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

The Role of Platelet Reactivity and Genotype Testing in the Prevention of Atherothrombotic Cardiovascular Events Remains Unproven

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

Clopidogrel is the second-leading drug sold worldwide, yet 13 years and millions of patients treated later, we are just beginning to understand the complexity of its metabolism and antiplatelet effects. There is a high degree of clopidogrel response variability in terms of pharmacokinetic and pharmacodynamic effects related to genetic and nongenetic factors.1–4 Several recent studies have shown that both high on-clopidogrel platelet reactivity5–8 and the cytochrome (CY) P2C19 reduced-function genotype9–11 are associated with an increased risk of cardiovascular events, including stent thrombosis (ST). Given the central role of clopidogrel in the management of acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI), physicians and regulatory bodies are tackling how best to integrate these data to guide patient care. These efforts are fueled by the motivation to identify people at risk of developing adverse outcomes on clopidogrel, to develop more precise estimates about prognosis, and to fine-tune treatment selection, thereby approaching a form of tailored or personalized medicine.

Response by Gurbel and Tantry on p 1303

On March 12, 2010, the US Food and Drug Administration (FDA) called attention to this issue via a boxed warning about the diminished effectiveness of the drug in patients with impaired ability to convert the drug into its active form as a result of genetic polymorphism in CYP2C19, one of the principal enzymes responsible for metabolic conversion of clopidogrel into its active form.12 In response to this boxed warning, the American College of Cardiology Foundation (ACCF)/American Heart Association released a clinical expert consensus document13 stating “the evidence base is insufficient to recommend either genetic testing or platelet function testing at the present time.” Since that statement, additional studies have been published that question the value of platelet function and genetic testing.14–18

In this commentary, we review the evidence linking platelet function and genetic testing to adverse cardiovascular outcomes, evaluate the current data supporting platelet function and genetic test–guided tailored treatment approaches, highlight the deficiencies in the empirical evidence base as a guide to future research, and provide our recommendations for the use of these tests in clinical practice. We organize our review in terms of 5 pragmatic questions:

1. Does antiplatelet resistance exist?
2. If it exists, can it be detected clinically?
3. If it can be detected, does it have prognostic importance?
4. If it has prognostic importance, is it subject to therapeutic modification?
5. If it can be modified, what is the optimal test/treatment strategy?
Does Antiplatelet Resistance Exist?

Definition

There is a lack of consensus regarding the definition of resistance to antiplatelet therapy. Terms that have been applied in the literature include nonresponsiveness, hyporesponsiveness, semiresponsiveness, low responsiveness, and suboptimal responsiveness. The optimal definition should encompass both the failure of the antiplatelet agent to inhibit the activity of its specific target (a laboratory phenomenon) and the failure to prevent atherothrombotic events (a clinical phenomenon). The ideal laboratory test to measure responses to antiplatelet therapy should use standardized techniques that can specifically, reliably, and reproducibly detect changes in activity of the target enzyme or receptor (eg, COX-1 for aspirin, P2Y12 for clopidogrel) before and after administration of therapy. However, pretreatment baseline platelet reactivity levels are difficult to ascertain in acute clinical practice settings. Thus, an absolute measure of platelet reactivity during treatment, ie, high on-treatment platelet reactivity (HPR), is generally used to define poor response instead. The use of arbitrary cutoff values and nonstandardized techniques to define HPR and thus to differentiate responders from nonresponders in the clinical setting remains challenging and controversial. Thus, not surprisingly, the reported prevalence of hyporesponsiveness to clopidogrel varies substantially, depending on the assay and methodology used, the definition applied, and the population group studied.

Prevalence

There is a large variability in the reported prevalence of HPR. A meta-analysis of 20 studies including 9187 patients reported a mean HPR of 32% (range, 6% to 80%). Several factors contribute to the observed variability, including the type of platelet function device (the greater the sensitivity of the assay, the higher the HPR prevalence); the selected platelet reactivity cutoff, which is inversely correlated with HPR; clopidogrel loading dose; the time of assessment from loading/last clopidogrel dose or after PCI; genetic variability in drug absorption and metabolism; presence of specific cardiovascular risk factors such as diabetes mellitus, smoking, obesity, renal dysfunction, and dyslipidemia; drug-related factors such as dosing regimens and drug-drug interactions; the underlying clinical presentation (higher prevalence in ACS patients or those undergoing PCI); and publication bias (ie, positive studies are more likely to be published than negative ones). Complicating matters further are the compelling data suggesting that compliance (which is difficult to measure systematically) may often contribute to HPR.

