Platelet-Mediated Thrombosis
From Bench to Bedside

Paul A. Gurbel, Young-Hoon Jeong, Eliano P. Navarese, Udaya S. Tantry

Abstract: The pivotal role that platelets play in thrombosis and resultant ischemic event occurrences in patients with high-risk coronary artery disease is well established. This role provides the fundamental basis for the current wide implementation of dual antiplatelet therapy with aspirin and a P2Y12 receptor inhibitor. The development of user friendly point-of-care methods to assess platelet reactivity to adenosine diphosphate has increased the frequency of platelet function testing in clinical practice. Recent large observational studies have established an independent relation between the results of point-of-care platelet function testing and clinical event occurrence in patients undergoing coronary artery stenting. However, prospective, randomized trials have failed to demonstrate that personalized antiplatelet therapy based on point-of-care assessment of platelet function is effective in reducing ischemic event occurrences. Important limitations were associated with these trials. In addition, the concept of a therapeutic window of P2Y12 receptor reactivity with an upper threshold associated with ischemic event occurrence and a lower threshold associated with bleeding has also been proposed. In the absence of strong prospective evidence to support personalized antiplatelet therapy, clinical decision making about antiplatelet therapy rests on the large body of observational data and the fundamental importance of platelet physiology in catastrophic event occurrence in patients with high-risk coronary artery disease. (Circ Res. 2016;118:1380-1391. DOI: 10.1161/CIRCRESAHA.115.307016.)

Key Words: antiplatelets ■ bleeding ■ platelets ■ platelet function inhibitors ■ platelet function testing ■ thrombosis

Overwhelming evidence exists that platelet-rich thrombus generation at the sites of plaque rupture and vessel wall erosion is a primary process causing ischemic events in patients with coronary artery disease (CAD). Plugging of the microvasculature with platelet aggregates was demonstrated in landmark studies of patients succumbing to acute myocardial infarction (MI). Atherectomy specimens of culprit plaques exhibit exuberant platelet-laden thrombi, and angioscopy studies further support the latter observations. Platelet thrombi were demonstrated at the site of coronary occlusion in histological studies and also at the distal site of active coronary disease in patients with unstable angina preceding death, suggesting a role for platelet activation during unstable angina. Finally, platelet activation has been reported in multiple
conditions of cardiovascular disease. These observations strongly suggest the pivotal roles of platelets in the development of thrombosis and subsequent ischemic complications.

After spontaneous plaque rupture in acute coronary syndrome (ACS) and induced rupture during percutaneous coronary intervention (PCI), platelets adhere to the injured vessel wall and undergo activation that is followed by the release of secondary agonists, thromboxane (Tx) A2, and adenosine diphosphate (ADP). Although TxA2 and ADP act synergistically during platelet aggregation, the ADP-P2Y12 receptor interaction plays a central role in sustaining the activation of glycoprotein IIb/IIIa receptors by amplifying the response to agonists, leading to stable platelet-rich thrombus generation. Simultaneously, large amounts of thrombin are generated on the procoagulant platelet surface, and thrombin is another strong platelet agonist. Thrombin converts fibrinogen to fibrin to further stabilize the platelet-fibrin clot (Figure 1). However, the relative contribution of each agonist-induced platelet activation pathway (ADP, TxA2, and thrombin) to the genesis of an in vivo stable thrombus is not clear at this time.

At this time, the direct inhibition of platelet P2Y12 or PAR-1 receptors and a pivotal enzyme associated with platelet agonist release (cyclooxygenase [COX]-1) has been exploited in clinical practice. However, various observations suggest an intrinsic variability in platelet response to various agonists in the healthy population not on antiplatelet agents. High response to agonists has been linked to increased risk for thrombotic events and lower risk for bleeding in selected patients on antiplatelet agents. Given these findings, the major potential clinical uses for platelet function measurement to bring the bench to bedside are the following:

1. Identification of subjects at increased risk for cardiovascular complications
2. Monitoring response to antiplatelet agents to assist in personalizing antiplatelet therapy
3. Assessment of pharmacodynamic effects to optimally time surgical procedures to reduce wait time and bleeding.

Assessment of Potential Risk Based on Platelet Measurements

The key revolution in the field of the assessment of platelet function occurred when a quantitative ex vivo method to measure platelet function, conventional/light transmittance aggregation, was developed in the Gustav Born laboratory in 1962. Born demonstrated that platelets underwent aggregation after the addition of ADP, and a decrease in optical density was recorded over time, which was proportional to the concentration of the platelets in plasma. In his landmark paper, Born concluded as follows: “If it can be shown that ADP takes part in the aggregation of platelets in blood vessels, it is conceivable that AMP or some other substance could be used to inhibit or to reverse platelet aggregation in thrombosis.” Since then, various laboratory methods have been used to explore the utility of measurement of platelet activation and aggregation in identifying high-risk patients as well as response to antiplatelet agents and its relation to recurrent ischemic event occurrences. In addition to mean platelet volume, conventional aggregation with various agonists, assessment of p-selectin, CD-40L, and activated glycoprotein IIb/IIIa receptor expression, intracellular vasodilator-stimulated phosphoprotein phosphorylation levels to measure P2Y12 receptor blocker response (a P2Y12-specific assay), and point-of-care methods—VerifyNow Assay, Platelet Mapping with Thrombelastography, and Multiplate analyzer—are the commonly used methods in the current era.

