Comparison of current platelet functional tests for the assessment of aspirin and clopidogrel response

A review of the literature

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Summary
The two most widely used antiplatelet drugs in the world are aspirin and clopidogrel. However, some patients on aspirin and/or clopidogrel therapy do not respond appropriately to either aspirin or clopidogrel. This phenomenon is usually called “aspirin/clopidogrel resistance”. Several platelet function tests have been used in various studies for the assessment of aspirin and clopidogrel resistance in healthy individuals and patients admitted in cardiology departments. An accurate assessment of platelet response to aspirin/clopidogrel could benefit patients by proposing tailored-antiplatelet therapy based on test results. However, there is a clear lack of standardisation of such techniques and their analytical variability may induce misinterpretation. After a quick report of the mechanisms responsible for aspirin/clopidogrel resistance, we describe the pre-analytical aspects and the analytical performances of current platelet function tests (Light-transmission aggregometry, whole-blood aggregometry, VerifyNow®, Platelet Function Analyzer®, thromboelastography, VASP assay) that are used for the assessment of aspirin/clopidogrel resistance in clinical studies. Considering the different variables that have to be taken into account with each of the platelet function tests, a particular attention should be paid when interpreting results.

Keywords
Antiplatelet agents, aspirin resistance, clopidogrel resistance, platelet function test, aggregometry

Introduction
The two most widely used antiplatelet drugs in the world are aspirin (or acetylsalicylic acid) and clopidogrel. Both are efficient for secondary prevention of cardiovascular diseases. Because they exhibit different mechanisms of action (▶ Figure 1), they can be prescribed either alone or in combination. Aspirin is a non-selective and irreversible cyclooxygenase (COX) inhibitor. It irreversibly inhibits intra platelet COX-1 isoform thus preventing the production of the potent platelet agonist thromboxane A2 (TXA2). Thromboxane A2 is an amplifying signal for other agonists, and so inhibition of COX-1 by aspirin modulates several pathways of platelet activation (1). Clopidogrel, a second generation thienopyridine, is a prodrug that needs to be converted into its active thiol metabolite by various hepatic cytochrome P450 enzymes, mainly CYP2C19 (2–4), to then bind irreversibly to the adenosine diphosphate (ADP)-P2Y12 receptor expressed on the platelet surface (5, 6). Despite a short half-life in blood after digestive absorption, both aspirin and clopidogrel are characterised by a long-lasting antiplatelet effect (7).

It is estimated that inhibition by aspirin has to be more than 95% in terms of platelet TXA2-forming capacity to be clinically efficient (8). Such a level of inhibition of platelet-dependent TXA2 is usually reached with regular low doses of aspirin. In contrast, a standard dose of clopidogrel will achieve incomplete P2Y12 antagonism, which translates into approximately 50% inhibition of ADP-induced platelet aggregation (9). Some patients on aspirin and/or clopidogrel therapy do not respond appropriately to these drugs. This phenomenon is usually described as “aspirin/clopidogrel resistance”. Interestingly, the prevalence of aspirin and clopidogrel resistance ranges from 0.4 to 83% (7, 10–13) and from 4–30% (14–19), respectively; the values are highly dependent on the assay used to assess platelet inhibition due to aspirin/clopidogrel therapy, cut-off values chosen, and population tested. Also, platelet inhibition by clopidogrel is both dose- and time-dependent (20), and patient-specific (16, 17). When interpreting the results of the various platelet function tests (PFTs) the mechanisms that could lead to aspirin/clopidogrel resistance are of interest. We believe that distinguishing extrinsic from intrinsic mechanisms may be relevant (▶ Table 1), in particular as extrinsic mechanisms are the most commonly identified reason for aspirin/clopidogrel resistance (7). Therefore the first step when evaluating a potentially aspirin/clopidogrel-resistant patient is to examine dosage, compliance, and possible drug interactions. The second step is to use PFTs to evaluate the inhibition of platelets as a result of antiplatelet therapy. Several laboratory methods have been proposed;
however, they have their own advantages and limitations (Table 2), and they exhibit significant intra- and inter-individual variability that will be highlighted in this review.

For this, the MEDLINE database was searched for relevant articles whose keywords included various combinations of “aspirin” or “clopidogrel” with following terms: “resistance”, “platelet function tests”, “light-transmission aggregometry”, “whole-blood aggregometry”, “VerifyNow”, “PFA 100”, and “VASP”. Progressively, we excluded i) articles that evaluated the prevalence of aspirin/clopidogrel resistance based on a single platelet function test, ii) articles that did not compare various platelet function tests with the gold-standard method (the light-transmission aggregometry, LTA, method), iii) articles that did not measure the coefficient of variation of the methods used before reporting results.

Aggregometry tests

**Light-transmission aggregometry (LTA)**

LTA, which was invented by Born (22), is the oldest available method evaluating platelet function. It is still considered as the “gold standard” to assess both aspirin and clopidogrel response, despite a lack of standardisation (Table 3) (23). It evaluates luminosity using an optical aggregometer with a fixed wavelength spectrophotometer as aggregation occurs in platelet-rich plasma (PRP) under stirring conditions following stimulation with a platelet agonist (24). Blood can either be drawn into a 3.8 % or a 3.2 % trisodium citrate tube, and preparation of PRP should be made within 2 hours (h) after blood sampling. Of note, citrate interferes with the involvement of calcium in platelet activation and aggregation by considerably reducing the normal plasma calcium concentration (25). Therefore, it provides misleading information on platelet reactivity and antiplatelet drug effects in vivo (20), notably by overestimating the platelet aggregation inhibitory activity of these drugs (26). As described in a recent survey from the International Society on Thrombosis and Haemostasis (ISTH), we noticed a wide range in LTA protocols among laboratories, notably in terms of preparation of PRP. Most of the laboratories centrifuge blood at 150 g for 10 minutes (min) to obtain PRP. Even though no significant difference