Genotypes and Their Prevalence

Recent efforts to understand clopidogrel hyporesponsiveness have focused on genetic factors that control its metabolism. Clopidogrel is an inactive prodrug that is oxidized to its active metabolite via the hepatic CYP450 system in a 2-step oxidation process. Hepatic bioactivation of clopidogrel is achieved via a number of different CYP isoenzymes, primarily the CYP2C19 isoenzyme. Current evidence suggests that there are 4 major variants of CYP2C19: CYP2C19*1, CYP2C19*2, CYP2C19*3, and CYP2C19*4. The CYP2C19*1 so-called wild-type allele has fully functional metabolism of clopidogrel (extensive metabolizers), whereas the CYP2C19*2 and CYP2C19*3 so-called loss-of-function alleles have no functional metabolism. These 2 alleles account for most of the loss-of-function alleles in white (85%) and Asian (99%) subjects classified as poor metabolizers. Poor metabolizers have 2 loss-of-function alleles; the prevalence in the general population is about 2% to 4%. Intermediate metabolizers have 1 copy of a loss-of-function allele and may have decreased active metabolite levels and reduced antiplatelet effects. The prevalence of intermediate metabolizers is about one third in the general population, ranging from 19% to 50% (higher in Chinese and blacks compared with whites and Mexican Americans). The CYP2C19*4, CYP2C19*5, CYP2C19*6, CYP2C19*7, CYP2C19*8, and other alleles are less common but may be associated with absent or reduced metabolism of clopidogrel. In contrast, there is mixed evidence that the CYP2C19*17 polymorphism (so-called gain-of-function allele) is significantly associated with enhanced response to clopidogrel and increased risk of bleeding (ultrarapid metabolizers).

Other genetic variations that may also affect the pharmacokinetic, pharmacodynamic, and clinical efficacy of clopidogrel include CYP3A4, CYP3A5, and genes encoding P-glycoprotein (an efflux transporter), ABCB1, and purinergic receptor P2Y12 (the active site for clopidogrel). Current evidence suggests that the adenosine 5'-triphosphate-binding cassette gene, ABCB1, may partly contribute to variability in clopidogrel responsiveness by affecting the intestinal absorption and oral bioavailability of clopidogrel. In contrast, no association of clopidogrel responsiveness with P2Y12 genotype has been found. Recently, another enzyme called paraoxonase-1 (PON1) was shown in 1 study but not in others to be crucial for activation of clopidogrel, with the rate of activation governed by the Q192R polymorphism.

Can Antiplatelet Resistance Be Detected Clinically?

Several platelet function tests have been developed that evaluate the ex vivo responsiveness of platelets to a variety of inhibitors. Furthermore, knowledge about genetic determinants of antiplatelet resistance has been translated into the development of tests designed to evaluate specific gene polymorphisms that regulate the metabolism of clopidogrel.

Platelet Function Tests

Platelet function testing plays an important role in determining responsiveness to antiplatelet therapy. Three categories of tests are commonly used to monitor the effects of clopidogrel. The aggregation-based tests that are commercially available include light transmission aggregometry (LTA) in
platelet-rich plasma, whole-blood impedance platelet aggre
ometry (multiplate analyzer or multielectrode aggregome
try [MEA]; Dynabyte, Munich, Germany), the VerifyNow P2Y
12 assay (Accumetrics, San Diego, CA), and Platelet
Works (Helena Laboratories, Beaumont, TX). Shear
dependent tests include the Impact cone and platelet analy
ser (Siemens Healthcare Diagnostics, Inc, Deerfield, IL), Platelet
Function Assay-100, and INNOVANCE PFA-P2Y. The third
category of platelet function assays uses flow cytometry
either to assess activation-dependent changes on platelet
surface membrane receptors such as P-selectin and glycopro
tein IIb/IIIa or alternatively to measure intracellular signaling
by vasodilator-stimulated phosphoprotein (VASP), which is a
specific biomarker for P2Y12 receptor activation. Other ap-
proaches such as the Thrombelastograph Platelet Mapping
System (Hemoscope, Niles, IL) have been used as a global
hemostatic assay. Of these tests, the currently available
point-of-care assays include VerifyNow, Thrombelastograph
Platelet Mapping, Multiple Platelet Function Analyzer (Mul-
tiple), Platelet Function Assay-100, and PlateletWorks.
These tests are not standardized and use different methodol-
dies and cutoff values to define platelet responsiveness.
Furthermore, correlations between assays are modest, and
concordance in defining suboptimal response is poor. So,
before one advocates the use of platelet function testing to
assess platelet responsiveness and guide therapy, one must
first prospectively define the method of testing to be used,
which agonist to use, which dose of agonist to use, and what
cutoff to use to distinguish hyporesponsiveness or hyperre-
sponsiveness from normal response.

