Dual antiplatelet therapy with aspirin and a thienopyridine is a cornerstone of treatment for patients with coronary artery disease. Compared with aspirin alone, the combination of aspirin and a thienopyridine significantly improves outcomes in patients with ST-elevation myocardial infarction (MI) and non–ST-elevation acute coronary syndrome (ACS). The pharmacodynamic effect of the thienopyridine clopidogrel varies substantially between individuals despite a clinical benefit across a range of disease states. Furthermore, a growing body of data supports an association between a lack of pharmacodynamic effect and ischemic events. These observations have spurred interest in the use of point-of-care platelet function assays to identify and treat at-risk patients. This review discusses the evidence that supports platelet function screening, describes the currently available assay devices, and outlines the future developments necessary for the adoption of routine platelet function testing in clinical practice.

The molecular target of the thienopyridines is the platelet P2Y₁₂ receptor. Binding of the P2Y₁₂ receptor by its agonist, ADP, activates the G-protein inhibitor secondary messenger system, which, through a complex series of events, mediates the completion and amplification of the aggregatory response. Ticlopidine and clopidogrel are prodrugs that are converted to an active metabolite through a 2-step process mediated by the hepatic cytochrome P450 (CYP) system. The active metabolite achieves its antiaggregatory effect by irreversibly binding and antagonizing the P2Y₁₂ receptor for the life of the platelet. This antiaggregatory effect can be measured ex vivo by light transmittance aggregometry (LTA). First described by Born in 1962, LTA assesses the aggregatory effect of an antiplatelet agent by measuring the transmission of light through platelet-rich plasma after exposure to a platelet agonist (in the case of thienopyridines, ADP) using platelet-poor plasma as reference. The antiaggregatory effect may then be described either by the absolute or relative change in aggregation before and after thienopyridine exposure or by the residual or on-treatment platelet reactivity, which requires only a single measurement after thienopyridine treatment has begun. (When used in this manner, the broad expression “residual reactivity” should not be confused with the technical term “residual platelet aggregation,” which specifically denotes the level of aggregation by LTA 5 to 6 minutes after induction with ADP.) The term “responsiveness” is often used to describe the effect of clopidogrel on either the change in aggregation or the level of residual reactivity, although this is not strictly correct because residual reactivity is an absolute value. In either case, the effect of clopidogrel has substantial interindividual variability that cannot be overcome even with high loading doses. The pharmacodynamic effect of thienopyridines also can be demonstrated by other basic research techniques, including flow cytometric measurement of the phosphorylation status of vasodilator-stimulated phosphoprotein, which is directly related to the activity of the P2Y₁₂ receptor.

Lack of Clopidogrel Effect as a Risk Factor for Adverse Outcome After PCI

To support platelet function screening, a poor response to thienopyridine treatment must be demonstrated to be a risk factor for cardiovascular events; i.e., a causal, rather than an indirect or chance, association should exist. Biological plausibility, a strong and consistent association across studies, temporal sequence (exposure to the factor preceding the adverse outcome), and evidence of a dose-response relationship lend support to a causal link. It is biologically plausible that the lack of a response and/or high residual reactivity despite thienopyridine treatment is associated with ischemic events, given that platelet activation and aggregation play a central role in the thrombosis that occurs after disruption or erosion of an atherosclerotic plaque during ACS and after the iatrogenic disruption of the vascular endothelium during PCI. Moreover, more powerful inhibition of the P2Y₁₂ receptor leads to a greater reduction in recurrent events after ACS. Prospective studies have demonstrated that the degree of clopidogrel responsiveness or residual platelet reactivity by LTA is significantly associated with short- and longer-term ischemic events in patients undergoing PCI for stable coronary artery disease and ACS and with cardiovascular events in chronically treated patients with coronary artery disease and diabetes mellitus. The relationship between clopidogrel effect and outcome also appears to have a dose-response relationship; studies that divide the population into quartiles of response generally demonstrate increasing...
event rates with progressively higher quartiles of residual reactivity (or lower quartiles of responsiveness). Although this observation is limited by the relatively small number of events in all of these studies, Hochholzer et al demonstrated that when considered as a continuous variable, platelet aggregation was an independent predictor of 30-day major adverse cardiac events after PCI.