Larger mean platelet volume may be an indicator of in vivo platelet activation. In this line, relation between mean platelet volume and platelet activation, platelet aggregation, TxB2 release, or adhesion molecule expression and relation between mean platelet volume and MI and cerebral infarction have been demonstrated. In a cohort of 149 patients, in vitro spontaneous platelet aggregation (in the absence of an agonist) measured 3 months after MI was shown to be predictive of coronary events and mortality in a 5-year follow-up. Similarly, platelet activation as indicated by elevated plasma and urinary TxB2 levels has been shown to be associated with spontaneous ischemia during unstable angina. In the Nortwick Park Heart study of 958 subjects, ADP-induced platelet aggregation was moderately associated with a history of ischemic heart disease in men and strongly associated with platelet fibrinogen concentration. The authors suggested that in addition to agonist-induced platelet aggregation, other important factors that influence platelet function, such as measurement of plasma fibrinogen concentration and thrombin generation, should also be included during the assessment of cardiovascular risk of the patient. A temporal association between significantly increased in vitro measured ADP- and epinephrine-induced platelet aggregation during morning hours (6–9 am) and increased frequency of MI and sudden cardiac death was also demonstrated in patients with CAD (Figure 2).

Sustained platelet activation after index myocardial event was demonstrated in some studies. The thrombolysis in myocardial infarction-12 trial demonstrated that platelet activation as indicated by increased expression of platelet-bound p-selectin expression during ACS was sustained for 28 days after ACS. Similarly, in vitro measured low-concentration ADP-induced glycoprotein IIb/IIIa expression was elevated during 90-day follow-up period in patients who experienced repeat revascularization after PCI compared with patients without revascularization (P=0.029). Same group also demonstrated an increased percentage of platelets expressing p-selectin in

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACS</td>
<td>acute coronary syndrome</td>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<tr>
<td>CAD</td>
<td>coronary artery disease</td>
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<td>COX</td>
<td>cyclooxygenase</td>
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<td>HPR</td>
<td>high platelet reactivity</td>
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<tr>
<td>MI</td>
<td>myocardial infarction</td>
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<td>PCI</td>
<td>percutaneous coronary intervention</td>
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<td>Tx</td>
<td>thromboxane</td>
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response to ADP in blood taken from the coronary artery versus coronary aorta from patients with ACS ($P=0.02$) but not in patients with stable symptoms ($P=\text{ns}$). These observations indicate a strong relation between elevated intrinsic platelet activation and reactivity and cardiovascular risk and are elevated after index event.

Variability in Platelet Response to Agonists and Its Relation to Heritability

In the general healthy population, platelet reactivity is influenced by various cardiovascular risk factors, including age, sex, obesity, and metabolic syndrome, and the majority of this variation in platelet aggregation is heritable. Based on the considerable interindividual and intra-individual variation in response to various agonists in a healthy population, it was hypothesized that subjects with maximal aggregation induced by low agonist concentrations may exhibit hyperreactive platelet phenotype that is associated with an increased risk for thrombotic complications. Faraday et al demonstrated that heritable factors indirectly related to COX-1 contribute prominently to variability in residual platelet function after aspirin therapy in 1880 asymptomatic subjects from families with premature coronary heart disease. They showed that the same factors that contribute to the variability in indirect COX-1 pathways (described later) during aspirin therapy also contribute to the variability before aspirin therapy. However, cardiovascular risk factors contributed modestly to the aspirin-response phenotype. The same group also demonstrated that >70% of variation in platelet aggregation in

Figure 1. Role of different platelet receptors in thrombotic event occurrences. After vascular injury, platelets adhere to injury site and undergo activation. Platelet activation results in the release of 3 important platelet agonists: thromboxane (Tx) $\alpha_2$, adenosine diphosphate (ADP), and thrombin, and thrombin protease-activated receptor-1, $\text{TxA}_2$, thromboxane receptor, and ADP-P2Y$_{12}$ receptor pathways amplify the response to platelets, resulting in sustained platelet aggregation via activated glycoprotein (GP)IIb/IIIa receptor. The ADP-P2Y$_{12}$ interaction plays a central role in platelet aggregation and subsequent ischemic event occurrences. Finally, formation of occlusive platelet-rich thrombus formation at the site of plaque rupture is mainly responsible for the occurrence of arterial thrombotic event occurrences, such as myocardial infarction, stent thrombosis, and stroke. ACS indicates acute coronary syndrome; COX, cyclooxygenase; HPR, high platelet reactivity; and PCI, percutaneous coronary intervention.

Figure 2. Time line in platelet-mediated thrombosis: from bench to bedside. ADP indicates adenosine diphosphate; CABG, coronary artery bypass grafting; CAD, coronary artery disease; and PCI, percutaneous coronary intervention.
African Americans and almost 60% of variation in European Americans is heritable.\textsuperscript{20} In a 6-year follow-up including 1699 subjects with a family history of early-onset CAD, greater collagen-induced platelet aggregation measured after 2 weeks of aspirin therapy was associated with a significantly increased risk for an ACS event (22 ACS events; 12 MI, 10 unstable angina with revascularization).\textsuperscript{21}

A genome-wide association study conducted in Caucasians identified 7 loci associated with agonist-induced platelet aggregation.\textsuperscript{22} In a cohort of 500 healthy subjects, 2 transcripts, \textit{COMMD7} and \textit{LRRFIP1}, were correlated with platelet responsiveness, and single nucleotide polymorphisms associated these 2 transcripts were correlated with platelet response and a modest association with MI in a cohort of 5145 MI/CAD cases and 6379 controls.\textsuperscript{23} The heritability of ADP-stimulated platelet aggregation was 33% at baseline and 73% in response to clopidogrel in a genome-wide association study conducted in 429 healthy Amish population. A cluster of 13 single nucleotide polymorphisms within chromosome 10q24 (out of \textasciitilde 400000 single nucleotide polymorphisms analyzed) was mostly associated with clopidogrel response, and cytochrome (CYP)2C19*2 variant (gene associated with enzyme involved in clopidogrel metabolism) was accounted for 12%, whereas increased age, body mass index, and triglyceride levels and decreased levels of high-density lipoprotein cholesterol account for \textasciitilde 10% of the variation. However, most of the heritability of clopidogrel response variability (\textasciitilde 80%) was unexplained in this study. In a replication study involving patients undergoing PCI, carriers of the \textit{CYP2C19}*2 allele had higher rate of cardiovascular event rates compared with non-carriers (hazard ratio [HR]: 2.42; \textit{P}=0.02).\textsuperscript{24}