Genotype Tests

Several commercial pharmacogenetic tests are available for
analysis of multiple single-nucleotide polymorphisms in
\textit{CYP2C19} for the purpose of predicting response to clopi
dogrel. These tests differ in genotyping methodology, sample
type required, and availability (direct to consumer or
physician-ordered). Genetic testing is technically demanding,
time-consuming, expensive, and generally not reimbursable
by insurance companies. Currently, \textit{CYP2C19} genotyping is
sent out to specialty laboratories at most institutions, and it
takes several days to receive results. There is limited avail-
ability of point-of-care assays: Verigene CYP2C19 Nucleic
Acid Test recently received a “nonapprovable” letter by the
FDA, and the Spartan RX \textit{CYP2C19} test has not yet been
submitted to the FDA, thus restricting its use in the acute
clinical care setting.

Does Antiplatelet Resistance Have Prognostic Implications?

Traditionally, the predictive performance of a risk factor has
focused on the strength of association using absolute or
relative risk increase (typically in the form of an odds ratio
[OR] or hazard ratio) as a measure of effect size and a \textit{P}
value to show that the risk factor is significantly associated with the
disease. The strength of statistical association is a necessary
component of model performance evaluation, but it may not
be the most informative in assessing the utility of a new
marker.\textsuperscript{32} Other indexes that might provide incremental
information include classification performance measures
such as sensitivity, specificity, likelihood ratio, predictive
accuracy (positive and negative predictive values), \textit{R}^2 (pro-
portion of explained variation), area under the receiver
operating-characteristic curve (AUC), calibration, and
reclassification.\textsuperscript{33–36}

A key measure of the clinical utility of a risk prediction
model is its ability to discriminate or to separate those who
will develop the event of interest (cases) from those who will
not (noncases). The AUC is a widely used metric to assess
discrimination. Unlike other indexes of test performance such
as sensitivity, specificity, likelihood ratio, and predictive
accuracy, the AUC has the advantage of being independent of
test criteria, verification bias, and prevalence.\textsuperscript{34} However, this
measure lacks an apparent intuitive interpretation and might
be insensitive for assessing improvement in risk prediction
because strong associations are required to cause minimal
improvement in AUC.\textsuperscript{34} Model performance measures be-
yond the AUC to evaluate the usefulness of risk prediction
include calibration (goodness of fit or the degree to which the
observed risk compares with the expected risk) and reclassi-
ication (reassignment of subjects into risk categories that
cross treatment thresholds or change management).\textsuperscript{35}

Prognostic Utility of Platelet Function Tests for
Thrombotic Events

Reduced platelet function has consistently been shown to be
associated with worse clinical outcomes in patients treated
with clopidogrel. The Do Platelet Function Assays Predict
Clinical Outcomes in Clopidogrel-Pretreated Patients Under-
going Elective PCI (POPULAR) trial was a single-center
observational study that compared 6 different methods of
measuring platelet function to predict major adverse cardiac
events (MACEs) at 1 year in 1069 patients undergoing
elective PCI.\textsuperscript{14} The principal finding was that platelet aggre-
gation tests (LTA-ADP, the VerifyNow-P2Y\textsubscript{12} assay, and
PlateletWorks) but not shear-dependent tests (IMPACT-R,
Platelet Function Assay collagen/ADP, and Innovanve PFA-
P2Y) were able to discriminate between patients with and
without MACEs. Table 1 summarizes the performance of the
3 platelet function tests that were prognostic of MACEs using
receiver operating-characteristic cutoffs. Despite the signifi-
cant strength of association (OR, 2.1–2.5; \textit{P}<0.001 to
<0.01), the impact of the aggregation tests on risk prediction
was quite small, as reflected in the modest likelihood ratios,
predictive accuracies (positive predictive value, 12% to 13%;
negative predictive value, 94%), and discriminant capacity
(AUC, 0.61–0.62). The addition of HPR to clinical and
procedural risk factors that were independently correlated
with clinical outcomes slightly but significantly improved the
AUC from 0.72 to 0.73 to 0.77 (\textit{P}<0.01). That HPR provides

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Table 1. Prognostic Performance of Platelet Function Tests