Requirements for a Screening Test to Assess Platelet Function

Although data support the assertion that the response to clopidogrel has a causal association with ischemic events, further conditions must be met to support the measurement of clopidogrel response in clinical practice. There are several widely accepted criteria to assess a candidate screening test: (1) acceptability for both the patient and the physician, (2) reliability, (3) validity (ie, the ability of the test to separate those patients at risk from those who are not), (4) cost, and (5) the ability to treat the condition adequately when discovered. Although a small randomized study of platelet function screening with vasodilator-stimulated phosphoprotein phosphorylation analysis in patients undergoing PCI demonstrated proof of principle, methods such as LTA and vasodilator-stimulated phosphoprotein phosphorylation analysis require substantial sample preparation, specialized equipment, and trained laboratory personnel. Integration of these techniques into routine practice is therefore challenging, especially when results are needed rapidly for clinical decision making. Several bedside or near-bedside platelet function assays are currently available in the United States (Table 1). The clinical utility of each of these assays must be interpreted in reference to their ability to fulfill the criteria required for an adequate screening test.

Platelet Function Assay-100

The Platelet Function Assay-100 (PFA-100 system; Dade Behring, Miami, Fla) consists of a disposable cartridge and an analyzer device. The system evaluates platelet function by determining the time to occlusion of an aperture in a membrane coated with collagen/ADP or collagen/epinephrine as whole blood flows under high shear stress conditions. Platelet aggregation causes aperture occlusion, and the time to occlusion is reported as closure time. The system provides a result in 5 to 8 minutes. Although the assay uses whole blood and therefore requires no sample preparation, the sample must be manually pipetted onto the cartridge, making this a near-bedside assay. The assay has received 510(k) clearance from the US Food and Drug Administration to detect platelet dysfunction in patients with a suspected disorder of primary hemostasis. Several studies have demonstrated that the PFA-100 collagen/ADP cartridge cannot detect platelet dysfunction in patients with a suspected disorder of primary hemostasis. Therefore, in its current form, this system is not a candidate for thienopyridine responsiveness screening.

### Table 1. Platelet Function Assays for the Effect of Thienopyridines Currently Available in the United States

<table>
<thead>
<tr>
<th>Assay</th>
<th>Components</th>
<th>Measurement</th>
<th>Assay Limitations</th>
<th>Clinical Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFA-100</td>
<td>Analyzer device, disposable cartridge</td>
<td>Time to occlusion of aperture in collagen/ADP-coated membrane under high shear stress conditions</td>
<td>Cannot distinguish thienopyridine effect</td>
<td>No</td>
</tr>
<tr>
<td>Plateletworks</td>
<td>ICHOR cell counter, EDTA tube, ADP tube</td>
<td>Difference in single platelet counts after stimulation with ADP vs baseline</td>
<td>Results highly dependent on time between sample collection and testing</td>
<td>No</td>
</tr>
<tr>
<td>VerifyNow P2Y12</td>
<td>Instrument, disposable cartridge</td>
<td>Agglutination of fibrinogen-coated beads by platelets in the presence of ADP and PGE1</td>
<td>Potential inaccuracy of surrogate measurement of percent inhibition without baseline preclopidogrel sample</td>
<td>Yes</td>
</tr>
<tr>
<td>TEG platelet mapping</td>
<td>Instrument, computer interface, software, reagents, disposable pin and cup</td>
<td>Platelet contribution to clot strength with ADP stimulation</td>
<td>Method requires substantial manual pipetting of several reagents and blood sample</td>
<td>Yes</td>
</tr>
</tbody>
</table>

TEG indicates thrombelastography.
support the adoption of this assay to screen patients on thienopyridine therapy in clinical practice.