**Figure 1: Mechanisms of action of clopidogrel and aspirin on platelet function.**

<table>
<thead>
<tr>
<th><strong>Aspirin “resistance”</strong></th>
<th><strong>Clopidogrel “resistance”</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrinsic mechanisms</td>
<td></td>
</tr>
<tr>
<td>• Non-compliance to treatment</td>
<td>• Non-compliance to treatment</td>
</tr>
<tr>
<td>• Under/inappropriate dosing of aspirin</td>
<td>• Under/inappropriate dosing of clopidogrel</td>
</tr>
<tr>
<td>• Drug interactions: NSAIDs (ibuprofen)</td>
<td>• Drug interactions: i.e. statins (HMG COA reductase inhibitors), calcium-channel blocker, omeprazole</td>
</tr>
<tr>
<td>Intrinsic mechanisms</td>
<td></td>
</tr>
<tr>
<td>• Genetic variables</td>
<td>• Genetic variables</td>
</tr>
<tr>
<td>• COX 1 A842&gt;G polymorphism (21)</td>
<td>• Polymorphisms of P2Y12 receptor: i.e. H2 haplotype</td>
</tr>
<tr>
<td>• Polymorphism on the gene encoding the aIIbβ3 P1 A2A2</td>
<td>• CYP2C19*2 polymorphism (3)</td>
</tr>
<tr>
<td>• Increased release of ADP</td>
<td>• Increased release of ADP</td>
</tr>
<tr>
<td>• Alternate pathways of platelet activation:</td>
<td>• Alternate pathways of platelet activation:</td>
</tr>
<tr>
<td>• Failure to inhibit catecholamine-mediated platelet activation (epinephrine)</td>
<td>• Failure to inhibit catecholamine-mediated platelet activation (epinephrine)</td>
</tr>
<tr>
<td>• Up-regulation of COX-independent pathways (thrombin, TXA2, collagen)</td>
<td>• Greater extent of P2Y1-dependent platelet activation</td>
</tr>
<tr>
<td>• High platelet turn-over: surgery, trauma, myeloproliferative syndrome</td>
<td>• Up-regulation of P2Y12-independent pathways (thrombin, TXA2, collagen)</td>
</tr>
</tbody>
</table>
was noted between 150 g and 200 g, it has been shown that the increase of maximum platelet aggregation induced by ADP (ADP$_{max}$) (27). Thus, the centrifugal force used to prepare PRP should be taken into account when interpreting the results of LTA, for the assessment of aspirin/clopidogrel response. Another concern is whether the platelet count in PRP should be adjusted or not. Taking into account that a wide interindividual variability exists in the native platelet count in PRP, many studies used adjusted PRP (Table 3) with autologous platelet-poor plasma (PPP), to achieve a platelet count in PRP of around 250,000/µl. However, it has been shown that adjusting PRP for the assessment of aspirin/clopidogrel responses, either in healthy subjects (28) or in patients undergoing percutaneous intervention (PCI) (29), may reduce both the peak level (29, 30) and the maximum velocity of aggregation (14) irrespective of the agonist and its concentration used for inducing platelet aggregation. The correlation between the platelet count measured in EDTA anticoagulated whole blood and platelet count measured in native PRP is usually excellent (29), supposing that platelet aggregation induced in native PRP might better reflect the in vivo platelet function. Considering that platelet count in adjusted PRP may falsify the individual responses to platelet agonists, by artificially increasing the prevalence of aspirin/clopidogrel resistance, and that platelet function can be compared between patients in native PRP, adjusting platelet count in PRP is no longer recommended.

Because aspirin does not completely inhibit platelet function not regulated by TXA2, an appropriate assay evaluating aspirin efficacy should be specific to the platelet COX pathway. For this reason LTA using 1-1.6 mM arachidonic acid (AA) as agonist is an excellent assay to detect platelet inhibition by aspirin (31–33). LTA efficacy should be specific to the platelet COX pathway. For this reason LTA using 1-1.6 mM arachidonic acid (AA) as agonist is an excellent assay to detect platelet inhibition by aspirin (31–33). LTA induced by low collagen (1 µg/ml), epinephrine (5 to 10 µM), or ADP (1 to 3 µM) concentrations, are also relatively dependent on TXA2 pathway (33, 34). In 2008, a study evaluated coefficients of variance (CV) of LTA in 20 healthy men, receiving aspirin treatment during 11 consecutive days (35). For duplicate tests the CVs were below 4.3 % for all agonists at baseline, and increased considerably during treatment with aspirin to reach 21.1 % when AA

### Table 2: Technical aspects of various platelet function tests used in laboratories for assessing aspirin and/or clopidogrel response.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Principle</th>
<th>POC</th>
<th>Platelet source</th>
<th>Sample volume</th>
<th>Sample temp.</th>
<th>Preincubation time</th>
<th>Per-acquisition time</th>
<th>Rheological conditions</th>
<th>Agonists to assess aspirin resistance</th>
<th>Agonists to assess clopidogrel resistance</th>
<th>Results expresion</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA</td>
<td>Optical aggregometry</td>
<td>No</td>
<td>PRP</td>
<td>380 µl</td>
<td>37 °C</td>
<td>3 to 5 min</td>
<td>6 to 10 min</td>
<td>Variable low shear stirring</td>
<td>AA ± ADP collagen</td>
<td>ADP, ADP + PGE1</td>
<td>MA (%)</td>
</tr>
<tr>
<td>Multiplate® (Roche Diagnostics, Meylan, France)</td>
<td>Impedance aggregometry</td>
<td>Yes</td>
<td>WB</td>
<td>37 °C</td>
<td>3 to 5 min</td>
<td>6 to 10 min</td>
<td>Stirling at 1000 rpm</td>
<td>Collagen/ EPI (10 µg) = Collagen/ADP (50mg) cartridges</td>
<td>AA</td>
<td>ADP</td>
<td>Ohms, AU, AUC</td>
</tr>
<tr>
<td>PFA-100® (Siemens Health-care Diagnostics Products GmbH, Marburg, Germany)</td>
<td>Aggregometry</td>
<td>Yes</td>
<td>WB</td>
<td>800 µl</td>
<td>37.9 ± 1 °C</td>
<td>&lt; 15 min</td>
<td>6 to 10 min</td>
<td>Whole-blood aspiration flow under high shear forces</td>
<td>Collagen/ Epi (10 µg) = Collagen/ADP (50mg) cartridges</td>
<td>-</td>
<td>Seconds</td>
</tr>
<tr>
<td>VerifyNow® (Accriva diagnostic, San Diego, CA, USA)</td>
<td>Optical aggregometry</td>
<td>Yes</td>
<td>WB</td>
<td>2 ml</td>
<td>37 °C</td>
<td>10–30 min</td>
<td>3 to 5 min</td>
<td>Fibrinogen-coated microparticles</td>
<td>AA</td>
<td>ADP (+ PGE1)</td>
<td>ARU/PRU</td>
</tr>
<tr>
<td>TEG® Platelet Mapping (Hae-mostscope Corp., Niles, IL, USA)</td>
<td>Elastography</td>
<td>Yes</td>
<td>WB</td>
<td>360 µl</td>
<td>37 °C</td>
<td>3 to 5 min</td>
<td>30 min</td>
<td>Shear elasticity</td>
<td>AA (1 mM)+ activator F</td>
<td>ADP (2 µM) + activator F</td>
<td>MA (mm)</td>
</tr>
<tr>
<td>Flow cytometry VASP assay (Biocytex, Marseille, France)</td>
<td>Cytometry</td>
<td>No</td>
<td>WB</td>
<td>30 µl</td>
<td>NA</td>
<td>NA</td>
<td>&gt; 3 h</td>
<td>NA</td>
<td>NA</td>
<td>ADP (+PGE1)</td>
<td>PRI</td>
</tr>
</tbody>
</table>

