[®] PFA P2Y and resistance for VerifyNow.

In conclusion, the sensitivity of INNOVANCE[®] PFA P2Y in detecting clopidogrel resistance is comparable to that of VerifyNow P2Y12 assay. Because the PFA-100[®] system is already widely used, the new test cartridge may be a useful tool for the assessment of clopidogrel effects. Additional clinical correlation studies are required to validate the effectiveness of INNOVANCE[®] PFA P2Y in predicting long-term clinical outcomes.

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