However, in the contemporary translation research arena, platelet response to widely used antiplatelet agents, P2Y\textsubscript{12} receptor inhibitor, and to some extent, aspirin is evaluated. Therefore, we focus on these 2 aspects here.

\textbf{Role of Platelet Function Testing in Assessing Response to Antiplatelet Agents and Its Clinical Relevance}

\textbf{Laboratory Assessment of Aspirin Responsiveness and Its Relation to Clinical Outcomes}

Earlier studies were attempted to demonstrate aspirin resistance by laboratory methods and its relation to clinical outcome.\textsuperscript{25-26} Laboratory methods, including point-of-care methods, use agonists, such as ADP, collagen, shear, and epinephrine, to stimulate platelets and indicate aspirin responsiveness. These methods do not solely indicate the level of COX-1 activity, the target of aspirin antiplatelet effect, and were considered COX-1 nonspecific methods. The optimal aspirin responsiveness/resistance should be based on the residual activity of the primary target of aspirin—the COX-1 enzyme. Measurement of arachidonic acid–induced platelet aggregation is the most widely used method to indicate COX-1 activity (COX-1-specific methods).\textsuperscript{27-28} There is wide variability regarding the prevalence of aspirin resistance (<1% to 57%) because of different ex vivo methods and criteria used to define aspirin resistance. However, it has been clearly established that in the presence of low-dose aspirin therapy, the prevalence of aspirin resistance as indicated by COX-1 activity is rare (<5%), and reports of a higher prevalence of aspirin resistance have been attributed to COX-1 nonspecific methods, noncompliance, or inadequate doses of aspirin in selected patients.\textsuperscript{27-29} The relation of aspirin resistance based on the VerifyNow aspirin assay to ischemic outcome was not demonstrated in a recent large-scale study.\textsuperscript{30} High urine 11-dh-TxB2 is the only marker of aspirin effect associated with clinical outcomes in 2 large clinical trials.\textsuperscript{31,32} A reliable and specific laboratory method to identify aspirin resistance has not yet been uniformly accepted by investigators. Other than in research trials, it is not currently recommended to test for aspirin resistance in patients or to change therapy based on such findings.\textsuperscript{33}

\textbf{The Platelet Hypothesis and the Importance of Platelet Function Measurement}

The first strong evidence about enhanced platelet inhibition by the addition of a P2Y\textsubscript{12} blocker to aspirin came from the work of Cadroy et al. These investigators demonstrated a significant decrease in ex vivo measured ADP-induced platelet aggregation when clopidogrel was added to aspirin for 10 days in healthy volunteers, and this result was further reflected by a decrease in arterial thrombus formation at arterial wall shear rates in a parallel-plate collagen–coated perfusion chamber assay.\textsuperscript{34} The relevance of this ex vivo observation of an enhanced thrombus inhibition resulting from dual pathway blockade to clinical outcomes was explored in large-scale clinical trials. These trials involved a broad spectrum of CAD patients, including stable disease, and provided a strong evidence for the platelet hypothesis. Platelet hypothesis states that pharmacological treatment strategies associated with the better reduction in platelet function determined ex vivo result in the lowest occurrence of clinical thrombotic events.\textsuperscript{35} Further evidence for the platelet hypothesis came from pharmacodynamic studies and clinical trials demonstrating superior platelet inhibition with more potent P2Y\textsubscript{12} inhibitor than clopidogrel is associated with superior anti-ischemic benefits.\textsuperscript{36-40} These results support an objective assessment of platelet function to ensure adequate inhibition to optimize outcomes in patients treated with DAPT. However, despite the evidence that unblocked P2Y\textsubscript{12} receptors play an important role in the genesis of thrombosis, physicians largely do not objectively assess the intensity of the ADP-P2Y\textsubscript{12} interaction in their high-risk patients treated with P2Y\textsubscript{12} receptor inhibitors and instead use a nonsensitive or one-size-fits-all approach.\textsuperscript{35}

\textbf{Ex Vivo Measurement of ADP-Induced Platelet Aggregation in Patients With CAD Treated With Dual Antiplatelet Therapy}

The following events triggered a resurgence in translational platelet function research in the recent years: (1) the development of percutaneous coronary artery revascularization (PCI)—a revascularization method that exerts a powerful thrombogenic stimulus, (2) extensive use of clopidogrel in patients undergoing PCI and subsequent demonstration of its major pharmacodynamic limitations, (3) enhanced
understanding of platelet receptor physiology, and (4) development of new point-of-care platelet function assays.

Järemo et al reported interindividual variability in the antiplatelet response to clopidogrel by measuring ADP-induced fibrinogen binding. At the same time, persistently increased platelet reactivity to ADP as assessed by conventional aggregometry following a 300 mg clopidogrel loading dose was demonstrated in patients undergoing successful PCI. In a subsequent prospective study, ADP-induced platelet aggregation and expression of p-selectin and activated glycoprotein IIb/IIIa were assessed serially for 30 days after stenting and administration of a 300 mg load/75 mg per day maintenance dose of clopidogrel. In this study, ≈30% of patients were resistant to clopidogrel at days 1 and 5 poststenting, and 15% were resistant at day 30. Resistance in these patients was defined as the absolute difference between maximal pre- and post-treatment platelet aggregation induced by 5 μM ADP ≤10%. The results of these studies have provided the strongest rationale for ex vivo quantification of the intensity of the ADP-P2Y12 interaction in patients treated with P2Y12 receptor blockers.