**VerifyNow P2Y12 Assay**

The VerifyNow System (Accumetrics Inc, San Diego, Calif) is a point-of-care turbidimetry-based optical detection system that measures platelet-induced aggregation. It has received 510(k) clearance from the Food and Drug Administration for measurement of the level of P2Y12 receptor blockade. The system consists of an analyzer instrument and a disposable assay device. The instrument controls all assay sequencing, the temperature, and reagent sample mixing and performs self-diagnostics. The assay device contains a lyophilized preparation of human fibrinogen–coated beads, platelet activators, and buffer. The patient sample is whole blood, which is automatically dispensed from the blood collection tube into the assay device by the instrument. The VerifyNow P2Y12 assay contains 20 μmol ADP and 22 nmol prostaglandin E1 (PGE1) to reduce the activation contribution from the ADP-induced platelet aggregation by LTA when either the ADP-PGE1 and iso-TRAP channels.

The VerifyNow P2Y12 assay has been well correlated with ADP-induced platelet aggregation by LTA when either the change in PRU before and after clopidogrel exposure or the posttreatment PRU is used to reflect the effect of clopido
grel. The dynamic range appears narrower than that of LTA; therefore, the assay may not be able to discriminate between very strong or between very weak levels of P2Y12 receptor inhibition. The percent inhibition reported by the device as a surrogate for the degree of P2Y12-mediated inhibition without a baseline clopidogrel sample may be inaccurate compared with the actual change in PRU before and after clopidogrel exposure, possibly because the iso-TRAP channel does not provide an adequate reflection of baseline ADP-induced aggregation.

Three prospective studies have tested the diagnostic performance of the VerifyNow P2Y12 assay using receiver-operating characteristics (ROC) curve analysis (Table 2). The ROC curve assesses how well a test discriminates, or separates, individuals into 2 classes such as those with and without clinical events. ROC analysis can be used to select an optimal threshold of a diagnostic test by determining the cutoff that maximizes the sum of its sensitivity and specificity. Patti et al prospec
tively examined the relationship between residual platelet reactivity measured by the VerifyNow P2Y12 assay and 30-day major adverse cardiovascular events in 160 clopidogrel-treated patients undergoing PCI. ROC curve analysis showed that PRU levels significantly discriminated between patients with and without 30-day major adverse cardiac events, which was driven primarily by periprocedural MI (area under the curve=0.69; P=0.016). A PRU value ≥240 was the optimal cutoff to predict 30-day outcome, providing a sensitivity of 81% and a specificity of 53%. Moreover, when the population was divided into quartiles based on PRU, there appeared to be a dose-response relationship, with increasing event rates across the first through fourth quartiles of residual reactivity. Price et al prospectively examined the prognostic value of residual platelet reactivity measured by the VerifyNow P2Y12 assay in 380 patients treated with aspirin and clopidogrel undergoing predominantly elective PCI with DES. The assay was able to discriminate between patients with and without cardiovascular death, nonfatal MI, or stent thrombosis at the 6-month follow-up (area under the curve=0.720; P=0.02). The optimal cutoff for the assay was a PRU ≥235, which identified approximately one third of the patient population and provided a sensitivity of 78% and a specificity of 70%. Marcucci et al assessed residual platelet reactivity with

**Table 2. Prospective Studies Examining the Relationship Between the VerifyNow P2Y12 Assay and Clinical Outcome After PCI**

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Procedure Indication</th>
<th>Primary End Point</th>
<th>Duration of Follow-Up</th>
<th>Diagnostic Cutoff</th>
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<tr>
<td>Buch et al</td>
<td>330</td>
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<td>Magnitude of CK-MB elevation</td>
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*Estimated inhibition provided by ratio of ADP and iso-TRAP channels.

CK-MB indicates creatine kinase-MB; ULN, upper limit of normal; CV, cardiovascular; TVR, target vessel revascularization; and ST, stent thrombosis.

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the VerifyNow P2Y12 assay in 683 patients treated with aspirin and clopidogrel undergoing PCI for ACS. The VerifyNow P2Y12 assay demonstrated a significant area under the curve on ROC curve analysis, and the optimal cutoff value to predict 12-month cardiovascular death or nonfatal MI was a PRU \( \geq 240 \), consistent with the findings of Patti et al.\(^{40}\) and Price et al.\(^{41}\) The association between ischemic events and residual reactivity above this cutoff remained significant after adjustment for baseline clinical, procedural, and angiographic characteristics.

