Do Platelet Function Testing and Genotyping Improve Outcome in Patients Treated With Antithrombotic Agents?

Platelet Function Testing and Genotyping Improve Outcome in Patients Treated With Antithrombotic Agents

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The only reason P2Y\textsubscript{12} inhibitors are administered in addition to aspirin is to improve the prevention of thrombosis. The clinical efficacy of adding clopidogrel to aspirin as a secondary prevention strategy in patients with high-risk coronary artery disease is well established.\textsuperscript{1} There are no effects of clopidogrel on any receptor other than P2Y\textsubscript{12} to explain the magnitude of the clinical benefit. All of the established clinical effects are attributed to reduced platelet responsiveness to ADP.\textsuperscript{2} Therefore, the patient with inadequate P2Y\textsubscript{12} inhibition determined by ex vivo testing logically has an increased risk for thrombosis. Persistent ischemic event occurrence and the irrefutable demonstration of clopidogrel antiplatelet response variability are 2 potent arguments against the widely practiced nonselective or one-size-fits-all strategy of administering clopidogrel therapy. Observational studies conducted in thousands of patients have led to an international consensus that high on-treatment platelet reactivity (HPR) to ADP is a major risk factor for post–percutaneous coronary intervention (PCI) ischemic event occurrence.\textsuperscript{3,4} Moreover, the recent 2011 American and European guidelines have given a Class IIb recommendation for platelet function testing or genotyping if the results of testing may alter management.\textsuperscript{5-7} Furthermore, the Society of Thoracic Surgeons gave a Class IIb recommendation for platelet function testing to determine the timing of surgery in patients on clopidogrel therapy (Level of Evidence C).\textsuperscript{8} These recommendations for personalizing antiplatelet therapy are unprecedented and acknowledge that a large body of data has accrued demonstrating the relation of HPR to ischemic risk in the PCI-treated patient. Finally, the evidence of diminished effectiveness of clopidogrel in poor metabolizers (those having 2 loss-of-function [LoF] cytochrome P450 [CYP] 2C19 alleles) has been recognized by the Food and Drug Administration (FDA) boxed warning about treatment with clopidogrel.\textsuperscript{9}

The Great Paradox: Reluctance to Quantify the ADP-P2Y\textsubscript{12} Interaction in Clinical Practice

Myocardial infarction (MI) and stent thrombosis are catastrophic events that occur in patients with coronary artery disease. Overwhelming evidence exists that thrombus generation resulting from platelet activation and aggregation at the sites of plaque rupture and endothelial cell erosion is the primary process involved in the occurrence of the latter clinical events.\textsuperscript{10} Although thromboxane A\textsubscript{2} and ADP act synergistically during platelet aggregation, the ADP-P2Y\textsubscript{12} receptor interaction plays a central role in sustaining the activation of glycoprotein IIb/IIIa receptors by amplifying the response to agonists. P2Y\textsubscript{12} activation also modulates platelet procoagulant activity, P-selectin expression, and inflammation (Figure 1).\textsuperscript{11} Numerous pharmacodynamic studies dem-
onstrated the strong relation between the occurrence of stent thrombosis and clopidogrel nonresponsiveness or HPR. The prothrombotic effects of the P2Y12 platelet receptor serve as the rationale for its pharmacological inhibition as a key strategy to prevent MI and stent thrombosis.

Because the development of all P2Y12 inhibitors is fundamentally based on a comparative assessment of ex vivo effects, we must ask why there is reluctance to use the same methodology to ensure that an optimal antiplatelet effect is present in patients. The most widely used method to assess platelet function during the development of P2Y12 inhibitors is conventional aggregometry. However, point-of-care assessment now allows rapid assessment of platelet reactivity, strongly correlates with aggregometry, and has been associated with clinical outcomes. Despite the fundamental importance of unblocked P2Y12 receptors in the genesis of thrombosis, physicians largely do not objectively assess the intensity of the ADP-P2Y12 interaction in their high-risk patients treated with clopidogrel, and instead use a nonselective or one-size-fits-all approach. The latter remains one of the most curious paradoxes in cardiovascular medicine. Aspirin and a P2Y12 inhibitor are arguably the most important pharmacological agents administered to the high-risk PCI/acute coronary syndrome (ACS) patient because of their intended actions to block platelet reactivity, yet there is reluctance to confirm that therapy is actually effective in the treated patient. The nonselective treatment approach with oral antiplatelet agents is unique compared with the objective assessments and adjustments made after administration of most drugs used to treat patients with cardiovascular disease. In fact, personalized parenteral antithrombotic therapy is widely administered for PCI. Weight-adjusted heparin and glycoprotein IIb/IIIa inhibitor administration is the standard of care, and activated clotting time is used to determine an optimal level of anticoagulation. Some examples of other drugs and assessments for personalizing therapy are β-blockers and heart rate/blood pressure, statins and cholesterol levels, antihypertensives and blood pressure, insulin and glucose levels, diuretics and renal function/patient weight, and warfarin and international normalized ratio.