Relation of Ex Vivo Platelet Function Measurement to Clinical Outcome

Barragan et al first demonstrated an association between a platelet reactivity index >50% measured by the vasodilator-stimulated phosphoprotein phosphorylation assay and the occurrence of thrombotic events. At the same time, Mateczny et al, using aggregometry, observed that patients undergoing primary PCI for ST-segment–elevation MI who were in the lowest quartile of clopidogrel responsiveness had the highest rates of ischemic events during follow-up. Subsequently, it was suggested that the level of on-treatment platelet reactivity is a better risk predictor compared with clopidogrel responsiveness.

Small early studies suggested that ischemic event occurrence was not linearly related to on-treatment platelet reactivity but rather occurred above a moderate level of platelet reactivity to ADP. The platelet reactivity in patients and recurrent events post-stenting (PREPARE POST-STENTING) study, the first prospective study linking a threshold of on-treatment platelet reactivity to post-PCI ischemic events, demonstrated that a threshold of ≈50% maximal postprocedural aggregation stimulated by 20 μmol/L ADP was associated with an increased occurrence of ischemic events within 6 months of PCI. Similarly, ADP-induced P2Y12 receptor assessed by various techniques was correlated with stent thrombosis and long-term post-PCI ischemic event occurrences and bleeding (Table 1). Some studies used receiver operating characteristic curve analysis to define an optimal cutoff of on-treatment platelet reactivity for predicting thrombotic risk. In 2010, a consensus statement proposed cutoff values based on receiver operating characteristic curve analysis for different platelet function assays to be used in future studies of personalized antiplatelet therapy.

Personalized Antiplatelet Therapy Trials

Prospective, albeit small, tailored antiplatelet therapy studies provided initial evidence that high platelet reactivity (HPR) may not just be a diagnostic marker but also a modifiable risk factor for post-PCI ischemic event occurrence. In 2 trials, tailored incremental loading doses of clopidogrel before PCI overcame HPR and were effective in reducing 30-day major adverse cardiac events. Similarly, 2 other studies demonstrated that selective glycoprotein IIb/IIIa receptor blocker administration to patients treated with PCI who had HPR after clopidogrel loading was effective in reducing subsequent periprocedural as well as long-term (1-year) ischemic outcomes. These studies were the first to suggest that the cutoff value used to identify PCI-treated patients at increased risk of thrombotic event occurrence was also useful to tailor therapy and led to an improved outcome.

There are 3 prospective investigation of personalized antiplatelet therapy, conducted in patients treated with coronary stents: Gauging Responsiveness with A VerifyNow assay—Impact on Thrombosis and Safety (GRAVITAS) trial (n=2214), Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel (TRIGGER-PCI) study (n=212), and The Assessment by a Double Randomization of a Conventional Antiplatelet Strategy Versus a Monitoring-Guided Strategy for Drug-Eluting Stent Implantation and of Treatment Interruption Versus Continuation One Year After Stenting (ARCTIC) study (n=2440; Table 2). These investigations failed to demonstrate the utility of platelet function testing in reducing the post-PCI ischemic risk. Major criticism for the latter neutral observation are the following: all these investigations used the VerifyNow P2Y12 assay to assess platelet reactivity to ADP and to identify HPR, these investigations included mostly low-risk patients undergoing PCI and had resultant low event rates irrespective of platelet reactivity. Given the postdischarge event rates observed in ARCTIC, it was estimated that 17,540 patients would have been necessary to refute the utility of personalized therapy. Finally, the results of all of these studies also suggest that high-dose clopidogrel is not an optimal strategy to overcome HPR and to improve clinical outcomes.

Although a major risk factor for post-PCI thrombotic event occurrence, HPR is not the sole factor responsible for these events. In contrast, the absence of HPR is the best reassurance thus far for a low likelihood of future ischemic events. The HPR cutoff values reported in many studies is associated with high negative predictive values and low positive predictive values. However, given the overall low prevalence of thrombotic events in these studies, the low positive predictive values and high negative predictive values are understandable. Other factors, including demographic, clinical, and angiographic factors, must be taken into consideration to optimally identify the patients at greatest risk. Along this line, recent studies have suggested that adding clinical variables and genotype to platelet reactivity measurements (a combined risk factor) may improve risk prediction.
Table 1. Studies Linking High On-Treatment Platelet Reactivity to ADP to Post-PCI Adverse Clinical Event Occurrence