Note: additional time has to be considered when LTA is used due to PRP and reagents preparation. POC: point of care test; PRP: platelet-rich plasma; WB: whole blood; MA: maximal amplitude; AUC: area under curve; Epi: epinephrine; ARU: aspirin reaction unit; PRU: P2Y12 reaction unit; MFI: mean fluorescence intensity; NA: non applicable.
Table 3: Studies using LTA to assess either aspirin or clopidogrel response and highlights the pre analytical discrepancies between laboratories.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population (n)</th>
<th>Sodium citrate concentration</th>
<th>Time between sampling and analysis</th>
<th>Centrifugation for PRP</th>
<th>PRP (adjusted/native)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lordkipanidze et al., 2007 (48)</td>
<td>201 patients with stable CAD</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>1000 rpm × 10 min</td>
<td>Adjusted 250–450 G/l</td>
</tr>
<tr>
<td>Madsen et al., 2008 (35)</td>
<td>20 healthy subjects</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>200 g × 6 min</td>
<td>Native</td>
</tr>
<tr>
<td>Pedersen et al., 2009 (49)</td>
<td>21 healthy subjects and 43 patients with CAD</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>100 g × 15 min</td>
<td>Native</td>
</tr>
<tr>
<td>Nielsen, et al., 2008 (50)</td>
<td>21 healthy subject and 40 patients with CAD</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>100 g × 15 min</td>
<td>Native</td>
</tr>
<tr>
<td>Harrison et al., 2008 (51)</td>
<td>?</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>250 g × 10 min</td>
<td>ND</td>
</tr>
<tr>
<td>Madsen et al., 2010 (36)</td>
<td>26 PCI</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>150 g × 12 min</td>
<td>Native</td>
</tr>
<tr>
<td>Santili et al., 2009 (52)</td>
<td>48 healthy subjects</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Native</td>
</tr>
<tr>
<td>Liang et al., 2012 (53)</td>
<td>82 patients with CAD</td>
<td>ND</td>
<td>ND</td>
<td>200g × 8 min</td>
<td>Native</td>
</tr>
<tr>
<td>Gaglia et al., 2011 (54)</td>
<td>200 patients with PCI</td>
<td>3.2 %</td>
<td>&lt;3 h</td>
<td>1000 rpm ×10 min</td>
<td>Native</td>
</tr>
<tr>
<td>Gremmel et al., 2011 (55)</td>
<td>288 patients with PCI</td>
<td>3.8 %</td>
<td>&lt;3 h</td>
<td>150 g × 10 min</td>
<td>Native</td>
</tr>
<tr>
<td>Linneman et al., 2010 (56)</td>
<td>40 patients with PAOD (peripheral artery occlusive disease)</td>
<td>3.2 %</td>
<td>&lt;3 h</td>
<td>140 g × 5 min</td>
<td>Native</td>
</tr>
<tr>
<td>Tsantes et al., 2012 (57)</td>
<td>90 patients with CAD and 20 healthy</td>
<td>3.8 %</td>
<td>&lt;2 h</td>
<td>200 g × 10 min</td>
<td>Adjusted 200–300 G/l</td>
</tr>
<tr>
<td>Kim et al., 2013 (58)</td>
<td>466 East Asian patients with PCI</td>
<td>3.2 %</td>
<td>ND</td>
<td>120 g × 10 min</td>
<td>Adjusted 250 G/l</td>
</tr>
<tr>
<td>Bagoly et al., 2013 (59)</td>
<td>114 patients with non-cardiogenic ischaemic cerebrovascular disease</td>
<td>3.2 %</td>
<td>&lt;4 h</td>
<td>150 g × 10 min</td>
<td>Adjusted 250 G/l</td>
</tr>
<tr>
<td>Tantry et al., 2005 (13)</td>
<td>6 healthy subjects + 203 patients with PCI + 20 patients with stent thrombosis</td>
<td>3.8 %</td>
<td>ND</td>
<td>120 g × 5 min</td>
<td>Native</td>
</tr>
<tr>
<td>Blais et al., 2009 (60)</td>
<td>45 healthy subject</td>
<td>ND</td>
<td>&lt;4 h</td>
<td>ND</td>
<td>Adjusted 200–300 G/l</td>
</tr>
<tr>
<td>Meen et al., 2009 (47)</td>
<td>79 patients with PCI + 16 healthy subjects</td>
<td>3.8 %</td>
<td>ND</td>
<td>190 g × 10 min</td>
<td>Adjusted 300 G/l</td>
</tr>
<tr>
<td>Varenhorst et al., 2009 (61)</td>
<td>?</td>
<td>3.8 %</td>
<td>&lt;3 h</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bidet et al., 2010 (62)</td>
<td>81 patients with ACS</td>
<td>3.8 %</td>
<td>ND</td>
<td>150 g × 10 min</td>
<td>ND</td>
</tr>
<tr>
<td>Hochholzer et al., 2007 (63)</td>
<td>27 patients with PCI</td>
<td>ND</td>
<td>ND</td>
<td>750 g × 2min</td>
<td>Adjusted 275–325 G/l</td>
</tr>
<tr>
<td>Dyszkiewicz-Korpanty et al., 2007 (64)</td>
<td>17 healthy subjects</td>
<td>ND</td>
<td>ND</td>
<td>170 g × 10 min</td>
<td>Adjusted 200–300 G/l</td>
</tr>
<tr>
<td>Bouman et al., 2010 (65)</td>
<td>20 patients with PCI</td>
<td>3.2 %</td>
<td>&lt;2 h</td>
<td>ND</td>
<td>Native</td>
</tr>
</tbody>
</table>

CAD = coronary artery disease; PCI = percutaneous coronary intervention; ACS = acute coronary syndrome; PAOD = peripheral artery occlusive disease; ND = non determined.
was used as agonist. The latter value may be explained by the low aggregation rates they obtained. A few years later, the same group found duplicate test CVs among 26 patients who underwent PCI were lower: 0.6% and 1.2% for LTA using AA as agonist (AA-LTA) at baseline and after long-term aspirin treatment, respectively (36). More recently, intraassay and interassay CVs of 1.5 mM AA-LTA from donors on daily aspirin therapy were found to be higher: 10.1% and 37.4%, respectively (37).