<table>
<thead>
<tr>
<th>Platelet Function Assay (Cutoff for HPR)</th>
<th>Clinical Scenario (n)</th>
<th>End Point</th>
<th>Event F/U</th>
<th>Rate, %</th>
<th>OR*</th>
<th>Sn</th>
<th>Sp</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>LR+</th>
<th>LR−</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTA 20 μmol/L ADP (64.5%)(^1)(^4)</td>
<td>Elective PCI (1069)</td>
<td>D, MI, ST, STroke</td>
<td>1 y</td>
<td>8.4</td>
<td>2.05</td>
<td>0.53</td>
<td>0.64</td>
<td>12</td>
<td>94</td>
<td>1.49</td>
<td>0.73</td>
<td>0.62</td>
</tr>
<tr>
<td>VerifyNow P2Y(_{12}) (236 PRU)(^1)(^4)</td>
<td>Elective PCI (1069)</td>
<td>D, MI, ST, STroke</td>
<td>1 y</td>
<td>1.2</td>
<td>3.85</td>
<td>0.69</td>
<td>0.63</td>
<td>2</td>
<td>99</td>
<td>1.88</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td>PlateletWorks (60.5%)(^1)(^4)</td>
<td>Elective PCI (1069)</td>
<td>D, MI, ST, STroke</td>
<td>1 y</td>
<td>8.7</td>
<td>2.53</td>
<td>0.59</td>
<td>0.63</td>
<td>13</td>
<td>94</td>
<td>1.62</td>
<td>0.64</td>
<td>0.62</td>
</tr>
<tr>
<td>VASP (53%)(^3)(^7)</td>
<td>NSTEMI PCI (195)</td>
<td>D, MI, ST, STroke</td>
<td>1 mo</td>
<td>8.9</td>
<td>2.22</td>
<td>0.61</td>
<td>0.59</td>
<td>13</td>
<td>94</td>
<td>1.47</td>
<td>0.66</td>
<td>0.61</td>
</tr>
<tr>
<td>MEA (416 AU)(^3)(^8)</td>
<td>DES PCI all comers</td>
<td>ST</td>
<td>1 mo</td>
<td>1.5</td>
<td>2.68*</td>
<td>0.67</td>
<td>0.57</td>
<td>2</td>
<td>99</td>
<td>1.55</td>
<td>0.58</td>
<td>0.66</td>
</tr>
<tr>
<td>LTA ADP + collagen (46% ADP, 69% collagen)(^3)(^9)</td>
<td>DES PCI all comers (1608)</td>
<td>ST</td>
<td>1 mo</td>
<td>0.9</td>
<td>7.30</td>
<td>0.64</td>
<td>0.80</td>
<td>3</td>
<td>100</td>
<td>3.26</td>
<td>0.44</td>
<td>0.79</td>
</tr>
<tr>
<td>LTA ADP + collagen (46% ADP, 69% collagen)(^3)(^9)</td>
<td>ST</td>
<td>24 hr after PCI</td>
<td>1 mo</td>
<td>0.6</td>
<td>4.03</td>
<td>0.50</td>
<td>0.80</td>
<td>2</td>
<td>100</td>
<td>2.51</td>
<td>0.62</td>
<td>0.77</td>
</tr>
<tr>
<td>LTA ADP + collagen (46% ADP, 69% collagen)(^3)(^9)</td>
<td>ST</td>
<td>ST</td>
<td>1 mo</td>
<td>2.7</td>
<td>0.80*</td>
<td>0.16</td>
<td>0.80</td>
<td>2</td>
<td>97</td>
<td>0.81</td>
<td>1.05</td>
<td>0.46</td>
</tr>
<tr>
<td>LTA ADP + collagen (46% ADP, 69% collagen)(^3)(^9)</td>
<td>ST</td>
<td>ST</td>
<td>6 mo</td>
<td>2.7</td>
<td>5.72</td>
<td>0.25</td>
<td>0.94</td>
<td>19</td>
<td>98</td>
<td>8.38</td>
<td>0.73</td>
<td>0.84</td>
</tr>
<tr>
<td>LTA ADP + collagen (46% ADP, 69% collagen)(^3)(^9)</td>
<td>ST</td>
<td>ST</td>
<td>6 mo</td>
<td>3.4</td>
<td>10.83</td>
<td>0.28</td>
<td>0.97</td>
<td>22</td>
<td>97</td>
<td>8.08</td>
<td>0.75</td>
<td>0.83</td>
</tr>
</tbody>
</table>

HPR indicates high on-treatment platelet reactivity; F/U, follow-up; OR, odds ratio; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; AUC, area under the receiver-operating characteristics curve; LTA, light transmission aggregometry; PCI, percutaneous coronary intervention; D, diabetes mellitus; MI, myocardial infarction; ST, stent thrombosis; PRU, P2Y\(_{12}\) reaction unit; VASP, vasodilator-stimulated phosphoprotein; STEMI, ST-segment–elevation MI; DES, drug-eluting stent; MEA, multielectrode aggregometry; and AU, arbitrary units. AUC <0.50 means no discriminant capacity.

\(^*\)P<0.05.

A clinically important incremental gain in prognostic information beyond clinical and procedural characteristics is somewhat debatable because the strongest determinant of on-treatment reactivity is pretreatment reactivity, which depends on a variety of clinical, environmental, genetic, and other variables. These findings raise questions about the utility of these tests to reliably predict clinical outcomes. Of note, the model was evaluated on the same sample on which it was developed in the POPULAR study. Ideally, the improvement in performance of a risk prediction model should be based on an independent validation or bootstrap resampling derived from the development set and not from a simple split sample thereof.