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (n)</th>
<th>Treatment</th>
<th>Methods</th>
<th>Definition</th>
<th>Clinical Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barragan et al26</td>
<td>PCI (46)</td>
<td>250 mg qd TLP or CLP 75 mg qd</td>
<td>VASP-PRI</td>
<td>&gt;50% VASP-PRI</td>
<td>↑ Stent thrombosis</td>
</tr>
<tr>
<td>Gurbel et al47</td>
<td>Elective PCI (192)</td>
<td>300 mg LD+75 mg qd CLP±EPT</td>
<td>5 μM ADP-LTA</td>
<td>HPR=75 percentile post-PCI aggregation</td>
<td>↑ 6 mo post-PCI events, OR=2.7</td>
</tr>
<tr>
<td>Matzesky et al43</td>
<td>PCI-STEMI (60)</td>
<td>300 mg LD+75 mg qd CLP±EPT</td>
<td>5 μM ADP-LTA</td>
<td>Reduction in platelet aggregation upper quartile</td>
<td>↑ 6 mo cardiac events</td>
</tr>
<tr>
<td>Geisler et al44</td>
<td>Stable CAD (206), ACS (173)</td>
<td>600 mg CLP LD &gt;6 h before PCI+5 mg qd</td>
<td>20 μM ADP-LTA</td>
<td>&lt;30% ADP-induced aggregation</td>
<td>↑ 3 mo MACE</td>
</tr>
<tr>
<td>Geisler et al45</td>
<td>CAD-PCI (1092)</td>
<td>600 mg CLP LD &gt;6 h before PCI+75 mg qd</td>
<td>20 μM ADP-LTA</td>
<td>Platelet aggregation above median</td>
<td>↑ 30 days MACE; OR=6.7</td>
</tr>
<tr>
<td>Price et al50</td>
<td>PCI (380)</td>
<td>600 mg CLP LD &gt;12 h before PCI or 75 mg qd &gt;5days</td>
<td>VerifyNow P2Y12 assay</td>
<td>HPR=post-treatment ≥235 PRU (ROC)</td>
<td>↑ 6 mo post-PCI events including ST</td>
</tr>
<tr>
<td>Gurbel et al52</td>
<td>Elective PCI (297)</td>
<td>300 or 600 mg LD/75 mg qd CLP±EPT</td>
<td>5 and 20 μM ADP-LTA</td>
<td>HPR=postprocedural (ROC) &gt;48% 5 μM ADP, &gt;50% 20 μM ADP</td>
<td>↑ 2-year ischemic events; 5 μM ADP OR=3.9; 20 μM ADP OR=2.8</td>
</tr>
<tr>
<td>Gurbel et al53</td>
<td>Stenting (120)</td>
<td>75 mg qd CLP &gt;5days</td>
<td>5 and 20 μM ADP-LTA</td>
<td>HPR &gt;75 percentile of platelet reactivity; 5 μM ADP=50%; 20 μM ADP=65%</td>
<td>↑ Stent thrombosis</td>
</tr>
<tr>
<td>Buonamici et al54</td>
<td>PCI-DES (804)</td>
<td>600 mg LD 75 mg qd for 6 mo</td>
<td>10 μM ADP-LTA</td>
<td>HPR ≥70% aggregation</td>
<td>↑ Stent thrombosis; HR=3.08</td>
</tr>
<tr>
<td>Bonello et al51</td>
<td>PCI-stenting (144)</td>
<td>300 mg LD &gt;24 h</td>
<td>VASP-PRI</td>
<td>&gt;50% PRI (ROC)</td>
<td>↑ 6 mo Post-PCI; MACE</td>
</tr>
<tr>
<td>Marucci et al56</td>
<td>PCI-ACS (683)</td>
<td>600 mg LD+75 mg qd</td>
<td>VerifyNow P2Y12 assay</td>
<td>HPR ≥240 PRU</td>
<td>12-month ischemic event; HR CV death=2.21; HR nonfatal MI=3.36</td>
</tr>
<tr>
<td>Sibbing et al57</td>
<td>PCI-DES (1608)</td>
<td>600 mg LD before PCI</td>
<td>6.4 μM ADP–multiplate analyzer</td>
<td>Upper quintile (&gt;468 AU min) (ROC)</td>
<td>↑ 1 mo definite stent thrombosis (OR=9.4)</td>
</tr>
<tr>
<td>Sibbing et al58</td>
<td>PCI-DES (2533)</td>
<td>600 mg LD before PCI</td>
<td>6.4 μM ADP–multiplate analyzer</td>
<td>&lt;188 AU min (ROC)</td>
<td>↑ Major bleeding adjusted OD=3.5</td>
</tr>
<tr>
<td>Cuisset et al59</td>
<td>NSTEMI-Stenting (598)</td>
<td>600 mg LD ≥12 h before PCI</td>
<td>10 μM ADP-LTA VASP-PRI</td>
<td>&gt;67% Aggregation (ROC)</td>
<td>↑ Stent thrombosis</td>
</tr>
<tr>
<td>Breet et al60</td>
<td>Elective PCI (1069)</td>
<td>75 mg qd &gt;5 days; 300 mg LD &gt;1 d; 600 mg LD &gt;4 h</td>
<td>5 and 20 μM ADP-LTA; VerifyNow P2Y12; 20 μM ADP-Plateletworks; Before PCI</td>
<td>OR for 1 y death, MI, ST, and stroke 5 μM ADP=2.09 20 μM ADP=2.05; VerifyNow=2.53; Plateletworks=2.22</td>
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<tr>
<td>Gurbel et al61</td>
<td>Elective PCI (225)</td>
<td>300 or 600 mg LD/75 mg qd CLP</td>
<td>MA-ADP TEG platelet mapping assay</td>
<td>&gt;47MA-ADP; ≤31 MA-ADP</td>
<td>3 y ischemic events (HR=10.3); 3 y bleeding</td>
</tr>
<tr>
<td>Campo et al62</td>
<td>PCI (300)</td>
<td>600 mg CLP ≥12 h before PCI</td>
<td>VerifyNow P2Y12</td>
<td>&gt;23 PRU; &lt;86 PRU</td>
<td>↑ 1 mo ischemic events; ↑ 1 mo bleeding</td>
</tr>
<tr>
<td>Bonello et al63</td>
<td>ACS patients undergoing PCI (301)</td>
<td>Prasugrel</td>
<td>VASP-PRI</td>
<td>&gt;53.5% VASP PRI; &lt;16%</td>
<td>↑ 1 y ischemic events; ↑ 1 y bleeding</td>
</tr>
<tr>
<td>Stone et al65</td>
<td>PCI-DES (8655)</td>
<td>Clopidogrel</td>
<td>VerifyNow P2Y12</td>
<td>&gt;208 PRU</td>
<td>↑ 1 y stent thrombosis HR=2.49; ↑ 1 y MI HR=1.42; ↓ 1 y bleeding HR=0.65</td>
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</table>