For clopidogrel, since it specifically inhibits P2Y12 ADP-receptor (38), ex vivo measurement of ADP<sub>max</sub> by LTA has been the most commonly used laboratory method to evaluate clopidogrel response and is considered to be the gold standard (16, 39, 40). The most common differences in the methods used include ADP concentration used to induce aggregation and the timing of analysis of aggregation curves. Some authors even recommend measuring the residual post-treatment P2Y12 activity by measuring ADP-induced platelet aggregation (ADP-LTA) before and after first intake of clopidogrel (41, 42). As the response to low concentrations of ADP is dependent on the generation of TXA2 and is inhibited by aspirin (7), higher concentrations of ADP such as 5-20 µM should be used to assess platelet response to clopidogrel. Indeed, a high concentration of ADP induces full and irreversible platelet aggregation, is insensitive to aspirin, but is inhibited by up to 90% in the presence of a P2Y12 antagonist. As clopidogrel does not inhibit the P2Y1 ADP-receptor, a first wave aggregation can be seen by stimulation with ADP. That is why the difference of aggregation before and after first intake may be more relevant to assess platelet response to ADP stimulation during clopidogrel treatment. Some authors found greater absolute response to clopidogrel treatment by measuring late platelet aggregation at 6 minutes after stimulation with ADP (ADP<sub>6min</sub>) than ADP<sub>max</sub> in healthy subjects (35, 43), as well as in coronary artery disease (CAD) patients (30). However, several studies have shown an excellent correlation and a high degree of agreement between ADP<sub>max</sub> and ADP<sub>6min</sub> either at baseline or during clopidogrel treatment (35, 36, 41, 44–46) and regardless of the ADP concentrations used, suggesting that both values are equally informative (41). In parallel, it has been demonstrated that treatment with clopidogrel increased the variability of ADP-LTA in comparison with baseline. Several studies found a more variable response when ADP<sub>6min</sub> was assessed in comparison with ADP<sub>max</sub> (46, 47). Intra-assay CVs for ADP<sub>max</sub> and ADP<sub>6min</sub> were ranging from 4.3% to 11.3% and from 9.7% to 17.1% at baseline, respectively, and reached 5.4–12.9% and 13.4–17.5% after clopidogrel treatment, respectively (37, 46, 47).

**Whole blood aggregometry (WBA)**

Whole blood aggregometry monitors the proportional increase of electrical impedance between two electrodes immersed in whole blood due to aggregation of agonist-stimulated platelets. A new fully computerised five-channel WBA instrument, which is called Multiple Platelet Function Analyser (Multiplate<sup>®</sup>,Roche Diagnostics, Meylan, France; ▶ Table 2) (66, 67), has become available with disposable ready-to-use cuvettes containing two independent sensor units, and with a range of different agonists for monitoring antiplatelet therapy (68). Several anticoagulants can be used: citrate, heparin, or hirudin. Whilst WBA is unaffected by icterus and lipidemia, haemolysis can still cause interference because it implies potential platelet activation. In case of thrombocytopenia with 50,000 to 100, 000/µl, samples may be assayed undiluted except when using ADP (69).

Several studies have shown that WBA is more sensitive to the effect of antplatelet drugs than is LTA, and could detect aspirin effects for a longer period of time in healthy controls (70, 71). Recently, Pedersen et al. (49) determined repeatability (with duplicate measurements) and day-to-day variation (calculated on the basis of duplicate measurements of platelet aggregation during four consecutive days) of Multiplate<sup>®</sup> versus LTA in 21 healthy subjects before and after treatment with aspirin, and in 43 patients suffering from CAD with long-term aspirin treatment. The repeatability was good in healthy individuals at baseline, with a CV value of 8%, whereas it was not good during aspirin treatment in both healthy individuals and patients, with a CV value at 46% each. The day-to-day variation of the Multiplate<sup>®</sup> was better than that of LTA among healthy individuals, (CV 12% vs 34%, respectively), and was comparable among CAD patients, with CV values about 24% each. Of note, both the median AUC measured by Multiplate<sup>®</sup> and maximal aggregation measured by LTA were reduced to a similar extent (by 90%) during aspirin treatment compared to baseline. However, the correlation between Multiplate<sup>®</sup> and LTA measurements in healthy individuals and patients, was quite low at both baseline and during aspirin treatment (Spearman’s correlation coefficient r=0.26). More recently a study comparing five PFTs also observed that intraassay and interassay CVs of the Multiplate<sup>®</sup> device considerably increased with the intake of aspirin treatment, from 5.2% to 16.3%, and from 9.7% to 24.7%, respectively (37).

It also has been reported that WBA was better than LTA to assess clopidogrel response in healthy subjects. Among 17 healthy subjects after 10 days of daily 75 mg clopidogrel treatment, WBA detected one subject that was totally non-responsive to clopidogrel, exhibiting the same results as in PRP, and using the same concentrations of ADP used as agonist for LTA (64). When comparing the platelet responses to clopidogrel by Multiplate<sup>®</sup> and LTA, in patients suffering from CAD, ADP<sub>max</sub> of both instruments were found to decrease significantly and to a similar extent (Spearman’s correlation coefficient of 0.71 and 0.73; p<0.0001) (63, 72, 73). One study evaluated the precision of both Multiplate<sup>®</sup> and LTA, measured as the CV determined by five consecutive measures per sample, in 10 healthy control individuals and 297 patients suffering from acute coronary syndrome (ACS) and receiving dual antiplatelet therapy, with 500 mg and 600 mg loading doses, followed by 325 mg and 75 mg daily of aspirin and clopidogrel, respectively (73). The same concentrations of agonists were used for both analyses. The mean CVs were higher in patients with ACS than in controls for each agonist: 3.9% and 6.2% for 10 µM ADP, 5.1% and 6.1% for 1 mM AA, and 4.5% and 6.6% for 2 µg/ml collagen, respectively. The same trend was observed for the CVs of LTA, which were similar. Significant correlations between the Multiplate<sup>®</sup> and LTA were observed after stimulation with ADP, AA, and collagen. By using ROC curve analysis, they determined proper

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cut-off values for both analyses. The cut-off values for Multiplate were as follow: 37 AU for ADP, 18 AU for AA, and 42 AU for collagen. They considered cut-off values for LTA, determining anti-platelet drug resistance, as being maximal aggregation >70%, >20%, and >56% for ADP, AA and collagen, respectively. Therefore, the sensitivity and specificity values of the Multiplate system by assuming LTA as the reference method and by using cut-off values obtained from ROC curves were determined. Whilst the sensitivity was low and highly variable depending on the agonist, ranging from 55% to 78% for AA and ADP, respectively, the specificity was satisfactory, being >95% for each agonist.

