Comparison of four tests to assess inhibition of platelet function by clopidogrel in stable coronary artery disease patients

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Aims  
We investigated the comparability of platelet function tests in quantifying platelet inhibition achieved by clopidogrel.

Methods and results  
This pre-specified substudy of a randomized, double-blind trial included 116 patients with stable coronary artery disease requiring diagnostic angiography. Patients received clopidogrel for 1 (300 or 600 mg) or 7 days (300 + 75 or 150 mg daily) before the procedure. Blood samples obtained before clopidogrel initiation and before diagnostic coronary angiography were assayed using light transmission aggregometry [adenosine diphosphate (ADP) 5 and 20 μM as the agonist], whole-blood aggregometry (ADP 5 and 20 μM), PFA-100® (Collagen-ADP cartridge), and VerifyNow® P2Y12. Although all assays studied were found sensitive to clopidogrel ingestion, none could distinguish categorically between patients who had, or not, ingested clopidogrel. Agreement between assays to identify patients with insufficient inhibition of platelet aggregation by clopidogrel was low.

Conclusion  
The assessment of platelet function inhibition by clopidogrel is highly test-specific. Decision to increase clopidogrel dosage may vary on the basis of the assay used, thus highlighting the need for unambiguous guidelines with respect to assay selection, as platelet function assays are not interchangeable. At present, platelet function testing evaluating clopidogrel efficacy cannot be recommended in routine clinical practice.

Keywords  
Platelet function testing • Platelet aggregation • Platelet analyzers • Clopidogrel • Responsiveness

Introduction  
Untoward platelet activation has been identified as an important pathophysiological component of coronary artery disease (CAD) and has led to the development of effective antiplatelet agents that are now considered mainstay therapy for the prevention of acute ischemic events.1 Adding clopidogrel to daily aspirin treatment is the basis of antiplatelet therapy in the context of percutaneous coronary interventions (PCIs), as it reduces the risk of stent thrombosis to ~1%.1–3 However, important interindividual variability in platelet response to clopidogrel has been reported, resulting in a significant proportion of patients displaying suboptimal inhibition of platelet aggregation and an increased risk of thrombotic complications.4
demonstrated. However, no method of quantification of platelet function inhibition by clopidogrel has consensually been recommended.

Although light transmission aggregometry (LTA), which measures luminosity as aggregation occurs in adenosine diphosphate (ADP)-stimulated platelet-rich plasma (PRP), is considered by many as the current gold standard in platelet function testing, the technique demands specific knowledge and skills, requires specialized equipments, and is labour-intensive. Fast and easy-to-use point-of-care methodologies are now widely available and often employed to assess platelet response to clopidogrel. However, little is known about the comparability or interchangeability of these tests.

Hence, we performed this study to evaluate the comparability of four major platelet function assays in assessing platelet inhibition provided by clopidogrel in patients with stable CAD requiring elective angiography.

**Methods**

**Patients**

Patients were recruited from the pre-angiography outpatient clinic of the Hôpital du Sacré-Cœur de Montréal in Canada and were included if they presented suspected CAD requiring an elective diagnostic coronary angiography. Exclusion criteria were major bleeding disorders or active bleeding; acute myocardial infarction, or unstable angina, with ST-segment changes ≥1 mm in at least two contiguous electrocardiographic leads at rest or a troponin level >0.06 μg/L, within 14 days of recruitment; stroke within the last 3 months; platelet count <150 × 10^9/L; prothrombin time >1.5 times control, haematocrit <35%, or haemoglobin level <100 g/L; alcohol or drug abuse; enrolment in other investigational drug trials within the previous month; the use of thienopyridines, glycoprotein IIb/IIIa inhibitors, warfarin, or acenocoumarol within the prior week; and allergic reaction or any contraindication to clopidogrel or aspirin. This study was approved by the Institutional Scientific and Ethics Review Board, and patients gave written informed consent for participation.