AA indicates arachidonic acid; ACS, acute coronary syndrome; ADP, adenosine diphosphate; AU, aggregation units; CAD, coronary artery disease; CLP, clopidogrel; DES, drug eluting stent; EPT, eptifibatide; HR, hazard ratio; LD, loading dose; LTA, light transmittance aggregometry; MACE, major adverse clinical events; MI, myocardial infarction; NSTEMI, non-ST-segment–elevation myocardial infarction; OR, odds ratio; PCI, percutaneous intervention; PCI-DES, percutaneous coronary intervention–drug-eluting stent; PRU, P2Y12 reaction units; qd, once daily; ROC, receiver operating characteristic curve; ST, stent thrombosis; STEMI, ST-segment–elevation myocardial infarction; TLP, ticlopidine; ULMD, unprotected left main disease; and VASP-PRI, vasodilator stimulated phosphoprotein–platelet reactivity index.
Table 2. Prospective Trials of Personalized Antiplatelet Therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (n)</th>
<th>Treatment Regimen to Address HPR</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonello et al64</td>
<td>Patients with coronary stenting, VASP-PRl &gt;50% on 600 mg clopidogrel (VASP-guided group=78 vs control=84)</td>
<td>Repeated CLP LD to decrease VASP-Index &lt;50%</td>
<td>1 y CV death, angiographically-confirmed ST, recurrent ACS: 0% vs10%, P=0.007; Major and minor bleeding: 5% vs 4%, P=1</td>
<td>Low number of patients</td>
</tr>
<tr>
<td>Bonello et al65</td>
<td>Patients undergoing stenting (50% ACS), VASP-PRl &gt;50% on 600 mg CLP LD (VASP-guided group=215 vs control=214)</td>
<td>Repeated CLP LD to decrease VASP-Index &lt;50%</td>
<td>&lt;30 day definite stent thrombosis: 0.5% vs 4.2%, P=0.01 CV death, recurrent ACS and urgent revascularization by coronary angioplasty or bypass surgery; 0.5% vs 8.9%; Bleeding, 2.8% vs 3.7%, P=0.8</td>
<td>Despite a 2400-mg LD of clopidogrel, 8% of patients in the VASP-guided group had VASP-PRl &gt;50%</td>
</tr>
<tr>
<td>Campo et al66</td>
<td>Patients undergoing PCI and treated with 600/300 mg clopidogrel, n=826 ARU; &gt;550=124% PI&lt;40%=278</td>
<td>GPI (tirofiban)</td>
<td>PP-MI was associated with poor responsiveness, HR=1.25. GPI reduced PP-MI in poor responders (21.2% vs 3.47%, P=0.02), not in responders (6.3% vs 6.5%, P=0.8).</td>
<td>Comparatively low-risk patients with silent ischemia, stable angina, and low-risk UA</td>
</tr>
<tr>
<td>Cuisset et al67</td>
<td>Elective PCI patients with 10 μM ADP-induced aggregation &gt;70%</td>
<td>GPI arm, n=74 Conventional arm, n=75</td>
<td>1 y any death, PP-MI, acute, or subacute definite or probable ST, and recurrent ACS, 19% vs 40%, P=0.006</td>
<td>Low number of patients</td>
</tr>
<tr>
<td>GRAVITAS68</td>
<td>Stable (60%) and NSTE-ACS (40%) patients with PRU&gt;230 on CLP</td>
<td>600 mg LD/150 mg MD vs or 75 mg MD for 6 mo</td>
<td>6 mo CV death, nonfatal MI, or stent thrombosis 2.3% vs 2.3%</td>
<td>Mainly low-risk patients, low event rate High dose clopidogrel is not sufficient to reduce HPR VerifyNow P2Y12 assay to determine HPR</td>
</tr>
<tr>
<td>TRIGGER-PCI69</td>
<td>Stable CAD patients undergoing stenting with &gt;208 PRU on 600 mg CLP</td>
<td>60 mg prasugrel (n=212) or placebo (n=212)</td>
<td>6 mo CV death, or MI, 0 vs 1 event; non-CABG TIMI major bleeding, 1.4% vs 0.5%</td>
<td>Stable CAD patients VerifyNow assay Prematurely terminated</td>
</tr>
<tr>
<td>ARCTIC70</td>
<td>2440 patients, 63% stable CAD+27% NSTEMI treated with DES</td>
<td>Monitoring group=1213 VerifyNow assay HPR=550 ARU or &gt;235 PRU (total =40%) GPI (30 during PCI)+additional ≥600 mg CLP (83%) or 60 mg prasugrel (2.3%)</td>
<td>1 y death, MI, ST, or UR (mainly driven by MI) 34% vs 31% P=0.1; No difference in bleeding</td>
<td>Mainly stable CAD patients. VerifyNow Assay. Twice more patients were lost to follow up in the conventional arm than in the monitoring arm (3.8% vs 1.9%). The event rate was mainly driven by periprocedural myocardial infarction that was assessed by nonuniform methodology postprocedure. Prasugrel was administered in only&lt;10% of patients.</td>
</tr>
</tbody>
</table>

CABG indicates coronary artery bypass grafting; CAD, coronary artery disease; CLP, clopidogrel; DES, drug eluting stent; GP, glycoprotein; HPR, high platelet reactivity; HR, hazard ratio; LD, loading dose; MI, myocardial infarction; NSTEMI, non–ST-segment–elevation myocardial infarction; PCI, percutaneous coronary intervention; PRU, P2Y12 reaction units; TIMI, thrombolysis in myocardial infarction; and VASP-PRI, vasodilator stimulated phosphoprotein–platelet reactivity index.

