Correlation of a new point-of-care test with conventional optical aggregometry for the assessment of clopidogrel responsiveness

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Dear Sir,
A variable response to clopidogrel is consistently observed when adenosine diphosphate (ADP)-induced platelet aggregation or vasodilator-stimulated phosphoprotein (VASP) phosphorylation is assessed in clopidogrel-treated patients (1–3). A fast, reliable and simple method to assess the degree of pharmacologic P2Y12 blockade would be of great value to assess the antiplatelet effect of clopidogrel and other P2Y12 antagonists. Such an assay would allow tailoring of P2Y12 antagonism to the individual needs of the patient. The VerifyNow™ P2Y12 assay (Accumetrics, Inc., San Diego, CA) has been developed for this purpose (4). The aim of this study was to correlate the results of the
VerifyNow™ P2Y12 assay and ADP-induced platelet aggregation assessed with conventional optical aggregometry before and four hours after administration of a clopidogrel loading dose.

Methods
In thirty consecutive patients of a recent trial comparing different clopidogrel loading doses (5) platelet function was determined with the VerifyNow™ P2Y12 assay and conventional optical aggregation before and four hours after clopidogrel administration. Out of the 30 patients included in the present study, ten received 300 mg, nine received 600 mg and 11 received 900 mg of clopidogrel. VerifyNow™ (formerly called “Ultegra Rapid Platelet Function Assay” or “RPFA”, Accumetries, Inc., San Diego, CA, USA) is a whole-blood, point-of-care assay that consists of an instrument and a single use assay device containing the biochemical reagents required to perform an assay (6). The VerifyNow™ P2Y12 assay has been developed to assess responsiveness to clopidogrel and other P2Y12 antagonists such as prasugrel. In addition to 20 µM ADP, 22 nM prostaglandin E1 (PGE1) are incorporated into the VerifyNow™ P2Y12 assay device to suppress intracellular free calcium levels and thereby to reduce the activation contribution from ADP-binding to P2Y1 receptors. In a separate channel in which iso-TRAP is used as an agonist, a baseline value (BASE) for platelet function is obtained. Iso-TRAP-induced platelet aggregation can occur independent of P2Y12 receptors; its effect is only partly mediated by secreted ADP. The VerifyNow™ P2Y12 assay reports patient results as P2Y12 Reaction Units (“PRU”), “% Inhibition” and “BASE” in less than 5 min. “% Inhibition” is calculated as (1-PRU/Base) * 100. The assay cannot be used to assess the response to P2Y12 inhibitors in the presence of glycoprotein IIb/IIIa inhibition. By measuring VASP phosphorylation in blood samples from five healthy volunteers (no drug use during the preceding 14 days), we were able to demonstrate that with the concentrations used in the VerifyNow™ P2Y12 assay inhibition of adenylyl cyclase by ADP (via P2Y12 receptors) cannot be overcome by PGE1. A commercially available VASP phosphorylation flow-cytometry assay (PLT VASP/P2Y12, Biocytex, Marseille, France) was used for this purpose in a way that the same concentrations of ADP and PGE1 were used as in the VerifyNow™ P2Y12 assay (Becton Dickinson FACSscan flow-cytometer). Mean fluorescence intensity (indicating VASP phosphorylation, arbitrary units) was 39.4 ± 5.4 after incubation with ADP (20 µM), 74.8 ± 8.5 after incubation with PGE1 (22 nM) and 41.0 ± 3.1 after incubation with ADP (20 µM) and PGE1 (22 nM) (P = 0.55, two-sided paired t-test for ADP versus ADP + PGE1; n = 5). Conventional optical aggregometry was performed on a Chrono-log lumi-aggregometer (Probe & go Labordiagnostica, Endingen, Germany) with a constant stirring rate of 1,000 rpm at 37°C. After baseline adjustment, ADP (final concentrations 5 or 20 µM) was added. Correlations between values obtained with the VerifyNow™ P2Y12 assays and those recorded with conventional optical aggregometry were assessed with simple linear regression.

Results
The correlation of P2Y12 Reaction Units (PRU) and maximal aggregation induced with 5 µM (A) and 20 µM (B) ADP. Filled circles represent individual measurements. Blue circles indicate that the measurements were performed before clopidogrel loading. Red circles indicate that the measurements were performed 4 h after administration of clopidogrel.

Implications
The main result of the present study is that P2Y12 reaction units obtained with the VerifyNow™ P2Y12 assay correlated with maximal ADP-induced platelet aggregation assessed with conventional optical aggregometry. The best correlation was observed in the presence of no or inadequate platelet function inhibition. In some of the patients, after administration of clopidogrel the VerifyNow™ P2Y12 assay indicated nearly complete inhibition of the P2Y12 pathway (low PRU values), whereas a modest level of ADP-induced platelet aggregation was still observed. This phenomenon could be related to variable responsiveness of the P2Y1 pathway that is suppressed in the assay. The VerifyNow™ P2Y12 assay may be useful in the rapid assessment of the response to clopidogrel.

References
5. von Beckerath N, Taubert D, Pogatsa-Murray G, et al. Absorption, metabolism, and antplatelet effects of 300-, 600-, and 900-mg loading doses of clopido-

Figure 1: Correlation between P2Y12 Reaction Units (PRU) and maximal aggregation induced with 5 µM (A) and 20 µM (B) ADP. Filled circles represent individual measurements. Blue circles indicate that the measurements were performed before clopidogrel loading. Red circles indicate that the measurements were performed 4 h after administration of clopidogrel.