Therefore, the VerifyNow P2Y12 assay is clinically practical, has adequate reliability, and, when residual platelet reactivity is used as a measure of clopidogrel effect, can identify patients undergoing PCI who at risk for periprocedural, medium-term, and longer-term cardiovascular events. Several studies have demonstrated that the optimal diagnostic cutoff appears to be \( \approx 235 \) and 240 PRU. The validity of the assay will be further examined by the Assessment of Dual Antiplatelet Therapy With Drug Eluting Stents (ADAPT-DES) registry (http://www.clinicaltrials.gov; identifier, NCT00638794), which is prospectively evaluating the relationship between the results of the VerifyNow P2Y12 assay and ischemic events, including stent thrombosis, in \( > 10 \, 000 \) patients undergoing PCI with DES.

Clinical trials are planned or are currently enrolling that will test whether modifying antiplatelet therapy based on the VerifyNow P2Y12 assay improves outcomes. The Dual Antiplatelet Tailored Therapy Based on the Extent of Platelet Inhibition (DANTE) trial (http://www.clinicaltrials.gov; identifier, NCT00774475) will randomize \( \approx 450 \) patients treated with PCI for ACS and with high residual platelet reactivity to either standard maintenance clopidogrel therapy (75 mg daily) or high-dose maintenance clopidogrel therapy (150 mg). The primary end point is the composite of cardiovascular death, nonfatal MI, or target vessel revascularization at the 6- and 12-month follow-up. The Gauging Responsiveness With a VerifyNow Assay—Impact on Thrombosis and Safety (GRAVITAS) trial (http://www.clinicaltrials.gov; identifier, NCT00645918) is an international, randomized, placebo-controlled clinical trial that will enroll \( \approx 2800 \) patients with stable angina/ischemia or non-ST elevation ACS undergoing PCI with DES. Patients with high residual platelet reactivity on clopidogrel therapy 12 to 24 hours after PCI will be randomized to standard maintenance clopidogrel therapy (75 mg daily) or high-dose clopidogrel therapy (additional loading dose followed by 150 mg daily) for 6 months. A random sample of patients without high residual reactivity will be followed and treated with standard clopidogrel therapy. The primary end point is the time to first occurrence of cardiovascular death, nonfatal MI, or definite/probable stent thrombosis. The clinical use of the VerifyNow P2Y12 assay to routinely screen clopidogrel-treated patients undergoing PCI may be supported only after these clinical trials are completed.

The VerifyNow P2Y12 assay also has been used to assess platelet functional recovery after discontinuation of clopidogrel therapy.\(^{10,43}\) These studies demonstrate that within a week after discontinuation, the effect of clopidogrel on a given day is explained more by an individual’s initial response than by the duration of time since the last dose was administered. This observation argues against the uniform approach recommended by current guidelines to defer surgery for 5 to 7 days in patients exposed to clopidogrel. However, although higher levels of platelet inhibition measured by LTA at the time of surgery are associated with greater blood product transfusion,\(^{44}\) the relationship between a particular level of inhibition measured by the VerifyNow P2Y12 assay and subsequent surgical bleeding has not been examined; therefore, further study is required to define the optimal cutoff of this assay to prevent hemorrhagic events in clopidogrel-treated patients who require surgery.