Degree of Ex Vivo Inhibition of Platelet Reactivity to ADP: A Predictor of Preclinical and Clinical Efficacy

Clopidogrel: Strong Support for the Platelet Hypothesis

Cadroy et al demonstrated that adding 75 mg/d clopidogrel to 325 mg/d aspirin for 10 days significantly decreased 5 μmol/L ADP–induced platelet aggregation compared with aspirin administration alone in healthy volunteers. The degree of platelet inhibition measured ex vivo was reflected by a decrease in arterial thrombus formation at arterial wall shear rates in a parallel-plate collagen-coated perfusion chamber assay. Furthermore, the antiplatelet and antithrombotic effects were more pronounced and appeared earlier (at 90 minutes) when a 300-mg clopidogrel loading dose was administered (Figure 2A and 2B). The work by Cadroy et al provided strong evidence that the ex vivo measurement of
platelet inhibition resulting from the addition of a P2Y₁₂ blocker predicted the ex vivo antithrombotic response. The clinical relevance of the ex vivo measured effects on platelet function resulting from clopidogrel plus aspirin therapy was put to the test in numerous trials involving patients with a broad spectrum of coronary artery disease, including non-ST-segment–elevation ACS and ST-segment–elevation MI. These trials demonstrated the superiority of adding clopidogrel to aspirin in reducing major adverse cardiac events. Finally, a meta-analysis performed in 79,262 patients enrolled in the above trials conclusively demonstrated that adding clopidogrel to aspirin is associated with a significant decrease in MI (odds ratio=0.82; P<0.001), stroke (odds ratio=0.82; P=0.002), and mortality (odds ratio=0.94; P=0.026; Figure 3). In these trials, the major driving factor for clinical efficacy was the significant reduction in MI observed with clopidogrel therapy. These trials provided further evidence for the platelet hypothesis, which states that pharmacological treatment strategies associated with a superior reduction in platelet function (by the addition a P2Y₁₂ inhibitor to aspirin) will result in superior clinical outcomes by reducing the occurrence of thrombosis.

Further evidence that ex vivo platelet function measurements correlate with clinical response comes from the PCI cohort of the Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events–Seventh Organization to Assess Strategies in Ischemic Syndromes (CURRENT OASIS-7) trial. Previously, it was demonstrated that a 600-mg clopidogrel loading dose produced greater platelet inhibition and was associated with less response variability and resistance compared with a 300-mg loading dose (Figure 4). Once again, the clinical response mirrored the pharmacodynamic response: A high loading dose followed by 7 days of 150-mg maintenance therapy was associated with a reduction in 30-day ischemic event occurrence, including stent thrombosis, in the PCI cohort of OASIS-7.

TRITON and PLATO: More Strong Support for the Platelet Hypothesis

Greater levels of active metabolite generation during prasugrel therapy (60-mg load/10 mg daily) were associated with greater platelet inhibition compared with clopidogrel therapy (600-mg load/75 mg daily). In a phase 2 trial, prasugrel was associated with a faster onset of action and greater platelet inhibition than high-dose clopidogrel. The clinical relevance of these superior inhibitory effects measured ex vivo was put to the test in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38 study. In that study, prasugrel therapy was associated with less major adverse cardiac event occu-
rence than clopidogrel therapy in high-risk ACS patients undergoing PCI. The effect of prasugrel therapy on the occurrence of stent thrombosis was dramatic (~50% reduction).25