**Study protocol and blood sampling**

The present study was a planned and pre-specified substudy of a randomized, prospective, double-blind, and placebo-controlled trial evaluating the effect on platelet aggregation of four different dosing regimens of clopidogrel given before elective diagnostic coronary angiography with or without PCI. From 20 September 2004 to 18 April 2006, 116 subjects were randomly assigned in a double-blind fashion to one of four groups of clopidogrel dosing regimens 1 week prior to PCI: 300 mg (n = 29) or 600 mg (n = 28) on the day prior to PCI; 300 mg followed by 75 mg daily (n = 31); or 150 mg daily (n = 28) started 1 week before PCI. In addition to the randomized study medication, all patients received 80 mg of enteric-coated aspirin daily for at least 7 days before the procedure and throughout the study period.

Four platelet function assays were assessed simultaneously in all patients: LTA and electrical impedance whole-blood aggregometry (WBA) after stimulation with ADP, VerifyNow® P2Y₁², and Platelet Function Analyzer (PFA-100®). Blood was drawn twice, before clopidogrel initiation and just before elective coronary angiography. The first 2 mL of blood, drawn by venipuncture through a 21 gauge needle, was discarded. Then, blood was drawn into evacuated tubes containing 3.2% sodium citrate. All blood samples were processed within 2 h of collection.

**Platelet aggregation assessment**

**Light transmission aggregometry**

The assessment of platelet function by LTA was considered the gold standard in this study. Platelet aggregation was assessed in PRP at 37 °C by LTA. PRP was obtained by the centrifugation of citrated whole blood for 10 min at 1000 c.p.m. (89 g). Platelet-poor plasma was obtained by the centrifugation of the remaining blood for 10 min at room temperature at 3000 c.p.m. (805 g). Platelet count in the PRP varied from 250 to 450 × 10^9/L. No adjustment of platelet count with PPP was performed, as it has been demonstrated that the adjustment of PRP with PPP could have deleterious effects on platelet function assessment. Aggregation measured with a ChronoLog Aggregometer (ChronoLog 540 model, Havertown, PA, USA) after stimulation with 5 and 20 μM of ADP (Sigma Aldrich, Oakville, Ontario, Canada), using platelet-poor plasma as reference. All analyses were performed by a single highly trained and experienced technician. Aggregation curves were recorded for 5 min and analysed according to international standards. The results are reported as either maximal platelet aggregation or platelet inhibition, defined as the relative change in platelet aggregation from baseline to the time of angiography. Platelet inhibition is calculated as (1 − residual aggregation/baseline aggregation) × 100.

**Whole-blood aggregometry**

WBA measures electrical impedance (maximal amplitude) between two electrodes immersed in whole blood 5 min after the addition of a platelet agonist (ADP 5 and 20 μM), using a ChronoLog Aggregometer (ChronoLog 560 model, Havertown, PA, USA). The results are reported as either residual platelet aggregation, measured as maximal amplitude of impedance (Ω), or platelet inhibition, defined as the relative change in impedance from baseline to the time of angiography. Platelet inhibition is calculated as (1 − residual impedance/baseline impedance) × 100.

**VerifyNow® P2Y₁₂**

The VerifyNow® P2Y₁₂ (Accumetrics, San Diego, CA, USA) point-of-care system is based on turbidimetric optical detection of platelet aggregation in whole blood. Blood was drawn into evacuated tubes containing 3.2% sodium citrate provided by the manufacturer. After withdrawal, whole blood was transferred into cartridges containing a combination of 20 μM of ADP and 22 nM of PGE₁, the latter being added to specifically measure the effect of clopidogrel following P2Y₁₂, but not P2Y₁, ADP receptor activation. As aggregation occurs, the system converts luminosity transmittance results into P2Y₁₂ reaction units (PRU). Results are reported as either residual platelet aggregation, measured in PRU, or platelet inhibition, defined as the relative change in platelet aggregation from baseline to the time of angiography. Platelet inhibition is calculated as (1 − residual aggregation/baseline aggregation) × 100.

**Platelet function analyser (PFA-100®)**

PFA-100® (Dade Behring, Deerfield, IL, USA) is a point-of-care assay that assesses platelet aggregation under high she, mimicking platelet-rich thrombus formation after injury to a small vessel wall under flow conditions. Whole blood was transferred into standard cartridges, and time necessary to occlude a microscopic aperture in a membrane coated with collagen and ADP was measured. The results are reported as closure time (s). As this technology does not measure platelet aggregation directly but rather assesses the time
required for clot formation, platelet inhibition cannot be calculated. Thus, the impact of clopidogrel administration is reported as the absolute prolongation of closure time (s) from baseline.