Also shown in Table 1 are the prognostic performance of 3 additional tests not evaluated in the POPULAR study: VASP,\(^7\) MEA-ADP,\(^8\) and LTA response to dual agonists (ADP and collagen).\(^3\)\(^9\) The VASP test is specific for P2Y\(_{12}\) receptor (the target for clopidogrel); accordingly, it demonstrates the highest sensitivity and negative likelihood ratio. In contrast, HPR in response to LTA (dual agonists) yields the highest specificity and positive likelihood ratio. Both tests yield good discrimination for ischemic thrombotic events. The HPR measured by MEA is associated with relatively high sensitivity and specificity, resulting in modestly large positive likelihood ratio, negative likelihood ratio, and AUC. The positive predictive accuracies are still modest given the low prevalence of the events.

There is a substantial interassay difference in the predictive values according to the clinical outcomes of interest. For example, in the POPULAR study, LTA-ADP 5 μmol/L had the highest OR for predicting death, LTA-ADP 20 μmol/L had the highest OR for ST, and the PlateletWorks device proved to be the best to predict myocardial infarction (MI).\(^1\)\(^4\) In the meta-analysis by Aradi et al,\(^3\) LTA-ADP had the highest prognostic value for cardiovascular death, whereas in the study by Sibbing et al,\(^8\) MEA-ADP predicted ST and MI >24 hours after PCI but had no prognostic value for MI <24 hours after PCI (OR, 0.8; LR, 0.81; negative likelihood ratio, 1.05; AUC, 0.46; Table 1). This may be due to the fact that peri-PCI MIs are related primarily to occlusion of side branches by atherothrombotic debris and washout of myocardial enzymes in ACS and are less likely to be influenced by antplatelet therapy. These findings suggest that there is not one superior device; instead, there are assays that are optimally suited to predict specific outcomes with variable degrees of clinical importance.

Figure 2 plots posttest probability versus pretest probability given values for specificity and sensitivity of different tests. The pooled estimate shows a statistically significant 3.8-fold greater risk of ST in those exhibiting HPR compared with those with normal platelet reactivity. There was no significant heterogeneity in risk associated with HPR across the studies. Two important observations are noteworthy. First, the positive predictive value (2% to 11%) tracks the prevalence of ST (Low positive predictive value is associated with low prevalence). Second, the AUC (0.56–0.93), which is independent of prevalence, is highly correlated with the strength of association (Higher risk ratios are associated with larger AUCs; \(r=0.80\)). This is consistent with the observation that strong associations are required to achieve reasonable discrimination into cases and noncases (AUC, 0.80–0.85).

Figure 2 plots posttest probability versus pretest probability given values for specificity and sensitivity of different tests.
platelet function tests for the prediction of ST. The prognostic performance is directly correlated with the area between the posttest probabilities based on a positive or negative test result, ie, the larger the area, the better the risk prediction performance (LTA to dual agonists [ADP plus collagen] and VASP > MEA > LTA [ADP] > VerifyNow). Unlike AUC, the posttest probabilities have intuitive interpretive appeal. However, they are highly dependent on the prevalence (pretest probability) of the outcome of interest. The lower limit of optimum pretest probability for the platelet function tests is 24% to 39%, which is ~10- to 20-fold higher than the actual prevalence of ST in clinical practice. These data show that even a highly accurate test can contribute little information when the pretest probability (prevalence) of disease is low.

Calibration is a measure of how well predicted risk estimates agree with actual observed risk across the spectrum of risk (eg, in deciles). Systematic assessment of calibration of platelet function tests is currently lacking. However, the results of 2 prospective randomized controlled trials, Gauging Responsiveness With a VerifyNow P2Y12 Assay: Impact on Thrombosis and Safety (GRAVITAS; observed MACE event rate of 2.3% compared with the expected 5%)18 and the Testing Platelet Reactivity in Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel (TRIGGER-PCI) trial, which was stopped early (MACE rate of <2.3% compared with the expected 7%),40 raise questions about the ability of these tests to predict outcomes accurately.

Finally, a relatively new (and controversial) criterion for the utility of a prognostic test is whether it leads to reclassifying subjects to different risk categories, resulting in different treatment decisions that improve outcomes. Currently, there are no systematic evaluations of reclassification performance and therefore clinical utility of platelet function tests.