Recently, the Multiplate Prostaglandin E1 (PGE1) reagent has become available, and has allowed an increase in the sensitivity of the ADP test to platelet inhibition by clopidogrel. Indeed, the addition of PGE1 reduces the platelet activation via the P2Y1-receptor and enables a more specific evaluation of the P2Y12-receptor pathway. The modified assay is named ADPtest HS (high sensitivity) and has shown strong correlation with the ADPtest in a recent cohort (74). Calculated optimal cut-off value for the prediction of ischemic events was ≥ 430 AU.min⁻¹. Intra-assay and day-to-day CVs for the ADPtest HS have not been published yet, and further studies have to be undertaken to determine if P2Y12-receptor specific testing has any advantage for prediction of clinical outcome.

**Point-of-care tests**

**VerifyNow™ assay**

The VerifyNow™ (Accriva Diagnostics, San Diego, CA, USA) has been specifically designed to detect antiplatelet drug resistance (75). Platelet aggregation in whole blood is monitored by light transmission through two duplicate reaction chambers in each cartridge (24, 32, 76). Specialised cartridges containing agonist, are available for the measurement of platelet responses to either aspirin (VerifyNow® Aspirin) or clopidogrel (VerifyNow® P2Y12).

The VerifyNow® Aspirin™ (VNA) detects agglutination of fibrinogen-coated beads in response to an agonist of COX-1 pathway by an increase of light transmission. If aspirin produced an antiplatelet effect, the beads do not agglutinate and light transmission is unchanged (34). The VNA cartridge originally contained propyl gallate (77–79) as agonist that has been replaced by AA (48, 80–82), with supposedly improved specificity.

Recently, a study conducted on 21 healthy volunteers and 40 patients with stable CAD treated with 75 mg daily aspirin examined the performance of the VNA system in comparison with AA-LTA (50). The VNA system showed a high degree of repeatability on duplicates, with a CV value of 0.5% at baseline and at 3% during aspirin treatment. The day-to-day variation during aspirin treatment was low in both healthy volunteers and patients; both had a CV of 3%. Both CVs for duplicate measurements and day-to-day variation were lower for the VNA system than for LTA, and this finding is consistent with the results of a recent study in both healthy individuals and donors on daily aspirin therapy that reported intra- and interassay CVs of VNA <5% (37). Using 1 mM AA-LTA as reference method to assess aspirin efficacy, and the cut-off value ≥ 550 ARU (according to manufacturer’s recommendation), Nielsen et al. found a poor sensitivity (30%) and a good specificity (91%). Surprisingly, sensitivity of the VNA system was found to be 100%, compared to 1.6 mM AA-LTA, in 201 patients with CAD (48). Santilli et al. (52), found a higher duplicate CV (19%) in 48 healthy subject receiving 100 mg daily of aspirin, and the correlation with AA-LTA was high (r=0.75; p<0.001).

For clopidogrel response assessment, the VerifyNow® P2Y12 (VNP) cartridge contains two chambers. One contains ADP that activates the platelets and PGE1 that suppresses platelet activation through P2Y1-receptor. The use of both ADP and PGE1 increases intracellular cyclic adenosine monophosphate (cAMP), theoretically enhancing the sensitivity and the specificity of the test for ADP-induced activation. VNP test results are usually expressed in arbitrary P2Y12 Reaction Units (PRU) and % P2Y12 inhibition thanks to the second chamber that contains thrombin receptor-activating peptide (TRAP) that stimulates platelets, producing a "BASE" value used for percent platelet inhibition as follows: [100-(BASE-PRU)]/BASE. It reflects the percent inhibition of the contribution from ADP-stimulated platelets to maximal clot strength (36).

Usually, sodium citrate is the standard anticoagulant used with the VNP assay but it has recently been demonstrated that hirudin provided more stable results over time. Results were strongly correlated with those obtained from citrated-blood samples (R=0.95; p<0.0001) (83, 84). It has been proved several times that the VNP results correlate reasonably well with ADP-LTA during clopidogrel therapy (36, 85, 86), with Spearman’s correlation coefficients of about 0.6 anytime, or Pearson’s correlation coefficient of about 0.8 (61). Intra- and interassay CVs of this method were recently measured; they were higher in donors under clopidogrel treatment (intra- and interassay CVs at 7.3% and 12.9%, respectively) than in healthy individuals (intra- and interassay CVs at 4.4% and 5.2%, respectively) (37). The VERITAS study conducted in 147 patients with multiple cardiovascular risk factors, demonstrated that the VNP assay is sensitive for the measurement of platelet inhibition with clopidogrel (87).

**PFA-100® and INNOVANCE PFA P2Y®**

The PFA-100® (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) assesses platelet aggregation in anticoagulated whole blood under high shear forces (5,000–6,000/seconds). It records the closure time (CT) of a 150 µm aperture collagen-coated membrane. Two other different coatings, considered as agonists, are available: 10 µg of epinephrine bitartrate (CEPI cartridge) and 50 mg of ADP (CADP cartridge) (35). When interpreting the results obtained with PFA-100®, many variables have to be taken into account, including von Willebrand factor level, haematocrit, and platelet count (88).

Duplicate and day-to-day CVs of PFA-100® were determined after aspirin intake in healthy individuals (35), and patients with CAD (73). Basically, duplicate CVs for the CEPI-CT were higher than duplicate CVs for the CADP-CT at baseline as well as during aspirin therapy, probably because the CEPI-CT in response to
aspirin is usually prolonged whereas the CADP-CT remains within the normal range (89, 90). Duplicate CVs for the CEPI-CT and for the CADP-CT were 13.5% and 10.6% at baseline, respectively. Both duplicate CVs increased during aspirin treatment, to reach 20.1% and 13.1%, respectively (35), which is concordant with previous findings attesting that ingestion of aspirin increases the CV of the CEPI cartridge in a dose-dependent manner (91). Day-to-day CVs for the CEPI-CT were also found higher than day-to-day CVs for the CADP-CT (35, 73), ranging from 5.4% to 14.2% and from 4.3% to 13.1%, respectively. It is also noticeable that both day-to-day variation CVs for the CEPI-CT and the CADP-CT were higher in patients with CAD, >9% each, compared to that found in healthy individuals (CVs around 5%) (73, 92). When compared with AA-LTA, better correlation was found with PFA-100® CEPI-CT (Spearman’s correlation coefficient r = 0.74; p < 0.0001), and sensitivity and specificity of CEPI-CT were 75% and 40%, respectively (48).

Unfortunately, the PFA-100®, even with the CADP cartridge, is unable to detect the clopidogrel effect on platelet function (14, 64, 86, 93–97). Therefore a new PFA-100® test cartridge, called INNOVANCE PFA P2Y®, has been developed to specifically assess platelet aggregation in response to clopidogrel treatment. It is coated with 20 µg ADP 5 ng PGE1, and 459 µg calcium chloride (56). The mean duplicate CVs in patients with cardiovascular diseases, range from 7.4% to 11.3% (36, 57). The authors concluded that INNOVANCE PFA P2Y® might be a suitable test to assess platelet response to clopidogrel but the clinical relevance of INNOVANCE PFA P2Y® results remains to be established.