**Sample size and statistical analysis**

Sample size was not calculated a priori to correlate platelet function results from different assays at the time of diagnostic coronary angiography. Post hoc calculations showed that the study had a power of 80%, with a two-sided $\alpha$-value of 0.05, to detect a correlation coefficient of at least 0.25 between any of the paired platelet aggregation data sets obtained from the different platelet function assays (PASS 2002, NCSS 2004 statistical software, Kaysville, UT, USA).

Normally distributed continuous variables are presented as mean (standard deviation), non-normally distributed continuous variables as median (inter-quartile range), and categorical variables as frequencies (percentages). Variables were analysed for a normal distribution with the Kolmogorov–Smirnov test. Continuous variables were compared using the paired t-test or the signed-rank test for comparison of paired samples (the Wilcoxon test), and categorical variables were compared using the $\chi^2$ or Fisher exact test, when applicable. To account for the randomization group, partial correlations between results obtained with the various assays were calculated. Agreement between assays to evaluate the inhibition of platelet aggregation from baseline (%inhibition) was assessed through the Bland–Altman agreement analysis. This analysis specifically measures bias, which can be defined as a systematic error responsible for either under- or over-estimation of a value, and sets limits agreement, similar to confidence intervals, which indicate the range of under- or overestimation of one reading in comparison with the other. Agreement among assays to identify patients with insufficient platelet aggregation, as recommended in current American guidelines, was assessed through the $\kappa$ statistic. A two-sided $P$-value of $<0.05$ was considered significant. Analyses were performed with SPSS 14.0 for Windows (SPSS Institute, Chicago, IL, USA) and GraphPad Prism 5 for Windows (GraphPad Software Inc., San Diego, CA, USA).

**Results**

**Patients**

Of the 116 patients studied, 92 (79.3%) were male. Mean age was 60.2 ± 9.0 years (range from 38 to 80 years). Eight (6.9%) were diabetic, 75 (64.7%) had hypertension, 100 (86.2%) suffered from dyslipidaemia, and 28 (24.1%) were active smokers. After diagnostic angiography, PCI was performed in 33 patients (28.4%). The inhibition of platelet aggregation was overestimated by 13% (95% CI 110 to 124%; $P = 0.006$ by paired t-test), indicating greater inhibition when 5 $\mu$M of ADP was used. However, the limits of agreement varied from −29 (95% CI from −34 to −25) to 38% (95% CI 34–42), indicating that the two ADP concentrations may significantly disagree in certain individuals (Figure 3).

Since the ADP concentration most commonly used in the literature is 20 $\mu$M, the extent of platelet inhibition measured by WBA and VerifyNow® P2Y12 was compared with that obtained with 20 $\mu$M ADP-induced LTA. Because the PFA-100® assay results cannot be converted into %inhibition, the assay has been excluded from agreement analysis.

The inhibition of platelet aggregation was overestimated by 13% (95% CI 2.9–24.0; $P = 0.01$, limits of agreement −97 (95% CI −112 to −82) to 124% (95% CI 109–139)) when 5 $\mu$M ADP-induced WBA was used and underestimated by 11% (95% CI −20.7 to −1.8; $P = 0.02$, limits of agreement −110 (95% CI −123 to −97) to 87% (95% CI 74–100)) when 20 $\mu$M ADP-induced WBA was used in comparison with 20 $\mu$M ADP-induced LTA (Figure 4A and B). When platelet inhibition assessed by the VerifyNow® P2Y12 assay was compared with 20 $\mu$M ADP-induced LTA, VerifyNow® P2Y12 overestimated WBA, and VerifyNow® P2Y12 was compared with that obtained with 20 $\mu$M ADP-induced LTA. Because the PFA-100® assay results cannot be converted into %inhibition, the assay has been excluded from agreement analysis.

Agreement among assays in measuring clopidogrel-induced platelet inhibition

The agreement between clopidogrel-induced platelet inhibition results among platelet function assays was studied by the Bland–Altman analysis of agreement. As ADP-induced LTA is the generally accepted gold standard in platelet function testing, we first compared platelet inhibition reported by both concentration of ADP with this methodology (Figure 3). The inhibition of platelet aggregation induced by ADP 5 and 20 $\mu$M showed a slight bias of 4.5% [95% confidence interval (95% CI) 1.3–7.7; $P = 0.006$ by paired t-test], indicating greater inhibition when 5 $\mu$M of ADP was used. However, the limits of agreement varied from −29 (95% CI from −34 to −25) to 38% (95% CI 34–42), indicating that the two ADP concentrations may significantly disagree in certain individuals (Figure 3).