Prognostic Utility of Platelet Function Tests for Bleeding Events

Although reduced response to clopidogrel has consistently been shown to be associated with future thrombotic events, evidence linking enhanced response to clopidogrel and bleeding is mixed. In the POPULAR study, none of the tests correlated with bleeding events.14 However, 3 recent studies,1 with a multiplate analyzer41 and 2 with the VerifyNow assay,42,43 have shown that an enhanced response to clopidogrel is associated with a higher risk of bleeding. All 3 studies suggested the possibility of a “therapeutic window” for P2Y12 receptor inhibition—189 to 467 arbitrary units for the VerifyNow assay—with patients within this range exhibiting low risk for both bleeding and ischemic events. The prognostic utility of these tests for the presence of a therapeutic window is shown in Table 2. The existence of a therapeutic window raises the possibility of tailoring the dose of antiplatelet therapy for optimization of the benefit-to-risk ratio in clinical practice. However, this awaits further investigation in larger prospective studies.

Prognostic Utility of Genetic Tests for Clopidogrel Responsiveness

Although genetic factors modulate platelet responsiveness to clopidogrel, both known genetic and nongenetic factors explain only a portion of the majority of variation. Shuldiner et al10 observed that CYP2C19 loss-of-function alleles accounted for only 12% of variability in clopidogrel platelet response in a homogeneous healthy Amish population. Similarly, Hochholzer et al44 reported that CYP2C19*2 carrier status accounted for only 4.6% of the variability in on-clopidogregel HPR in patients undergoing elective drug-eluting
stent implantation. Together, patient demographic, clinical, and genetic factors accounted for only 11.5% of the variability in HPR. In the study by Shuldiner et al., a gene-dose effect on platelet reactivity was demonstrated whereby *1/*1 individuals (extensive metabolizers) had the greatest reduction in platelet aggregation (41% residual aggregation), *1/*2 individuals (intermediate metabolizers) had intermediate reduction in platelet aggregation (47% residual aggregation), and homozygous variant *2/*2 individuals (poor metabolizers) had the greatest residual platelet reactivity (65% residual aggregation) in response to clopidogrel load. In 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) and 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) studies, the influence of CYP2C19 genotype on platelet reactivity in clopidogrel-treated patients was most evident during maintenance therapy compared with after loading and was most effectively demonstrated by the VerifyNow P2Y12 assay.

**Figure 2.** Plots of posttest probability as a function of pretest probability for 5 platelet function tests based on positive (thick lines) and negative (thin line) test results for prediction of stent thrombosis (ST) based on the Academic Research Consortium criterion for definite or probable ST. Predictive utility for ultrasensitive troponin used for diagnosis of acute myocardial infarction is shown for comparison. The figure shows that prevalence (pretest probability) of ST can affect posttest probabilities (positive predictive value) to a great extent. The predictive performance is directly correlated with the area between the posttest probabilities based on a positive or negative test result: the larger the area, the better the risk prediction performance. LTA indicates light transmission aggregometry; VASP, vasodilator-stimulated phosphoprotein; AUC, area under the curve; and MEA, multielectrode aggregometry.

**Prognostic Utility of Genetic Tests for Clinical Outcomes**

Several lines of evidence derived primarily from cross-sectional and registry studies that used both candidate gene-related and genome-wide association approaches have shown a significant association between CYP2C19 loss-of-function variants and clopidogrel treatment outcomes. Three meta-analyses summarizing the association in cross-sectional studies have been published. The results show that among patients on clopidogrel therapy, those carrying the CYP2C19*2 allele had a greater risk of cardiovascular events compared with those with the *1/*1 genotype. The risk appears to be independent of clinical and procedural risk factors and platelet reactivity and is outcome and indication specific, with the strongest association observed with the risk of ST early after stenting. A gene-dose relationship is
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immediately before PCI

600-mg clopidogrel dose and
Platelet function test done after
mellitus; and MI, myocardial infarction.

after PCI

Platelet function test done 1 mo

The beneficial effect of clopidogrel in
an association between
tive analyses of randomized controlled trials have not found
CYP2C19*2
allele exhibiting greater risk. There is some

specific, which makes comparison across studies challenging.

In contrast to the nonrandomized observations, retrospec-
tive analyses of randomized controlled trials have not found
an association between CYP2C19 genotype and cardiovascu-
ar outcomes.15–17 The beneficial effect of clopidogrel in
improving clinical outcome in Clopidogrel in Unstable An-
gina to Prevent Recurrent Events (CURE) and Atrial Fibril-
lation Clopidogrel Trial With Irbesartan for Prevention of
Vascular Events (ACTIVE A) was consistent regardless of
the loss-of-function CYP2C19 carrier status.15 Similarly, the
beneficial effect of ticagrelor over clopidogrel in Platelet
Inhibition and Patient Outcomes (PLATO) was observed in
both carriers and noncarriers of the loss-of-function
CYP2C19*2 allele.17 Interestingly, an association between
CYP2C19 genotype and MACE outcome in clopidogrel-
treated group was observed at 30 days but not at the end of the
12-month follow-up period. Of note, the populations in these
randomized studies differed from many of the other reported
populations in the frequency of PCI use. For example, only 15.5% in CURE, 66% in PLATO, and none in ACTIVE A received stents, thereby suggesting that the major effect of CYP2C19*2 on clopidogrel response may be indication specific, which makes comparison across studies challenging.