In the Multi-Centre Randomized, Double-Blind, Double-Dummy Parallel Group Study of the Onset and Offset of Antiplatelet Effects of AZD6140 Compared With Clopidogrel and Placebo With Aspirin as Background Therapy in Patients With Stable Coronary Artery Disease (ONSET/OFFSET) study, ticagrelor, a direct-acting P2Y12 receptor blocker, was associated with a more rapid onset of action, a greater level of inhibition during maintenance therapy, and a more rapid offset of pharmacodynamic effect than clopidogrel.26 In the Randomized, Double-Blind, Outpatient, Crossover Study of the Anti-Platelet Effects of AZD6140 Compared With Clopidogrel in Patients With Stable Coronary Artery Disease Previously Identified as Clopidogrel Non-Responders or Responders (RESPOND) study, ticagrelor therapy was associated with greater platelet inhibition compared with clopidogrel in both clopidogrel responders and nonresponders. Ticagrelor was extremely effective in reducing the prevalence of HPR within 30 minutes of therapy.27 The superior pharmacodynamic effects observed in the ONSET/OFFSET and RESPOND studies were mirrored by the superior clinical efficacy of ticagrelor observed in the Platelet Inhibition and Patient Outcomes (PLATO) trial.28

The results of the ex vivo platelet function measurements during prasugrel and ticagrelor therapy in conjunction with the results of the TRITON and PLATO trials lend powerful support for the platelet hypothesis. In trials enrolling more

Figure 3. Enhanced in vivo antithrombotic effect by the addition of clopidogrel to aspirin observed across a wide spectrum of coronary artery disease in large-scale randomized trials and a meta-analysis of these trials. ASA indicates aspirin; CLP, clopidogrel; MI, myocardial infarction; OR, odds ratio; NSTE ACS, non-ST-segment–elevation acute coronary syndromes; STEMI, ST-segment–elevation myocardial infarction; CAD, coronary artery disease; CURE indicates Clopidogrel in Unstable Angina to Prevent Recurrent Events; CREDO, Clopidogrel for the Reduction of Events During Observation; CLARITY, Clopidogrel as Adjuvant to Reperfusion Therapy; COMMIT, Clopidogrel and Metoprolol in Myocardial Infarction Trial; and CHARISMA, Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization Management and Avoidance. Data derived from Gurbel et al1 and Helton et al.20

Figure 4. Greater ex vivo platelet inhibition and less response variability after a 600-mg clopidogrel load compared with a 300-mg load in percutaneous coronary intervention patients measured at 24 hours after loading. Resistance is defined as absolute change in maximal aggregation from pretreatment (ΔA) ≤10%. Adapted from Gurbel et al,22 with permission of the publisher. Copyright © 2011, Elsevier.
than 100,000 high-risk patients, the clinical response to an antiplatelet regimen has always reflected the ex vivo response to that regimen.20,25,28 Thus, an objective assessment of platelet function to ensure adequate inhibition and to optimize outcomes in clopidogrel-treated patients is logical.

Understanding the Mechanisms Responsible for the Variable Antiplatelet Effect of Clopidogrel and the Role of the CYP2C19 LoF Allele: The Rationale for Genetic Testing

The unpredictable antiplatelet response to clopidogrel was reported nearly a decade ago in *Circulation*: ∼30% of PCI patients were resistant (≥10% decrease in platelet aggregation from baseline) at 24 hours after a 300-mg load, and this prevalence of resistance persisted at 5 days and fell to ∼15% at 30 days after PCI during 75-mg/d maintenance therapy.29 Similar observations have been made in numerous subsequent studies involving thousands of PCI patients.4 In a recent study reported in this journal, ADP-induced platelet aggregation in ∼42% of stable CAD patients after aspirin plus 600-mg clopidogrel loading dose was in the same range observed in ∼50% of patients treated with 75–100 mg per day aspirin alone. These patients have also been defined as having high on-treatment platelet reactivity (see Reference 4). Adapted from Gurbel et al.26