**Therapeutic Window Concept of P2Y<sub>12</sub> Receptor Reactivity**

In addition to the upper threshold for ischemic risk (ie, HPR) described earlier, the relation of low platelet reactivity to bleeding was demonstrated in small translational research studies. The concept of a therapeutic window of P2Y<sub>12</sub> receptor reactivity associated with both ischemic event occurrence (upper threshold) and bleeding risk (lower threshold) has been proposed. A consensus document highlighting the above observations with a therapeutic window concept with updated cutoffs for HPR and low platelet reactivity in the setting of P2Y<sub>12</sub> inhibitor therapy has been recently published (Table 3). This approach is more meaningful while treating patients with more potent P2Y<sub>12</sub> receptor blockers that are known to be associated with increased incidences of bleeding.30,58,61–63

In the recent multinational prospective registry, platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES), platelet reactivity was assessed in 8583 patients (52% ACS patients) after successful drug-eluting stenting with the VerifyNow assay. This study reinforced the independent association between HPR and definite/probable stent thrombosis. In this study, HPR (>208 P2Y12 reaction units) was independently associated with 30-day definite/probable stent thrombosis (HR=3.0; P=0.005), 1-year definite/probable stent thrombosis (HR=2.49; P=0.001) and MI (HR: 1.42; P=0.01), and 2-year definite/probable stent thrombosis (adjusted HR=1.84; P=0.009) and MI (HR=1.33; P=0.01). In addition, >208
P2Y12 reaction units was independently associated with a lower incidence of bleeding at 1 year (HR=0.73; P=0.002) and also at 2 years (HR=0.82; P=0.02).36,37 The largest body of data to support a therapeutic window comes from these ADAPT-DES data.

Evaluating the Clinical Usefulness of Platelet Function Testing As Follows

HPR is consistent with the proposed Wilson–Jungner requirements for a meaningful risk marker6 because (1) it has biological plausibility because it is a measure of the potential for platelet aggregation in vivo, which is the physiological process being targeted by the P2Y12 inhibitor, (2) there is a strong, consistent association of HPR with worse outcome across multiple studies, including observational studies, analyses of randomized clinical trials, and meta-analyses, (3) HPR precedes the event, and (4) there is evidence of a dose–response relationship between the degree of platelet reactivity and outcome.74 Despite these associations, the clinical usefulness of platelet function testing has been disputed, frequently based on misapplication of statistical measures that are more appropriately suited for the evaluation of diagnostic tests, such as sensitivity and specificity, not prognostic indicators, such as odds ratios or HR, which describe the multiplicative risk of the condition or hazard.75

Multivariate models are frequently used to evaluate whether the test is an independent predictor of the outcome. However, simply demonstrating that the test result is significantly associated with the risk for the outcome is not sufficient for concluding that the test is clinically useful. For example, small differences may achieve statistical significance in studies with large populations, but not all statistically significant associations are clinically meaningful. Other statistical techniques have been used to demonstrate that the test has additive value to other established risk assessment indices and improves the overall risk assessment. Net reclassification improvement is useful in demonstrating the ability of a new test to improve risk stratification. In the meta-analysis by Reny et al, the net reclassification improvement for survival data was computed to quantify the contribution of platelet reactivity testing for the prediction of the 6-month risk of major adverse coronary events in patients with increasing numbers of traditional risk factors.76 The strength of the association between the risk of major adverse coronary events and platelet reactivity increased significantly with the number of risk factors present (age >75 years, ACS at inclusion, diabetes mellitus, and hypertension). Measurement of platelet reactivity allowed the reclassification of 44% of the total population to a different risk level for the outcome of major adverse coronary events, mostly in intermediate or high-risk patients. In patients experiencing major adverse coronary events in the first 6 months of follow-up, the risk predicted by the combination of platelet reactivity and risk factors was on average increased compared with the risk predicted from risk factors only: the net reclassification improvement was 0.39 (95% confidence interval [CI] 0.23–0.62).76

Platelet Reactivity in Medically Managed ACS Patients

Thus far, the majority of studies that demonstrated a strong association between HPR and clinical outcomes were conducted in patients undergoing PCI. Recently, 9326 patients with medically managed unstable angina or non–ST-segment–elevation myocardial infarction were randomly assigned to treatment with either clopidogrel or prasugrel in the The Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes (TRILOGY) study and 27.5% were included in the platelet function sub-study (prasugrel, n=1286 and clopidogrel, n=1278).77 Platelet function was measured serially ≤30 months after randomization by VerifyNow assay. In this study, prasugrel therapy was consistently associated with lower platelet reactivity than clopidogrel, irrespective of age, weight, and dose. However, there was no significant independent association between platelet reactivity and the occurrence of the primary ischemic end point. In addition, in the Antiplatelet Drug Resistances and Ischemic Events (ADIRE) study that enrolled 771 stable cardiovascular outpatients, platelet reactivity was not associated with 3-year major cardiovascular adverse events.78