In contrast, a significant association of CYP2C19*2 with
MACEs (53% increased risk) and ST (3-fold increased risk) was reported in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel (TRITON) among patients treated with clopidogrel.16 The genetic substudy of GRAVITAS also reported a similar worse clinical outcome in patients homozy-
gous for the CYP2C19*2 loss-of-function gene, although the
total number of events was only 14.46

In contrast to the meta-analysis by Bauer et al,47 the prior meta-
alyses included only 15 studies that combined both nonrandomized and randomized studies described above reported that carriers of at least 1 loss-of-function allele show a modest but statistically significant increased risk of ST but not MACEs.47 However, the association of ST with a loss-of-function genotype was subject to bias, and adjustment for the bias tended to abolish the association.47 The authors concluded that the low overall epidemiological credibility of the asso-
ciations makes all effect estimates and clinical inferences uncertain. Thus, there is no robust evidence to recommend individualized clopidogrel treatment driven by CYP2C19 genotype.47

The findings of the meta-analysis by Bauer et al are
inconsistent with those observed in a previous meta-
analysis by Mega et al,16 who reported a significant higher risk of
MACEs (OR, 1.57 versus 1.11) and ST (OR, 2.67 versus 1.77) in carriers of loss-of-function CYP2C19 gene variants. Two factors could potentially explain the discrepancies. First, the phenomenon of regression to the mean, ie, decreasing strength of association with increasing number of studies, is a possible explanation. The previous meta-analysis included a

Table 2. Platelet Function Tests and Evidence of a Therapeutic Window

<table>
<thead>
<tr>
<th>Platelet Function Assay (Cutoff for HPR)</th>
<th>Clinical Analytic Substudy</th>
<th>Scenario (n)</th>
<th>End Point</th>
<th>F/U</th>
<th>Event Rate, %</th>
<th>OR</th>
<th>Sn</th>
<th>Sp</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>LR+</th>
<th>LR−</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA (≥68 AUC) poor responders (&lt;20%)</td>
<td>Elective PCI (2533)</td>
<td>ST (definite, probable)</td>
<td>1 mo</td>
<td>0.9</td>
<td>6.04</td>
<td>0.55</td>
<td>0.83</td>
<td>3</td>
<td>100</td>
<td>3.29</td>
<td>0.54</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>MEA (≥188 AUC) enhanced responders (40%)</td>
<td>Elective PCI (2533)</td>
<td>TIMI major bleeding</td>
<td>1 mo</td>
<td>1.3</td>
<td>2.62</td>
<td>0.62</td>
<td>0.62</td>
<td>2</td>
<td>99</td>
<td>1.62</td>
<td>0.62</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>MEA (189–467 AUC) normal responders (40%)</td>
<td>Elective PCI (2533)</td>
<td>ST, bleeding</td>
<td>1 mo</td>
<td>2.2</td>
<td>2.46</td>
<td>0.75</td>
<td>0.45</td>
<td>3</td>
<td>99</td>
<td>1.36</td>
<td>0.55</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Platelet function test done 1 mo after PCI</td>
<td>Elective PCI (300)</td>
<td>D, MI, stroke</td>
<td>1 y</td>
<td>7.0</td>
<td>27.6</td>
<td>0.81</td>
<td>0.92</td>
<td>43</td>
<td>98</td>
<td>9.82</td>
<td>0.21</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>VerifyNow P2Y₁₂ (≥239 PRUs) poor responders</td>
<td>Elective PCI (300)</td>
<td>Bleeding</td>
<td>1 y</td>
<td>6.3</td>
<td>11.3</td>
<td>0.79</td>
<td>0.79</td>
<td>20</td>
<td>98</td>
<td>3.70</td>
<td>0.27</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>VerifyNow P2Y₁₂ (≥85 PRUs) enhanced responders</td>
<td>Elective PCI (300)</td>
<td></td>
<td>1 y</td>
<td>13.3</td>
<td>0.11</td>
<td>0.85</td>
<td>0.69</td>
<td>30</td>
<td>97</td>
<td>2.73</td>
<td>0.22</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>VerifyNow P2Y₁₂ (86–238 PRU) normal responders</td>
<td>Elective PCI (300)</td>
<td></td>
<td>1 y</td>
<td>13.3</td>
<td>0.11</td>
<td>0.85</td>
<td>0.69</td>
<td>30</td>
<td>97</td>
<td>2.73</td>
<td>0.22</td>
<td>0.85</td>
<td></td>
</tr>
</tbody>
</table>