**Flow cytometric VASP phosphorylation assay**

Flow cytometry is another laboratory test for assessing platelet response to antiplatelet therapy. AA- or ADP-induced CD62P (P-selectin) externalisation can be used to monitor aspirin-mediated inhibition of platelet COX-1 (109) or to identify clopidogrel responsiveness (16, 110). However, to date, the most specific assay measuring clopidogrel effects, relies on flow cytometric measurement of the level of vasodilator-stimulated phosphoprotein (VASP) phosphorylation (111). The level of VASP phosphorylation has been linked to the degree of platelet fibrinogen binding and integrin αIIbβIIIa activation through ADP-dependent P2Y12 receptor pathways (112). VASP is an intraplatelet actin protein that is non-phosphorylated at basal state. Its phosphorylation is enhanced by PGE1 via the increase of the cAMP level. In contrast, ADP inhibits PGE1-induced VASP phosphorylation through P2Y12 receptors, an effect that cannot be detected in platelets isolated from patients receiving clopidogrel treatment. The platelet VASP/P2Y12 kit from Biocytex (Marseille, France) is usually used in trials (53, 58, 113, 114). Basically, PGE1 either alone or in combination with ADP is added to anticoagulated whole blood. Platelets are then labelled with a phosphospecific monoclonal antibody directed against serine 239-phosphorylated VASP, such as 16C2. Mean fluorescence intensity (MFI) corresponding to each experimental condition, resting (PGE1 alone) and activated
platelets (ADP and PGE1) is determined to establish a ratio that directly correlates with the VASP phosphorylation state and is expressed as a mean percentage of platelet reactivity. The magnitude of platelet activation is expressed as the platelet reactivity index (PRI) in percentage, which is calculated using the following formula:

$$\text{PRI} (%) = 100 \times \left( \frac{\text{MFI PGE1} - \text{MFI PGE1} + \text{ADP}}{\text{MFI PGE1}} \right)$$

Clopidogrel strongly attenuates the inhibitory effect of ADP on PGE1-stimulated VASP phosphorylation (115), thus being responsible for the persistence of VASP phosphorylation induced by PGE1 even with the simultaneous addition of ADP. Importantly, this method allows to specifically detect the effects of clopidogrel irrespective of concomitant antiplatelet therapy, such as aspirin or integrin αIIbβIII inhibitors (116). Comparison of the VASP assay with LTA shows that the level of inhibition is higher in the flow cytometry assay because non-specific aggregation can occur via ADP stimulation of P2Y1 in LTA (58, 114). Indeed, the study conducted among 466 Asian patients undergoing PCI (58) demonstrated a median PRI of 58.1 % after a 600 mg-clopidogrel loading dose, compared to median values of 5–20 μM ADPmax-LTA at 39 % and 55 %, respectively. Data regarding correlation between VASP assay and LTA vary among studies. Spearman’s correlation coefficients are usually slightly above 0.5 (58, 95, 117), and the degree of correlation seems to depend on the concentration of ADP used for LTA, increasing with higher concentration (i.e 20 μM) (58), and on the PRI levels. Sensitivity and specificity using classic 5 μM ADP-LTA cutoff (<46 %) have been calculated at 84.5 % and 71.7 %, respectively. Using the cut-off value of <39 % ADPmax after 20 μM ADP-LTA, the sensitivity increased to 90.6 %, and the specificity of VASP assay remained similar at 70.2 % (58). A lower volume of reagents could be used since high correlation was observed between full volume and half or quarter volume of reagent used in the VASP kit assay (113); the reproducibility was good (CVs around 14%). They further confirmed that the geometric mean fluorescence intensity (FI) should be used for calculation of PRI, as stated by the manufacturer’s instructions, because PRI was found 4 % and 6 % higher than when calculated with mean FI in healthy individuals and patients with CAD with clopidogrel therapy. As many studies prefer to use the mean FI (118, 119) or the median FI (114, 120), it appears that it should be recommended to report which kind of FI was used for calculating the PRI.

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<table>
<thead>
<tr>
<th>Studies</th>
<th>Population</th>
<th>Loading/daily doses of aspirin (mg)</th>
<th>Clopidogrel concomitant treatment</th>
<th>[AA] (mM)</th>
<th>Parameter</th>
<th>Cut-off values</th>
<th>Other PFTs used in comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lordkipanidze et al., 2007 (48)</td>
<td>201 patients with stable CAD</td>
<td>-80</td>
<td>-</td>
<td>1.6</td>
<td>MA</td>
<td>≥20%</td>
<td>WBA, PFA-100®&lt;sup&gt;®&lt;/sup&gt;, VN Aspirin</td>
</tr>
<tr>
<td>Madsen et al., 2008 (35)</td>
<td>20 healthy subjects</td>
<td>300/75</td>
<td>-</td>
<td>1.5</td>
<td>MA</td>
<td>≥20%</td>
<td>PFA 100®&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pedersen et al., 2009 (49)</td>
<td>18 healthy subjects and 43 patients with CAD</td>
<td>-75</td>
<td>-</td>
<td>0.5</td>
<td>MA</td>
<td>≥20%</td>
<td>WBA</td>
</tr>
<tr>
<td>Nielsen et al., 2008 (50)</td>
<td>21 healthy subject and 40 patients with CAD</td>
<td>-75</td>
<td>-</td>
<td>1</td>
<td>MA</td>
<td>≥20%</td>
<td>VN Aspirin</td>
</tr>
<tr>
<td>Harrison et al., 2008 (51)</td>
<td>72 patients with CAD</td>
<td>ND</td>
<td>-</td>
<td>1</td>
<td>MA</td>
<td>≥20%</td>
<td>PFA 100®&lt;sup&gt;®&lt;/sup&gt;, VN Aspirin</td>
</tr>
<tr>
<td>Madsen et al., 2010 (36)</td>
<td>26 patients with PCI</td>
<td>-325</td>
<td>+</td>
<td>1</td>
<td>MA</td>
<td>≥20%</td>
<td>VN Aspirin, TEG&lt;sup&gt;®&lt;/sup&gt; Platelet Mapping (AA)</td>
</tr>
<tr>
<td>Tantry et al., 2005 (13)</td>
<td>6 healthy subjects + 203 patients with PCI + 20 patients with stent thrombosis</td>
<td>-325</td>
<td>+/-</td>
<td>1</td>
<td>MA</td>
<td>≥20%</td>
<td>TEG&lt;sup&gt;®&lt;/sup&gt; Platelet Mapping</td>
</tr>
<tr>
<td>Blais et al., 2009 (60)</td>
<td>45 healthy subjects</td>
<td>-80</td>
<td>-</td>
<td>1.6</td>
<td>MA</td>
<td>≥20%</td>
<td>VN Aspirin</td>
</tr>
</tbody>
</table>