Clopidogrel is a prodrug that requires metabolic activation by CYPs to a reactive metabolite (Figure 6).30 CYP isoenzyme activity is influenced by single-nucleotide polymorphisms primarily of the gene encoding the CYP2C19 isoenzyme and interactions with other drugs. These influences contribute to variable and, in some cases, insufficient active metabolite generation, leading to resistance.4 Common LoF polymorphisms of CYP2C19 and CYP2C9 have been associated with decreased clopidogrel active metabolite exposure and less platelet inhibition.31 Less plasma active metabolite exposure (34% relative reduction; *P*<0.001) and a reduction in platelet aggregation (9% absolute reduction; *P*<0.001) were demonstrated in healthy carriers of at least 1 CYP2C19 LoF allele compared with noncarriers.32 In the first genome-wide association study, conducted in healthy subjects, CYP2C19*2 was the only single-nucleotide polymorphism associated with clopidogrel response variability. In a replication study of PCI patients, carriers of the CYP2C19*2 allele had an ∼2.4-times-higher cardiovascular event rate compared with noncarriers.33 Elsewhere, it was reported that patients carrying 2 LoF alleles had a 1.98-times-higher rate of cardiovascular events than noncarriers among acute MI patients, and the risk was even higher (3.6 times) among patients who underwent PCI.34 In a collaborative meta-analysis of clinical trials involving primarily patients who underwent PCI (91%), an increased risk of the composite end point of cardiovascular death, MI, or stroke among carriers of 1 LoF allele (1.6 times) and carriers of 2 LoF alleles (1.8 times) compared with noncarriers was reported. A significantly increased risk of stent thrombosis in both carriers of 1 LoF allele (2.7 times) and 2 LoF alleles (4 times) compared with noncarriers was also observed.35

Subsequent retrospective analyses of trials involving mainly non-PCI patients failed to demonstrate a significant association between CYP2C19 LoF allele carriage and adverse clinical outcomes. The relation of the gain-of-function allele (CYP2C19*17) carrier status and ABCB1 genotype (gene encoding the intestinal transporter protein that participates in clopidogrel absorption) to the antiplatelet response of clopidogrel and clinical outcomes in clopidogrel-treated patients is inconclusive at this time.32–38 Most recently, a single-nucleotide polymorphism of the gene encoding paraoxonase-1 was linked to the pharmacokinetic and pharmacodynamic effects of clopidogrel and stent thrombosis.39 However, in a substudy of the Gauging Responsiveness With A VerifyNow Assay—Impact on Thrombosis and Safety (GRAVITAS) trial and another study, there was no influence of the paraoxonase-1 polymorphism on platelet reactivity during clopidogrel therapy.40,41

Taken together, these findings show that LoF allele carrier status is an important independent predictor of the pharmacodynamic response to clopidogrel and the outcomes of high-risk clopidogrel-treated patients who have undergone PCI. The strong relation of carriage to the occurrence of stent thrombosis is noteworthy and is a major rationale for determining the genotype of the PCI patient being considered for or already treated with clopidogrel.

Addressing the Problem of CYP2C19 LoF Allele Carriage: High-Dose Clopidogrel Is Not the Best Remedy

In May 2009, the FDA first added information about poor metabolizers to the Plavix drug label.9 Another pharmacody-
namic study prompted the addition of a boxed warning. In this randomized crossover study, the pharmacokinetic and pharmacodynamic effects of clopidogrel were examined in 40 healthy subjects, 10 from each of the genetically predicted metabolizer groups (ultrarapid, extensive, intermediate, and poor metabolizers). Subjects were randomized to treatment with either a 300-mg load followed by 75 mg daily for 5 days or a 600-mg load followed by 150 mg/d clopidogrel for 5 days. Compared with the other groups, poor metabolizers had the lowest peak plasma concentrations of clopidogrel active metabolite and the least platelet inhibition regardless of dose. Among the poor metabolizers, active metabolite concentration and platelet inhibition were greater with the higher-dose regimen compared with the lower-dose regimen.42 On the basis of these results, the FDA noted that healthcare professionals should be aware that tests are available to determine genotype and that the antiplatelet response in poor metabolizers is increased by high-dose clopidogrel. The FDA also recommended the use of other antiplatelet medications or alternative dosing strategies for clopidogrel in poor metabolizers.9

However, other studies indicated the limited influence that high-dose clopidogrel has in normalizing platelet reactivity in patients carrying the LoF allele and that the antiplatelet effect of 150 mg/d clopidogrel was negligible in poor metabolizers.43,44 The largest (n = 1152) combined assessment of genotype and serial platelet function was reported in a recent substudy of the GRAVITAS trial. In that analysis, the CYP2C19 LoF allele was significantly associated with high post-PCI platelet reactivity during high-dose clopidogrel treatment (odds ratio = 1.62 for 1 LoF and 11.2 for 2 LoF alleles). LoF homozygotes with HPR treated with high-dose clopidogrel had the same poor antiplatelet response as LoF homozygotes with HPR treated with standard-dose clopidogrel.40 All of these data suggest that the recommendation implied by the FDA that poor metabolizers be treated with a high-dose clopidogrel regimen that was based on a healthy volunteer study is not relevant for CAD patients treated with stents.