**Thrombelastography Platelet Mapping**

Thrombelastography platelet mapping (Hemoscope Corp, Niles, Ill) is a modification of the original thrombelastography assay that enables a quantitative analysis of platelet function. This 510K-cleared near-bedside system consists of an analyzer instrument, a computer interface module, software, several reagents, and disposable cups and pins. Thrombelastography measures the physical properties of a forming clot by the use of an oscillating cup that holds a sample of whole blood. Suspended within the blood is a pin; a mechanical-electrical transducer monitors the motion of the pin. The torque of the rotating cup is transmitted to the immersed pin as fibrin-platelet bonding links the cup and pin together. The magnitude of pin motion is directly related to the strength of fibrin-platelet bonding as the clot is formed. Maximal hemostatic activity is measured by a kaolin-activated whole-blood sample treated with citrate. Heparin is used as an anticoagulant to eliminate thrombin activity in the sample. Reptilase and factor XIIIa (activator F) are used to generate a crosslinked fibrin clot to isolate the fibrin contribution to clot strength. The contribution of P2Y\(_{12}\) receptor activity to clot formation is measured by the addition of 10 \( \mu \)mol ADP (final concentration, 2 \( \mu \)mol). The effect of thienopyridine therapy also can be estimated by comparing the unmodified thrombelastography curve (ie, kaolin-activated whole-blood sample) with the ADP-stimulated thrombelastography curve.\(^{45}\) The method has been shown to be reliable with low analytical variation.\(^{46}\) This assay requires manual pipetting of the various reagents, and therefore, performance of the assay requires an appropriately trained technician with proper laboratory equipment.

In a small prospective study of 100 patients on chronic dual antiplatelet therapy undergoing elective stenting, ADP-induced platelet aggregation by thrombelastography platelet mapping was correlated with LTA, and high ADP-induced platelet aggregation by thrombelastography platelet mapping before PCI was associated with an increased risk for subsequent ischemic events over 1 year of follow-up.\(^{20}\) In the same study, ROC curve analysis demonstrated that the thrombelastography assay could discriminate between patients with and without subsequent events (area under the curve=0.881, \( P=0.0001 \)). A predefined threshold of \( \geq 70\% \) ADP-induced aggregation by thrombelastography provided a positive predictive value of 73%. Clot strength by unmodified thrombelastography also has been shown to be associated with ischemic events after PCI.\(^{18}\)
Thrombelastography platelet mapping is therefore a potentially useful screening test to identify patients who may be at risk for ischemic events after PCI. However, the validity of the assay must first be confirmed in additional studies, and clinical trials must determine whether altering management of PCI-treated patients on the basis of the results of the assay can improve outcomes cost-effectively.

Thrombelastography platelet mapping also can be used to determine the effect of thienopyridine therapy in patients who require cardiac surgery. The use of the unmodified thrombelastography can reduce transfusion requirements in patients undergoing cardiac and liver surgery; the additional value of thrombelastography platelet mapping is still undefined.

The Role of Platelet Function Screening and Newer Antiplatelet Agents

Several new compounds provide powerful and consistent inhibition of the platelet P2Y\textsubscript{12} receptor. Prasugrel, a third-generation thienopyridine, reduces the rate of ischemic events compared with clopidogrel in ACS patients treated with PCI at the cost of increased major bleeding. The active metabolite of prasugrel is generated more efficiently than clopi-
dogrel, and prasugrel therapy provides significant platelet inhibition in clopidogrel nonresponders. The safety and efficacy of the nonthienopyridines AZD6140 and cangrelor, which also provide greater and more consistent inhibition than early-generation thienopyridines, are currently being examined in phase III clinical trials. The future availability of these agents may affect the role of platelet function testing in 2 ways. First, these agents may provide a more effective therapy compared with increasing the clopidogrel dose in patients who are nonresponsive to standard clopidogrel treatment. Second, treatment with a consistent and powerful P2Y\textsubscript{12} receptor antagonist in all eligible post-PCI patients challenges the rationale for platelet function screening by minimizing the phenomenon of nonresponsiveness and/or high posttreatment residual reactivity. However, post-PCI ischemic events are rare in clopidogrel-treated patients with periprocedural platelet aggregation <50% by LTA with 20 µmol ADP, implying that only a modest antiaggregatory effect is needed to achieve clinical benefit. Indeed, ischemic events on clopi-
dogrel therapy tend to cluster within the upper quartile of residual reactivity; event rates in the other quartiles are relatively low, especially below the median level of the population. The negative predictive value of high residual reactivity measured by the VerifyNow assay is 96%, suggesting that ACS patients treated with PCI who have residual reactivity below the diagnostic cutoff for the assay (approximately two thirds of presenting patients) are at low risk for recurrent events. Although the exact relationship between the level of platelet aggregation and the risk of bleeding has not been fully elucidated, more aggressive platelet inhibition has consistently been associated with increased bleeding rates in large clinical trials. Prasugrel provides a greater level of inhibition than almost the entire range of response to clopidogrel. Therefore, for many patients, the absolute risk reduction provided by potent P2Y\textsubscript{12} inhibitors compared with clopidogrel may be relatively small, at the cost of an increased bleeding hazard, because the relationship between residual reactivity and risk appears curvilinear, patients with a modest clopidogrel effect appear to have low event rates, and more aggressive platelet inhibition is generally associated with increased bleeding (the Figure). If this hypothesis is confirmed, it argues against using a “one size fits all” approach with next-generation P2Y\textsubscript{12} antagonists.