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inhibition by a mean of 6.3% (95% CI 1.6–14.2%, \( P = 0.117 \)), with wide limits of agreement \([-54.4 (95\% \text{ CI} -64 \text{ to } -44) \text{ to } 67.0\% (95\% \text{ CI } 57–77)\], Figure 4C), indicating poor accord between assays.

**Agreement among assays to identify patients with insufficient platelet inhibition**

Current guidelines by ACC/AHA/SCAI without any supporting evidence state that adjusting the clopidogrel dose may be considered if <50% inhibition of platelet aggregation is detected on clopidogrel therapy.\(^3\) Results were reanalysed seeking to investigate whether clinical conduct in such a context would be influenced by the platelet function-testing methodology chosen. Insufficient platelet inhibition (<50%) was found in 55% of patients by 5 \( \mu \text{M} \) ADP-induced LTA, 66% of patients by 20 \( \mu \text{M} \) ADP-induced LTA, 71% of patients by 5 \( \mu \text{M} \) ADP-induced WBA, 47% of patients by 20 \( \mu \text{M} \) ADP-induced WBA, and 61% of patients by VerifyNow\(^\text{® P2Y}_{12}\). Overall, agreement was poor, as assessed by the \( \kappa \) statistic and presented in Table 3. Although WBA failed to select the same patients as candidates for intensified therapy as either LTA or VerifyNow\(^\text{® P2Y}_{12}\), the latter showed fair agreement with LTA, with 20 \( \mu \text{M} \) of ADP yielding better results. The only results displaying strong agreement were the different ADP concentrations

**Table 1** Correlation (95% confidence interval) between platelet function tests at baseline

<table>
<thead>
<tr>
<th>Platelet function test</th>
<th>LTA ADP 20 ( \mu \text{M} )</th>
<th>WBA ADP 5 ( \mu \text{M} )</th>
<th>WBA ADP 20 ( \mu \text{M} )</th>
<th>PFA-100(^\text{®} )</th>
<th>VerifyNow(^\text{® P2Y}_{12})</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA ADP 5 ( \mu \text{M} )</td>
<td>0.766* (0.679–0.832)</td>
<td>0.229* (0.049–0.394)</td>
<td>0.326* (0.153–0.479)</td>
<td>0.102 (−0.081 to 0.279)</td>
<td>0.167 (−0.067 to 0.383)</td>
</tr>
<tr>
<td>LTA ADP 20 ( \mu \text{M} )</td>
<td>0.249* (0.070–0.412)</td>
<td>0.309* (0.135–0.465)</td>
<td>0.076 (−0.107 to 0.254)</td>
<td>0.134 (−0.100 to 0.354)</td>
<td></td>
</tr>
<tr>
<td>WBA ADP 5 ( \mu \text{M} )</td>
<td></td>
<td>0.732* (0.635–0.806)</td>
<td>−0.197* (−0.366 to −0.016)</td>
<td>0.052 (−0.181 to 0.280)</td>
<td></td>
</tr>
<tr>
<td>WBA ADP 20 ( \mu \text{M} )</td>
<td></td>
<td>0.036 (−0.147 to 0.216)</td>
<td>0.006 (−0.225 to 0.237)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFA-100(^\text{®} )</td>
<td></td>
<td></td>
<td></td>
<td>−0.020 (−0.212 to 0.250)</td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at \( P < 0.05 \).
used in LTA, thus suggesting that ADP concentration plays a minor role in quantifying platelet response to clopidogrel when assessed optically.