In a randomized study of patients with stable CAD, the CYP2C19 genotype and single-nucleotide polymorphisms of genes encoding other isoenzymes did not affect prasugrel active metabolite formation or the magnitude of platelet inhibition during either the loading or maintenance phase of treatment.45 Furthermore, in a subanalysis of the TRITON-TIMI 38 trial, carriers of the LoF allele treated with clopidogrel had higher rates of the primary outcome (12.1% versus 8.0%; hazard ratio = 1.53; P = 0.01) and definite/probable...
stent thrombosis (2.6% versus 0.8%; hazard ratio = 3.09; 95% confidence interval = 1.19–8.00; P = 0.02) compared with noncarriers. However, among prasugrel-treated patients, LoF carrier status was unrelated to outcomes.\textsuperscript{35,46} The \textit{CYP2C19} LoF genotype significantly influenced the antiplatelet effect of clopidogrel but not ticagrelor, a direct-acting P2Y\textsubscript{12} receptor blocker, in stable coronary artery disease patients.\textsuperscript{47} Platelet reactivity in ticagrelor-treated patients was consistently lower than in clopidogrel-treated patients regardless of \textit{CYP2C19} genotype.\textsuperscript{47} Furthermore, in the genetic substudy of the PLATO trial, ticagrelor was associated with a reduced occurrence of cardiovascular events compared with clopidogrel regardless of genotype.\textsuperscript{37}

The Escalating Clopidogrel by Involving a Genetic Strategy (ELEVATE–TIMI 56 trial, conducted in PCI patients, demonstrated that triple-dose clopidogrel (225 mg daily) achieved, on average, the same platelet inhibitory effect in heterozygous LoF allele carriers that standard-dose clopidogrel (75 mg daily) achieved in noncarriers. Daily doses up to 300 mg were not able to produce a similar level of platelet inhibition in poor metabolizers.\textsuperscript{48} Taken together, these most recent studies indicate that higher clopidogrel doses can overcome the diminished pharmacokinetic/pharmacodynamic effects observed in carriers of 1 LoF allele during standard-dose clopidogrel therapy, whereas prasugrel and ticagrelor are better alternatives than high-dose clopidogrel in carriers of 2 LoF alleles (poor metabolizers).

The fundamental reason for genotyping clopidogrel-treated patients is to identify the high-risk phenotype, ie, HPR. However, clopidogrel metabolism is influenced by other administered drugs and agents that share metabolic pathways with clopidogrel such as proton pump inhibitors, calcium channel blockers, and cigarette smoke (Figure 6). In addition, on-treatment platelet reactivity to ADP is influenced by the coronary artery disease state, age, sex, diabetes mellitus, and obesity.\textsuperscript{4} The net cumulative effect of all of these influences is reflected in the final platelet reactivity phenotype. Although the genotype is permanent, the cumulative influence of other factors on platelet reactivity is dynamic. Therefore, assessment of platelet function may be more appropriate than genotyping to indicate the risk for ischemic event occurrence.

\textbf{Rationale for Platelet Function Testing: The Overwhelming Body of Evidence Linking HPR to Ischemic Event Occurrence}

Over 30 translational research studies involving thousands of patients have evaluated the relation of platelet reactivity during clopidogrel treatment to the risk of post-PCI ischemic event occurrence.\textsuperscript{4} Each of these studies has reached the identical conclusion: Patients treated with PCI who have HPR are definitely at increased risk for ischemic events, including stent thrombosis. These overwhelmingly concordant findings from studies conducted at centers around the world provide the strongest rationale for ex vivo quantification of the intensity of the ADP-P2Y\textsubscript{12} interaction in patients treated with PCI and clopidogrel. Barragan et al\textsuperscript{49} first demonstrated an association between a platelet reactivity index >50% measured by vasodilator-stimulated phosphoprotein phosphorylation and the occurrence of thrombotic events in a case-control study. At the same time, Matezky et al,\textsuperscript{50} 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. Recent meta-analyses of studies using the VerifyNow point-of-care assay lend strong support for the prior observations.\textsuperscript{51}