Personalized Antiplatelet Therapy in Patients Undergoing Surgery

The major rationale for 5- to 7-day discontinuation of P2Y12 receptor inhibitor treatment recommended by the guidelines in patients undergoing coronary artery bypass grafting was to allow platelet function recovery, thereby avoiding excessive perioperative bleeding. In a small prospective study, it was demonstrated that clopidogrel-treated patients undergoing first-time on-pump coronary artery bypass grafting had similar bleeding (24-hour chest tube output and number of transfused red blood cells) as clopidogrel naïve patients when surgery was scheduled on the basis of a preoperative assessment of platelet reactivity to ADP. Moreover, the individualized timing

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Table 3. Platelet Reactivity Cutoff Associated With Ischemic and Bleeding Events (Therapeutic Window)

<table>
<thead>
<tr>
<th>Test</th>
<th>Cutoff Associated With Ischemic Event Occurrences</th>
<th>Cutoff Associated With Bleeding Event Occurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>VerifyNow PRU Assay (PRU)</td>
<td>&gt;208 (30, 66)</td>
<td>&lt;85 (66)</td>
</tr>
<tr>
<td>Multiplate Analyzer ADP-induced aggregation, AU min</td>
<td>&gt;46 (58)</td>
<td>&lt;19 (58)</td>
</tr>
<tr>
<td>Thrombelastography Platelet Mapping Assay ADP-induced Platelet-fibrin clot strength, mm</td>
<td>&gt;47 (61)</td>
<td>&lt;31 (61)</td>
</tr>
<tr>
<td>VASP-PRI</td>
<td>≥50% (62)</td>
<td>&lt;16% (62)</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; ADP, adenosine diphosphate; AU, arbitrary aggregation units; PRU, P2Y12 reaction units; and VASP-PRI, vasodilator-stimulated phosphoprotein phosphorylation-platelet reactivity index.

---
of surgery reduced the overall preoperative waiting period by ≈ 50% as compared with the time recommended in the guidelines. Preoperative platelet reactivity to ADP was measured by thrombelastography (with Platelet Mapping). Surgery in patients treated with clopidogrel was scheduled within 24 hours of the last dose of clopidogrel in those with a maximum amplitude (MA\textsubscript{ADP}) > 50 mm, within 3 to 5 days of the last dose in those with an MA\textsubscript{ADP} 35 to 50 mm, and 5 days after the last dose in those with an MA\textsubscript{ADP} < 35 mm.59

Guidelines
In 2012, updated American and European practice guidelines have issued a Class IIb recommendation for platelet function testing to facilitate the choice of P2Y\textsubscript{12} receptor inhibitor in selected high-risk patients treated with PCI.80-84 In the 2012 update to the Society of Thoracic Surgeons guideline on use of antiplatelet drugs in patients having cardiac and noncardiac operations, there is a Class IIa recommendation for platelet function testing in clopidogrel-treated patients to shorten the preoperative waiting period.85 Similarly, there is Class IIb recommendation to consider platelet function testing in shortening the time window to coronary artery bypass grafting after P2Y\textsubscript{12} inhibitor discontinuation in the 2015 ESC Guidelines for the management of ACSs in patients presenting without persistent ST-segment elevation.86

Conclusions
Platelet activation and aggregation resulting in thrombus generation at the site of plaque rupture is a primary underlying factor responsible for the development of ischemic events in patients with cardiovascular disease. Strong evidence indicates that the ADP-P2Y\textsubscript{12} receptor interaction plays a central role in modulating platelet reactivity and arterial thrombotic event occurrence. HPR is associated with poorer clinical outcomes in high-risk clopidogrel-treated patients who have undergone PCI. The development of more user-friendly methods to assess platelet reactivity to ADP has resulted in a large body of data supporting a strong relation between the results of the ex vivo platelet function testing and clinical event occurrence. Based on observational studies conducted in thousands of patients, an international consensus is that HPR is a major risk factor for post-PCI ischemic event occurrence. The concept of a therapeutic window of P2Y\textsubscript{12} receptor reactivity associated with both ischemic event occurrence (upper threshold) and bleeding risk (lower threshold) has also been proposed. However, 2 large prospective trials using VerifyNow have thus far failed to demonstrate that personalized antiplatelet therapy is effective in reducing ischemic event occurrences. Potential explanations for the neutral results may be related to the assay itself. Platelet agglutination to fibrinogen-coated beads in anticoagulated blood in the VerifyNow P2Y12 assay may not optimally discriminate high-risk patients. In this line, other point-of-care assays such as the Multiplate analyzer which is based on impedance aggregometry and thrombelastography by TEG6s which measures platelet–fibrin clot strength may be more effective in discriminating risk.59 In addition, the risk of the patient groups studied in GRAVITAS and ARCTIC were overall low and led to underpowering to demonstrate the utility of platelet function testing in personalizing antiplatelet therapy. The potency of the strategy chosen to overcome HPR was mostly a doubling of the standard clopidogrel dose; a strategy much less effective than therapy with a new P2Y\textsubscript{12} inhibitor. Finally, HPR may not be a modifiable risk factor.

In the ongoing Testing Responsiveness to Platelet Inhibition on Chronic Antiplatelet Treatment for Acute Coronary Syndromes (TROPICAL-ACS) study (n=2600), a Multiplate analyzer-guided approach to personalize antiplatelet therapy in ACS patients treated with PCI is being evaluated. In the absence of strong prospective evidence to support personalized antiplatelet therapy, the bench is brought to the bedside by 3 major advances in the last 20 years: (1) our understanding of the central role that the P2Y\textsubscript{12} receptor plays in platelet-mediated thrombosis and (2) observational data strongly demonstrating that poor P2Y\textsubscript{12} blockade is an independent risk factor in patients treated with coronary artery stents and that (3) the magnitude of the association between platelet reactivity and outcomes seems influenced by the level of cardiovascular risk.

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References


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