**Discussion**

Ability to identify patients with insufficient platelet inhibition by clopidogrel using different platelet function assays varies greatly according to the platelet function assay used, and correlation between the tests is poor. Although all assays studied were found sensitive to clopidogrel therapy, none could distinguish categorically patients who were treated with clopidogrel from those who were not. Comparability between assays was low, both at baseline and after clopidogrel intake, as demonstrated by poor agreement through the Bland–Altman analysis of clopidogrel-induced platelet inhibition. Recommendation to increase clopidogrel dosing if insufficient platelet inhibition by clopidogrel is demonstrated, as suggested in the current ACC/AHA/SCAI

![Figure 2](image_url)  
*Figure 2* Partial correlation (controlling for the randomization group) between aggregation results obtained by the various platelet function assays. (A) Correlation between tests at baseline. (B) Correlation between tests after clopidogrel ingestion. ADP, adenosine diphosphate; LTA, light transmission aggregometry; WBA, whole-blood aggregometry.

<table>
<thead>
<tr>
<th>Platelet function test</th>
<th>LTA ADP 5 μM</th>
<th>WBA ADP 5 μM</th>
<th>WBA ADP 20 μM</th>
<th>VerifyNow® P2Y12</th>
<th>PFA-100®</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA ADP 5 μM</td>
<td>0.902a (0.862–0.931)</td>
<td>0.255b (0.077–0.417)</td>
<td>0.307a (0.133–0.463)</td>
<td>−0.270a (−0.438 to −0.102)</td>
<td>0.370a (0.152–0.554)</td>
</tr>
<tr>
<td>LTA ADP 20 μM</td>
<td>0.291a (0.112–0.449)</td>
<td>0.382a (0.215–0.527)</td>
<td>−0.274a (−0.434 to −0.097)</td>
<td>0.496a (0.299–0.652)</td>
<td></td>
</tr>
<tr>
<td>WBA ADP 5 μM</td>
<td>0.981a (0.833–0.916)</td>
<td>−0.139 (−0.313 to 0.044)</td>
<td>0.187 (−0.046 to 0.401)</td>
<td></td>
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</tr>
<tr>
<td>WBA ADP 20 μM</td>
<td>−0.150 (−0.322 to 0.033)</td>
<td>0.293a (0.066–0.491)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFA-100®</td>
<td>−0.334a (−0.525 to −0.111)</td>
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</tbody>
</table>

*Correlation is significant at P < 0.05.
guidelines, would benefit from a revision to specify platelet function assay selection, as the current available assays are clearly not interchangeable.

A number of studies have looked at the correlation between various assays in defining platelet response to clopidogrel.\(^{11-13}\) Although the use of correlation to report on the association between two measurements is widespread, it is often inappropriate as it does not imply agreement between methods, nor does it evaluate bias.\(^{14}\) To weigh one method against another, or to assess how closely two measurements are related, the Bland–Altman agreement analysis was used in this study to overcome the shortcomings of correlation. Thus, this study expands on our current understanding of how each assay measures up against the rest.

LTA is considered by many as the gold standard in platelet function testing.\(^{5}\) In addition to having been used extensively in the last 50 years, it remains to this day the most widely used assay in the assessment of platelet response to clopidogrel, and it targets, with the use of ADP as the agonist, the platelet activation pathway inhibited by clopidogrel, namely the purinergic pathway. Even though the assay has become better automated, it remains time- and labour-intensive, requires technical expertise, and thus is restricted to specialized laboratories. Its major drawback however is the lack of standardization, which renders the assay results hard to compare between research teams.\(^{15}\) As this study is a single-centre study and all analyses were performed by a single highly trained and experienced technician, the latter limitation of LTA could be avoided, thus minimizing the variability of LTA and resulting in more precise measurements. Predisposing conditions that can create disparities in results between laboratories include different blood specimen temperatures, choice of anticoagulant agent, varying delays of testing from blood collection, adjustment of platelet count to a pre-specified level in the PRP specimen, varying ADP concentrations, and quantification of platelet aggregation at maximal levels vs. at the end of the experiment.\(^{15,16}\) Although each of these variables affects the outcome of aggregometry measurement and interpretation, LTA remains among the leading platelet function testing tools to evaluate platelet response to clopidogrel.