\textbf{Evidence for a Threshold}

Small early studies demonstrated that ischemic risk was not linearly related to on-treatment platelet reactivity but rather occurred above a moderate level of platelet reactivity to ADP. In the Platelet Reactivity in Patients and Recurrent Events Poststenting (PREPARE POST-STENTING) study, a threshold of \(\approx50\%\) maximal postprocedural aggregation (20 \(\mu\)mol/L ADP) was associated with the occurrence of an ischemic event within 6 months.\textsuperscript{52} Similarly, in the Clopidogrel Effect on Platelet Reactivity in Patients With Stent Thrombosis (CREST) study, \(\approx40\%\) aggregation (20 \(\mu\)mol/L ADP) was associated with stent thrombosis occurrence.\textsuperscript{53} In a third study, \(\approx40\%\) preprocedural platelet aggregation (5 \(\mu\)mol/L ADP) among patients receiving clopidogrel and aspirin therapy before stenting was associated with an ischemic event occurring within 12 months.\textsuperscript{54} Subsequent studies have provided evidence for a threshold of platelet reactivity associated with ischemic event occurrence. The threshold concept also has significant implications for reducing bleeding risk because achieving levels of platelet reactivity below the threshold may not further reduce ischemic risk.\textsuperscript{3,4}

Cutoff values for HPR determined by receiver-operating characteristic curve analysis have been associated with a high negative predictive value for the occurrence of major adverse cardiac events.\textsuperscript{4} In the large Do Platelet Function Assays Predict Clinical Outcomes in Clopidogrel-Pretreated Patients Undergoing Elective PCI (POPULAR) study, multiple assays were used to measure platelet reactivity in PCI patients who were then followed up for ischemic event occurrence within 1 year.\textsuperscript{13} As expected from the overall low prevalence of event rates, the positive predictive value was low for all assays used in the POPULAR study and in all the aforementioned studies. Receiver-operating characteristic curve analysis identified an association of the VerifyNow assay, light transmittance aggregometry, and single platelet counting results with the occurrence of the composite primary end point, with an area under the curve of \(\approx0.62\) for each assay. The addition of HPR as measured by the noted platelet assays to more classic clinical and procedural risk factors resulted in a statistically significant improvement of the area under the curve to \(\approx0.73\).\textsuperscript{13}

The HPR threshold in the consensus statement was determined by receiver-operating characteristic curve analysis and is applicable only to the PCI population.\textsuperscript{4} However, on the
basis of the group of patients from GRAVITAS treated with standard-dose clopidogrel, an even lower threshold (≈170 P2Y12 reaction units [PRUs]) was associated with much greater sensitivity for ischemic event occurrence. It was suggested that this “immunity to thrombosis” cutoff should be considered the new therapeutic target in the PCI patient because a major goal of the treating physician who performs platelet function testing is to ensure that the patient is out of the danger zone for stent thrombosis.\textsuperscript{55,56} During the early phase of ACS and/or PCI, disease activity is greatest, and the prevalence of clopidogrel nonresponsiveness level is higher. At that time, a potent antiplatelet regimen may provide the greatest net clinical benefit (reduction in ischemic events that outweighs the risk of bleeding events), whereas at time points further downstream from the ACS event, less intense antiplatelet effects may be desirable. The optimal HPR threshold at ≈30 days may therefore differ from the acute threshold during the index ACS hospitalization.

**HPR Is Not Just a Risk Predictor But a Modifiable Risk Factor: Current Evidence From Prospective Studies**

The large body of consistent data from observational studies is strong evidence that HPR is a risk predictor; a single post-PCI measurement predicted both short-term and long-term (up to 2 years) clinical outcomes.\textsuperscript{3,4} Subsequent prospective, albeit small, studies provided evidence that HPR is not just a risk predictor but a modifiable risk factor. In these prospective trials, tailored incremental loading doses of clopidogrel overcame HPR and were effective in reducing major adverse cardiac events.\textsuperscript{57,58} Similarly, 2 other studies demonstrated that selective glycoprotein IIb/IIIa receptor blocker administration to PCI patients with HPR after clopidogrel loading was effective in reducing subsequent 30-day and 1-year post-PCI ischemic event occurrence.\textsuperscript{59,60} These studies were the first to suggest that the cutoff value identifying patients at increased risk of thrombotic events was useful in tailoring therapy and led to an improved outcome. This is in contrast to clinical trials such as TRITON-TIMI 38 and PLATO, in which the treatment strategy was to nonselectively provide more potent P2Y\textsubscript{12} inhibitors to all ACS patients. This strategy was associated with a significant increase in major bleeding.\textsuperscript{25,28} To date, a strategy of intensified antiplatelet therapy in only patients with HPR has not been associated with a significant increase in bleeding.