ND = non determined; MA = maximal aggregation; VN = VerifyNow, WBA = whole blood aggregometry.
Table 5: Studies using LTA as gold standard to established comparison between various PFTs for the assessment of platelet response to clopidogrel. The table shows the variability of clopidogrel dosage, evaluates the possible influence of aspirin on clopidogrel responsiveness assessment, the various concentration of ADP used as agonist for LTA and the various cut-off values used.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Population</th>
<th>Loading/daily doses of clopidogrel (mg)</th>
<th>Dual antiplatelet therapy with aspirin</th>
<th>[ADP] (µM)</th>
<th>Parameters</th>
<th>Cut-off values</th>
<th>Other PFTs used for comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madsen et al., 2008 (35)</td>
<td>20 healthy subjects</td>
<td>600/75</td>
<td>-</td>
<td>5</td>
<td>MA</td>
<td>LA &gt; 50 %</td>
<td>PFA 100®</td>
</tr>
<tr>
<td>Madsen et al., 2010 (36)</td>
<td>26 patients with PCI</td>
<td>600/75</td>
<td>+</td>
<td>5</td>
<td>MA; LA</td>
<td>MA &lt;10 % absolute decrease compare to baseline or LTA LA &gt; 50 %</td>
<td>VN P2Y12, TEG (ADP)</td>
</tr>
<tr>
<td>Cuisset et al., 2010 (117)</td>
<td>70 patients with PCI</td>
<td>600/150</td>
<td>+</td>
<td>10</td>
<td>MA</td>
<td>MA &gt; 70 %</td>
<td>VASP, VN P2Y12</td>
</tr>
<tr>
<td>Gaglia et al., 2011 (54)</td>
<td>200 patients with PCI</td>
<td>600/75</td>
<td>-</td>
<td>5; 20</td>
<td>MA</td>
<td>MA (ADP5) &gt;46 %; MA (ADP20) &gt;60 %</td>
<td>VASP, VN P2Y12</td>
</tr>
<tr>
<td>Linneman et al., 2010 (56)</td>
<td>40 PAOD</td>
<td>300/75</td>
<td>+/-</td>
<td>2; 5</td>
<td>LA</td>
<td>LA (ADP2) ≥42.9 %; LA (ADP5) ≥72.1 %</td>
<td>Innovance PFA-P2Y12</td>
</tr>
<tr>
<td>Tsantes et al., 2012 (57)</td>
<td>90 CAD + 20 healthy</td>
<td>-75</td>
<td>+</td>
<td>20</td>
<td>MA</td>
<td>MA &gt;63 %</td>
<td>Innovance PFA-P2Y12, VASP, WBA</td>
</tr>
<tr>
<td>Kim et al., 2013 (58)</td>
<td>466 east Asian patients undergoing PCI</td>
<td>600/- or 300/-</td>
<td>-</td>
<td>5; 20</td>
<td>MA</td>
<td>-MA (ADP5) &gt;46 %; MA (ADP20) &gt;59 %</td>
<td>VASP</td>
</tr>
<tr>
<td>Bagoly et al., 2013 (59)</td>
<td>114 patients with non-cardiogenic ischemic cerebrovascular disease</td>
<td>-/75</td>
<td>+</td>
<td>5; 20</td>
<td>MA</td>
<td>-MA (ADP5) &gt;39.5 %; MA (ADP20) &gt;56.8 %</td>
<td>VASP, VN P2Y12, specific ADP aggregation test</td>
</tr>
</tbody>
</table>

ND = non determined; MA = maximal aggregation; LA = late aggregation; VN = VerifyNow, WBA = whole blood aggregometry.

Discussion and conclusion

Both aspirin and clopidogrel are efficient antiplatelet drugs for secondary prevention of cardiovascular events, but their clinical efficacy may vary among individuals. Having reliable PFTs to assess individual platelet response to aspirin or clopidogrel treatment could lead to tailored-antiplatelet therapy that may improve clinical outcomes (121, 122). Despite many efforts undertaken to standardise platelet function tests and most-particularly light-transmission aggregometry (123) among laboratories, the variability of methods and the variability of their technical accomplishment remain and many published studies assessing aspirin/clopidogrel resistance do not report CVs of the methods they used. That is why the widespread use of correlation to report an association between two PFTs shows variable results (46), that have to be considered with precaution. Usually, results of platelet aggregation in whole blood and PRP are not closely correlated. In a study comparing six PFTs among 201 patients with CAD (48), the best correlation was between WBA and AA-LTA, but still the correlation was weak (r=0.24). The correlation between VNA and AA-LTA results was poor in patients with CAD (48), whereas in healthy subjects it was found to be good (r=0.793) (60). Interestingly, the sensitivity of VNA reported in the literature varied widely from 38 % to 95 % (48, 75, 79). When a high sensitivity was found, the authors suggested that either AA-LTA or the VNA assay might be considered optimal because both assays are sensitive to detect aspirin effects to a similar degree.

However, poor correlation exists between PFA 100® and either AA- or ADP-LTA, in the assessment of platelet response to aspirin or clopidogrel treatment, respectively, independently of the cartridge used (12, 48, 77, 86). It appears that PFA-100® is not suitable for detection of aspirin or clopidogrel resistance (14, 86, 93, 95–97). However, the novel specific cartridge INNOVANCE PFA P2Y containing ADP and PGE1 allows a slight improvement of the correlation with ADP-LTA, with Spearman’s correlation coefficient r=0.51 (p<0.001) (57).
A similar situation has also been reported when evaluating these various PFTs as predictive tools for clinical outcome. Indeed, Breet et al. evaluated the capability of multiple PFTs to predict clinical outcome in 1069 consecutive patients undergoing PCI and taking clopidogrel (124). After defining their own cut-off values using ROC curve analysis, they reported that clinical thrombotic events occurred more frequently in patients with clopidogrel resistance when assessed by ADP-LTA and VNP assays, whereas the PFA-100 system (with both PFA-100® with ADP cartridge and INNOVANCE PFA P2Y®) was not able to discriminate between patients with and without clinical events. However, predictive values were modest with all PFTs tested.