Genetic Testing

Several genetic polymorphisms have been identified that may affect clopidogrel absorption and metabolism. Loss-of-function polymorphisms of the CYP isoenzymes CYP2C19, CYP2C9, and CYP2B6 are associated with decreased area under the plasma concentration-time curve of the active metabolite and diminished platelet inhibition after clopi-
dogrel administration. Carriers of CYP2C19 loss-of-function polymorphisms treated with clopidogrel have higher rates of major adverse cardiovascular events after PCI for ACS or acute MI compared with noncarriers. A variant
of the drug-efflux transporter gene ABCB1 also is associated with an increase in cardiovascular events in clopidogrel-treated patients, especially in the presence of 2 CYP2C19 reduced-function alleles.

Because CYP2C19 polymorphisms do not affect the generation of the active metabolite of prasugrel, the development of point-of-care genetic screening tests could potentially help the clinician individualize treatment with the most appropriate thienopyridine. In the subgroup of clopidogrel-treated patients with genetic testing in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38), noncarriers of the loss-of-function CYP2C19 allele undergoing PCI for ACS had an ≈33% relative reduction in the risk of cardiovascular death, nonfatal MI, or stroke compared with carriers.

In the overall trial, patients randomized to prasugrel had only an ≈19% relative risk reduction compared with clopidogrel. Further studies must determine whether clopidogrel therapy provides ischemic outcomes similar to those with prasugrel therapy in patients without the CYP2C19 polymorphism. A significant proportion of carriers of the loss-of-function CYP2C19 allele do not have high postclopidogrel residual platelet reactivity, and high residual reactivity is present in many patients with wild-type alleles. Moreover, the interactions between multiple genetic polymorphisms and environmental factors (eg, concomitant medications or smoking) on the functional effect of thienopyridines are undoubtedly complex and cannot be captured by genetic testing. Assessment of platelet function with point-of-care assays will therefore likely be useful even if genetic screening tests are successfully developed.

Conclusions

Despite the clinical benefit of clopidogrel in a broad range of patients with cardiovascular disease, ex vivo laboratory measurements demonstrate substantial interindividual variability in the effect of clopidogrel on platelet aggregation and P2Y₁₂ receptor activity. Data support the assertion that poor responsiveness and/or high residual reactivity on clopidogrel therapy is a risk factor for ischemic events in PCI-treated patients. Point-of-care or near-bedside platelet function tests offer the possibility of integrating the measurement of clopidogrel response variability into clinical practice.

Such tests must be reliable and valid, with well-defined diagnostic cutoff values, and treatment decisions based on such tests should cost-effectively improve patient outcome. No point-of-care or near-bedside platelet function test currently fulfills all of these criteria. Early studies demonstrate the potential value of thrombelastography platelet mapping. Several prospective, observational studies have demonstrated that the VerifyNow P2Y₁₂ assay can provide prognostic information in clopidogrel-treated patients undergoing PCI. The ADAPT-DES registry will provide more data on the incremental value of this information in addition to traditional risk factors, and planned and currently enrolling clinical trials such as GRAVITAS will examine whether routine screening of PCI patients with the VerifyNow P2Y₁₂ assay provides clinical benefit. Platelet function testing could in the future help determine which patients would derive the most benefit from newer, more powerful P2Y₁₂ inhibitors.

Disclosures

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