**Recent Studies**

The GRAVITAS trial was the first large-scale investigation of personalized antiplatelet therapy in the elective PCI patient.\textsuperscript{61} Patients with HPR were treated with either a 600-mg extra loading dose given the day after stenting followed by doubling the standard dose of clopidogrel maintenance therapy or standard-dose clopidogrel therapy for 6 months. In addition, a group of patients without HPR were treated with standard-dose clopidogrel therapy. High-dose clopidogrel treatment was ineffective in reducing 6-month composite ischemic event occurrence (cardiovascular death, nonfatal MI, and stent thrombosis); both HPR groups had an unexpectedly low event rate (2.3%). There are several reasons for the neutral results of GRAVITAS. The most unlikely is that HPR identified after PCI is only a risk indicator and not a modifiable risk factor. A credible argument against the latter comes from the results from large-scale clinical trials of ACS patients demonstrating that treatment with P2Y\textsubscript{12} inhibitors associated with more potent platelet inhibition than clopidogrel produces lower ischemic event rates than clopidogrel treatment. Another possible explanation is that high-dose clopidogrel was a suboptimal remedy to overcome HPR. In GRAVITAS, high-dose clopidogrel reduced the prevalence of HPR at 30 days in only 60% of patients. In a study with an event rate as low as GRAVITAS, only treatment with a highly effective remedy to reduce HPR would have had a chance to produce positive clinical trial results. Finally, the cutoff for HPR may have been too high.\textsuperscript{62} Subsequent analyses from GRAVITAS have demonstrated the clustering of events above and below the cutoff of 230 PRUs and that “responders” had events that clustered just below 230 PRUs. In a time-covariate Cox regression analysis of on-treatment platelet reactivity, PRUs <208 was an independent predictor of event-free survival at 60 days (hazard ratio = 0.23; 95% confidence interval = 0.05–0.98; P = 0.047) and strongly trended to be an independent predictor at 6 months (hazard ratio = 0.54; 95% confidence interval = 0.28–1.04; P = 0.06).\textsuperscript{63} These findings are particularly important given the very low event rate observed in GRAVITAS.

In the Testing Platelet Reactivity in Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel (TRIGER-PCI) study conducted in stable elective PCI patients (non-ST-segment–elevation and ST-segment–elevation MI excluded), the HPR cut point of >208 PRUs was used.\textsuperscript{64} Instead of a 150-mg daily dose of clopidogrel, a 10-mg daily dose of prasugrel was used in the active arm and was highly effective in reducing the prevalence of HPR, only ≈6% of patients had HPR after 90 days of prasugrel therapy. However, the study was terminated early because of futility. There was only 1 occurrence of the primary end point among 236 patients who completed 6 months of follow-up. In addition, ≈30% of the enrolled patients declined randomization after being identified as having HPR.\textsuperscript{64}

The Assessment of Dual Antiplatelet Therapy With Drug-Eluting Stents (ADAPT-DES) trial was large-scale, prospective, multicenter registry examining the relationship between platelet reactivity and stent thrombosis after drug-eluting stent implantation. In this much-higher-risk population (≈50% had ACS), those with >208 PRUs had a 3-fold adjusted hazard for the occurrence of 30-day stent thrombosis.\textsuperscript{65} However, a relative risk ratio of ≈2 for hypercholesterolemia currently serves as a rationale for testing and altering therapy.\textsuperscript{66}
Limitations of Diagnostic Test Statistics to Describe Prognostic Test Utility

The occurrence of future ischemic events is influenced by many other variables besides platelet reactivity. These variables influence the exposure of platelets to factors that stimulate activation in vivo, the vessel vulnerability. The simultaneous presence of vulnerable vessel and vulnerable blood (HPR) is required for the occurrence of a thrombotic event. The unreasonable expectation of high specificity in the latter condition markedly differs from the requirement of high specificity for a marker to identify an evolving event or one that has already occurred. An example is the comparison of platelet function testing to identify patients destined to have post-PCI ischemic events compared with measuring ultra-sensitive troponin to identify a patient with an evolving MI. Although it is possible to express prognostic test utility with diagnostic test statistics such as sensitivity, specificity, predictive values, and receiver-operating characteristic curve analysis, one must exercise caution in the interpretation of these measures. Diagnostic tests are used in situations when the event has already occurred but its presence is unknown to the physician. In contrast, prognostic tests are indicators of risk for events that have not yet occurred. Thus, the future status is unknown, and prognostic test results can be interpreted only as the probability or risk of occurrence. Prognostic tests should not be expected to have the same performance as diagnostic tests when evaluated with diagnostic test statistics. Although a major determinant of 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 of the low likelihood of future ischemic events.