Increasing the specificity for the detection of clopidogrel effect by using ADP and PGE1, which suppress the contribution of P2Y1-receptor to ADP-induced platelet aggregation, might explain why most studies comparing various PFTs have found best correlation between VASP assay and VNP (48, 62, 65, 117). The correlation among VASP, VNP assay, and ADP-LTA in different studies ranged from 0.50 to >0.80 but were mainly around 0.60 (44, 54, 62, 65, 73, 117) and seems to improve when higher concentration of ADP were used as agonist (54). Some studies demonstrated that flow-cytometric VASP-assay and VNP system were the PFTs most correlated with peak plasma levels of active metabolite of clopidogrel (53, 61, 65), concluding that both VASP- and VNP-assays are the most reliable PFTs to evaluate in vivo clopidogrel effects on platelet, as well as the Multiplate® device (125, 126). Based on the increase of specificity by using PGE1 to suppress the contribution of P2Y1 receptors, Bagoly et al. (59) have developed a new P2Y12 receptor-specific ADP aggregation test for the detection of clopidogrel effects, where PRP is pre-incubated with 0.31 µM PGE-1 prior to ADP-LTA. The major advantage of this test is that it is not influenced by the intake of concomitant treatment with aspirin and thus may be suitable to monitor clopidogrel response in patients on dual antiplatelet therapy. Cut-off values determining clopidogrel resistance in healthy subjects were 9.1 % maximal aggregation induced by the ADP (PGE1)-LTA. This test was well correlated to VASP assay (r=0.79, r²=0.62, p<0.0001).

More clinically relevant measures of agreement in the setting of an accepted "gold standard" would be sensitivity and specificity. However, a reasonable "gold standard" for measuring platelet response to antiplatelet drugs has yet to be established since LTA methods have never been standardised and vary greatly between individual laboratories. Furthermore, the generally disappointing degree of correlation between different PFTs should not be surprising given the varying methodology of each test. It has already been described above that huge discrepancies concerning preanalytical aspects between laboratories and between laboratory methods may influence results and thus comparison between PFTs. What is also important is the cut-off values used for detection of aspirin/clopidogrel resistance, because it impacts on the calculation of sensitivity and specificity of the different PFTs, as well as on correlation and agreement between PFTs. Table 4 and Table 5 describe some studies comparing various PFTs to assess aspirin or clopidogrel response, respectively, and highlight the varying observed within analytical aspects and cut-off values of LTA used as gold standard. Usually, LTA aspirin resistance is defined as AA-LTA ≥20 % and/or ADPmax-LTA ≥70 % (13, 35, 36, 48–51, 60).

Similarly, proportions of clopidogrel non-responsive patients in different studies cannot be compared unless the ADP

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Table 6: Resume of intra-assay coefficient variation (intra-CV) and day-to-day CV of various platelet function assay in the assessment of aspirin or clopidogrel resistance, as found in the literature.

<table>
<thead>
<tr>
<th>Evaluation of aspirin resistance</th>
<th>Evaluation of clopidogrel resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra CV</td>
</tr>
<tr>
<td>LTA</td>
<td>0.6–21.1 %</td>
</tr>
<tr>
<td>Multiplate® (Roche Diagnostics,</td>
<td>5.1–16.3 %</td>
</tr>
<tr>
<td>Meylan, France)</td>
<td></td>
</tr>
<tr>
<td>PFA-100® (Siemens Healthcare</td>
<td>CEPI: 13.5–20.1 %</td>
</tr>
<tr>
<td>Diagnostics Products GmbH,</td>
<td>CADP: 10.6–13.1 %</td>
</tr>
<tr>
<td>Marburg, Germany)</td>
<td></td>
</tr>
<tr>
<td>VerifyNow® (Accriva diagnostics</td>
<td>0.5–19 %</td>
</tr>
<tr>
<td>San Diego, CA, USA)</td>
<td></td>
</tr>
<tr>
<td>TEG® Platelet Mapping (Haemoscope</td>
<td>3.5–6.6 %</td>
</tr>
<tr>
<td>Corp., Niles, IL, USA)</td>
<td></td>
</tr>
<tr>
<td>Flow cytometry VASP assay</td>
<td>NA</td>
</tr>
<tr>
<td>(Biocytex, Marseille, France)</td>
<td></td>
</tr>
</tbody>
</table>

NA = not applicable; ND = non determined.
concentrations and the recorded ADP parameter (ADP\textsubscript{max} or ADP\textsubscript{emin}) are the same. Authors have used empirically defined cut-off values varying between >10% to >40% to segregate non-responders from responders (16–18, 127). Most recently, an absolute cut-off value of >50% ADP\textsubscript{emin} is considered as being useful to detect clopidogrel resistance (35, 36, 128); some authors also consider the delta aggregation after stimulation with ADP before and after treatment with clopidogrel < 10% (36, 128). Cut-off values might be lower when patients are under concomitant treatment with aspirin. Recently, Tantry et al. published guidelines presenting cut-off values of multiple platelet function tests to be used in future clinical studies (125). According to these consensus guidelines, high on-treatment platelet reactivity should be defined as a value >208 PRU for the VNP assay, > 46 AU for the Multiplate® analyser, MAADP>47mm by TEG, or PRI ≥50% with the VASP assay. If we take into account all the factors that may affect results of PFTs, we believe that cut-off values have to be determined by each lab instead of using arbitrary cut-off values based on the literature. The question remains whether the chosen cut-off values have to be determined compared to healthy subjects (considered as controls) or calculated for each patients from pre-drug values. It is certain that multiple assessments of aspirin/clopidogrel response for a single patient should preferably be performed in the same laboratory. Tantry et al. also suggest that it would be more reliable to use more than a single PFT to reflect the effect of aspirin/clopidogrel platelet response in all patients, and that adding clinical variables and genotype to PFTs may improve risk prediction of thrombotic events, if we consider PFTs could be useful as a prognostic marker (129).

In conclusion, the non-standardised use of these PFTs and the absence of a formal definition explain much of the disparity reported in the literature with regards to the prevalence of aspirin/ clopidogrel resistance. Considering the wide variability of preanalytical and analytical aspects of various PFTs, comparing assay results to those of a gold standard such as LTA seems to be irrelevant. Instead all assays should be tested to determine cut-off values that best predict clinical outcomes. This also highlights the major role of knowledge groups such as the International Society on Thrombosis and Haemostasis, which can help define the best clinical and laboratory strategies to adopt for reducing the risk of ischaemic events due to aspirin/clopidogrel resistance. PFTs should rather be included into a risk algorithm, along with biomarker testing and clinical factors to better predict the risk of thrombotic events and to ease personalisation of antiplatelet therapy. To summarise, PFTs are not interchangeable, and much work focused on standardisation of the different methods has to be undertaken.

Conflicts of interest
C. Negrier has received honoraria or acts as a paid consultant for Baxter, Biogen Idec/SOBI, CSL Behring, LFB, NovoNordisk, and Pfizer. He has received grants/research support from Baxter, Bayer, CSL Behring, Inspiration, NovoNordisk, Octapharma, and Pfizer. None of the other authors declares any conflicts of interest.

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