Although WBA is seldom used in the literature to evaluate platelet response to clopidogrel (like LTA, it remains restricted to specialized laboratories), the poor correlation found between WBA and LTA results in the current study is consistent with a recent report, which described a similar correlation \((r = 0.257)\) between 20 \(\mu\)M ADP-induced LTA and WBA in 27 patients scheduled to undergo PCI.\(^{17}\) Similarly, in 17 healthy volunteers, 20 \(\mu\)M ADP-induced LTA and WBA disagreed significantly in identifying patients with >50% inhibition of platelet aggregation.\(^{18}\) In comparison with LTA, aggregometry measured in whole blood requires less manipulation of the specimen, thus making the assay less prone to artefactual platelet aggregation during preparatory steps.\(^{19,20}\) It also offers the advantage of a more physiologically relevant milieu for platelets, said to be more sensitive to the antiplatelet effect of clopidogrel.\(^{21,22}\) However, the presence of other blood constituents alters platelet aggregation profiles and produces less consistent results than those obtained in PRP.\(^{23}\) Erythrocytes and leukocytes have been shown to actively regulate the availability of ADP in blood, and higher doses of ADP are believed to be necessary to elicit platelet aggregation in whole blood.\(^{21,24}\) This particularity may explain the important variability detected in whole blood clopidogrel-induced platelet inhibition in our study and the subsequent lack of agreement with LTA results obtained with the same agonist concentrations.

In an effort to make platelet function testing widely available outside of specialized laboratories, several point-of-care assays have been commercialized. The VerifyNow\(^\text{®}\) P2Y\(_{12}\) assay was developed and Food and Drug Administration-approved to specifically evaluate the effect of P2Y\(_{12}\) receptor blockade on platelet aggregation. This technology offered the novelty of isolating the effect of ADP stimulation on the P2Y\(_{12}\) receptor from that of the P2Y\(_{1}\) receptor, by incorporating the effects of PGE\(_{1}\) in addition to ADP.\(^{25}\) This design aimed at limiting the variability of response to clopidogrel by removing the contribution of the P2Y\(_{1}\) ADP receptor, which is unaffected by clopidogrel administration. However, our results and others published recently detected significant variability in platelet aggregation measured with this device, with an important overlap between pre- and post-clopidogrel values.\(^{26}\) In comparing these results with those obtained through LTA, we found weak-to-moderate correlation, which contrasts with recent reports describing a much stronger correlation \((0.73 < r < 0.86)\) between these methodologies.\(^{11,12}\) Moreover, we found that agreement between these methodologies in selecting patients with insufficient platelet inhibition was low, concordant with a recent report on 1267 patients admitted for acute coronary syndrome.\(^{13}\) We further compared the VerifyNow\(^\text{®}\) P2Y\(_{12}\) device results with those obtained through WBA, as the VerifyNow\(^\text{®}\) P2Y\(_{12}\) device also requires a whole-blood specimen. It is worth mentioning that the disadvantages of whole-blood specimens apply to the VerifyNow\(^\text{®}\) P2Y\(_{12}\) technology as well. The association between the VerifyNow\(^\text{®}\)
P2Y\textsubscript{12} results and WBA was not improved in comparison with LTA. It also resulted in a complete lack of agreement in selecting patients with insufficient clopidogrel-induced platelet inhibition.

The PFA-100\textsuperscript{w} is another point-of-care device intended for the detection of platelet dysfunctions. However, our results are in agreement with several reports describing its inability to monitor clopidogrel therapy.\textsuperscript{27–30} As described previously, although a statistical difference could be detected, clopidogrel ingestion did not result in a major inhibitory shift of platelet aggregation. Furthermore, the PFA-100\textsuperscript{w} results lacked meaningful correlation with all other platelet function assays studied, in accordance with previous studies.\textsuperscript{28,30} As the assay cannot quantify the inhibition of platelet aggregation by clopidogrel from baseline, it fails to highlight patient populations requiring intensified therapy and, as such, is inadequate to evaluate the effect of clopidogrel.

Limitations

As this report is a substudy, some limitations are inherent to the study design. Mainly, the sample size was not determined beforehand, and the analysis was conducted post hoc of the parent randomized, double-blind, placebo-controlled trial.\textsuperscript{7} However, power calculations demonstrated sufficient power to detect