The HPR cutoff values reported in many studies are 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. Indeed, the low positive predictive value of platelet function tests has been used as an argument against their clinical utility. Prognostic markers such as platelet function tests are better described by relative risk or hazard ratios. The current data indicate that platelet reactivity plays a major role in ischemic event occurrence (up to 50% of the attributable risk of 30-day stent thrombosis in ADAPT-DES). However, 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 (combined risk factor) will improve risk prediction.

Finally, 2 important facts should be remembered. First, the development of all antiplatelet agents has been based on an ex vivo assessment of platelet inhibition. Second, all of the established benefit of P2Y12 blockade is attributed to inhibition of platelet function; there are no other proven mechanisms linked to outcomes. Therefore, it is logical to assess the antiplatelet drug effect in the individual patient to determine thrombotic risk; patients without a measurable drug effect would logically be at greatest risk.

Conclusions

Nonelective administration of antiplatelet therapy contradicts common practice in cardiovascular medicine when a measurable drug effect is mandated, and if the response is suboptimal, an alternative strategy is warranted. It is indisputable that HPR and LoF carrier status are associated with a significant increase in ischemic risk in PCI patients treated with clopidogrel, and this is not surprising given the central role of the ADP-P2Y12 interaction in the genesis of coronary thrombosis. The data from large-scale trials demonstrating that treatment with P2Y12 inhibitors associated with more potent ex vivo inhibition than clopidogrel is resulted in lower ischemic event rates constitute a powerful argument against HPR being just a risk indicator and not a modifiable risk factor.

The primary goal of platelet function testing is to identify the patient who is suboptimally responsive and to adjust therapy accordingly to reduce the risk for the catastrophic events of MI and stent thrombosis. Genotyping predicts who is at risk of being suboptimally responsive but does not replace platelet function testing. It is unreasonable to expect that tests predictive of complex future events such as the occurrence of ischemic events in patients with cardiovascular disease will perform with the same sensitivity and specificity as tests diagnostic of events that have already occurred or are evolving at the time of testing. Predictive test results can be expressed only as a probability of risk. Statistical analyses that have been used to assess the utility of diagnostic tests will always result in less favorable results when applied to predictive tests.

There is conclusive pharmacodynamic evidence recognized by the FDA, American College of Cardiology, American Heart Association, and European Society of Cardiology that clopidogrel has a suboptimal effect in a substantial proportion of patients and that increasing the dose has limited efficacy in selected patients. Given the cost of clopidogrel therapy and the evidence presented, we should ask why clopidogrel has been and continues to be administered non-selectively. If we conservatively estimate the prevalence of nonresponsiveness at 10%, on the basis of an analysis of sales in 2009, nearly $1 billion dollars was spent on a drug with minimal or no pharmacodynamic effect. And we must remember that pharmacodynamic analyses are the primary method used to assess the efficacy of all antiplatelet agents during their development. Although we do not yet have conclusive evidence from a definitive large-scale randomized trial that personalized antiplatelet therapy improves patient outcomes, the evidence is strong enough now to recommend genotyping and phenotyping in the high-risk PCI patient. Moreover, conducting the “definitive” randomized personalized antiplatelet therapy trial now may be delicate. Investi-
gators may be reluctant to randomly assign their patients to a less pharmacodynamically effective therapy.

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References


Response to Gurbel and Tantry

Vamsi Krishna, MD; George A. Diamond, MD; Sanjay Kaul, MD

Drs Gurbel and Tantry offer thoughtful arguments favoring a strategy of personalized antiplatelet therapy. Ideally, individualizing antiplatelet therapy should be based on a biomarker that precisely measures platelet responsiveness, accurately characterizes low- and high-risk patients, and reliably guides treatment decisions to optimize outcomes. Unfortunately, the quest for such a marker remains elusive, because none of the currently available platelet function or genetic tests are endowed with these desirable attributes. Their claim that high-platelet reactivity is a modifiable risk factor is not supported by results of 3 large randomized trials (Gauging Responsiveness With a VerifyNow Assay-Impact on Thrombosis and Safety) trial. Talk presented at the American Heart Association 2010 Scientific Sessions; November 13–17, 2010, Chicago, IL.


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