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**Figure 4** Bland–Altman analysis of agreement to assess how closely two measurements are related to each other, with limits of agreement depicting the range of variability in agreement between the two measures. Bias is a measure of a systematic error leading to over- or underestimation of a known value (in this case 20 \textmu M ADP-induced LTA) by alternative measurements made with (A) 5 \textmu M ADP-induced WBA. Solid red line represents bias (13%), dashed blue lines represent 95% confidence intervals (2.9–24.0), dotted green lines represent limits of agreement (−97 to 124), and dashed grey line represents 95% confidence intervals of limits of agreement. (B) 20 \textmu M ADP-induced WBA. Solid red line represents bias (−11%), dashed blue lines represent 95% confidence intervals (−20.7 to −1.8), dotted green lines represent limits of agreement (−110 to 87), and dashed grey line represents 95% confidence intervals of limits of agreement. (C) VerifyNow\textsuperscript{w} P2Y\textsubscript{12}. Solid line represents bias (6.3%), dashed blue lines represent 95% confidence intervals (−1.6 to 14.2), dotted green lines represent limits of agreement (−54 to 67), and dashed grey line represents 95% confidence intervals of limits of agreement. ADP, adenosine diphosphate; LTA, light transmission aggregometry; WBA, whole-blood aggregometry.
clinically meaningful correlations between assay results. Although clopidogrel metabolites were not measured to ensure that patients took clopidogrel, compliance was verified by pill count and personal interview. The cut-off value of 50% to indicate inadequate clopidogrel-induced platelet inhibition is arbitrary, and no strong evidence indicates that this cut-off is a predictor of adverse cardiovascular events. However, this cut-off value is recommended by the current American guidelines as an indicator of insufficient platelet inhibition by clopidogrel and has therefore been selected as most relevant herein. It should be noted that this study was not designed to evaluate the clinical utility of platelet inhibition measurements. The small sample size precludes us from stating that either test is better suited to do so. However, the current study highlights that the available platelet function test assays are not interchangeable and thus the recommendation to use available platelet function tests interchangeably to screen non-responders to clopidogrel is premature.

Several other platelet function assays are available to evaluate clopidogrel-induced platelet inhibition. Some examples include the flow cytometric measurement of the vasodilator-stimulated phosphoprotein (VASP) phosphorylation status, the Thromboelastograph®, and Plateletworks®. As these techniques were not evaluated in the current study, their comparability with present platelet function assays cannot be discussed. However, because the VASP index is highly P2Y12-specific, it may prove more sensitive to the evaluation of clopidogrel-induced platelet inhibition.

### Conclusion

At present, no platelet function assay can be acclaimed as optimal for quantifying the inhibition of platelet aggregation by clopidogrel. Although most studied assays were sensitive to clopidogrel-induced platelet inhibition, the results showed only weak association among platelet function test results, and agreement between tests to select patients requiring intensified clopidogrel therapy was accordingly low. Consequently, before implementing guidelines influencing clinical decision as recommended by recent American guidelines, the assay and cut-off values that should be used to direct medical conduct in terms of clopidogrel therapy need to be specifically evaluated. At the present time, we believe that more work needs to be done to better understand the shortcomings of various platelet function assays. Large prospective clinical trials are necessary to determine the clinical value of platelet function tests, and their use in a clinical setting should therefore be avoided as long as the results of such studies are pending.

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### Conflict of interest

none declared.

### References


### Table 3 Agreement to identify patients with insufficient platelet inhibition (≤50%) among assays as assessed by the κ statistic

<table>
<thead>
<tr>
<th>Platelet function test</th>
<th>LTA ADP 20 μM</th>
<th>WBA ADP 5 μM</th>
<th>WBA ADP 20 μM</th>
<th>VerifyNow® P2Y12</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA ADP 5 μM</td>
<td>0.679a</td>
<td>–0.117</td>
<td>0.057</td>
<td>0.295a</td>
</tr>
<tr>
<td>LTA ADP 20 μM</td>
<td>–0.187a</td>
<td>0.101</td>
<td>0.364a</td>
<td>–0.047</td>
</tr>
<tr>
<td>WBA ADP 5 μM</td>
<td>0.308a</td>
<td>–0.047</td>
<td>0.132</td>
<td>–0.117 0.057 0.295a</td>
</tr>
<tr>
<td>WBA ADP 20 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is generally accepted that a κ statistic of 0–0.2 translates into slight agreement, 0.2–0.4 into fair agreement, 0.4–0.6 into moderate agreement, 0.6–0.8 into substantial agreement, and 0.8–1 into almost perfect agreement.

*Agreement (κ statistic) is significant at *P* < 0.05.