HPR indicates high on-treatment platelet reactivity; F/U, follow-up; OR, odds ratio; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; AUC, area under the receiver-operating characteristics curve; PCI, percutaneous coronary intervention; MEA, multielectrode aggregometry; ST, stent thrombosis; TIMI, Thrombolysis in Myocardial Infarction; PRU, P2Y₁₂ reaction unit; D, diabetes mellitus; and MI, myocardial infarction.

reported in most studies, with carriers of 2 compared with 1 CYP2C19*2 allele exhibiting greater risk. There is some uncertainty with regard to the impact of clopidogrel loading dose (300 or 600 mg) on the association. However, caution must be observed in drawing definitive conclusions from these studies because none of them was randomized; thus, the possibility of bias and confounding (by overrepresentation of clopidogrel nonresponders) cannot be excluded.

In contrast to the nonrandomized observations, retrospec-
tive analyses of randomized controlled trials have not found
an association between CYP2C19 genotype and cardiovascu-
ar outcomes.15–17 The beneficial effect of clopidogrel in
improving clinical outcome in Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) and Atrial Fibril-
lation Clopidogrel Trial With Irbesartan for Prevention of
Vascular Events (ACTIVE A) was consistent regardless of
the loss-of-function CYP2C19 carrier status.15 Similarly, the
beneficial effect of ticagrelor over clopidogrel in Platelet
Inhibition and Patient Outcomes (PLATO) was observed in
both carriers and noncarriers of the loss-of-function
CYP2C19*2 allele.17 Interestingly, an association between
CYP2C19 genotype and MACE outcome in clopidogrel-
treated group was observed at 30 days but not at the end of the
12-month follow-up period. Of note, the populations in these
randomized studies differed from many of the other reported
populations in the frequency of PCI use. For example, only 15.5% in CURE, 66% in PLATO, and none in ACTIVE A received stents, thereby suggesting that the major effect of CYP2C19*2 on clopidogrel response may be indication specific, which makes comparison across studies challenging.
smaller number of studies published before 2010 (9 trials comprising a total of 9,685 patients) that generally show stronger effects compared with the subsequently published studies included in the newer meta-analysis (15 trials comprising a total of 19,328 patients). Second, only presuppecified clinical events that conformed to standardized (and unbiased) definitions were included in the newer meta-analysis, thereby minimizing the possibility of confounding resulting from clinician-driven or less accurately defined events or outcomes.

Less is known about the other CYP2C19 genotypes. Some studies have indicated that patients who carry the common CYP2C19*1/*1 gain-of-function allele have lower ADP-induced platelet aggregation and consequently a higher bleeding risk compared with *1/*1 individuals. In contrast, in CURE, the CYP2C19*1/*17 gain-of-function allele was not associated with increased bleeding risk. Of note, CURE found a greater reduction in adverse cardiovascular outcomes among *17 carriers than noncarriers. Shuldiner et al did not observe any difference in outcomes by *17 carrier status that was not accounted for by *2 carrier status. The ONSET/OFFSET and RESPOND genotype studies did not show any difference in platelet function between CYP2C19*17/*1 gain-of-function ultrarapid metabolizers and CYP2C19*1/*1 extensive metabolizers. The meta-analysis by Bauer et al found no association of CYP2C19*17 gain-of-function allele with MACEs or ST.

Controversy also exists about whether the ABCB1 3435C→T variant is associated with clopidogrel treatment outcomes. ABCB1 is an efflux pump involved in the transport of clopidogrel and may affect the bioavailability of the drug. However, recent studies have reported conflicting results. An analysis of the TRITON–Thrombolysis in Myocardial Infarction (TIMI) 38 study found that individuals with the TT gene variant associated with low activity treated with clopidogrel were at increased risk of MACEs but not ST compared with individuals exhibiting a CC gene variant associated with high activity, whereas Shuldiner et al and PLATO found no association between ABCB1 genotype and treatment outcomes.

Conflicting results have also been reported with PON1 gene polymorphism. In patients with coronary artery disease who underwent stent implantation and received clopidogrel, PON1 QQ192 homozygous individuals had significantly lower PON1 plasma activity, lower plasma concentrations of clopidogrel active metabolite, lower platelet inhibition, and higher risk of ST than RR192 homozygous individuals. This remains the only single study to date to conclusively link the presence of a loss-of-function genetic polymorphism, suboptimal clopidogrel active metabolite generation (pharmacokinetic measurement), decreased clopidogrel responsiveness (pharmacodynamic measurement), and adverse clinical outcomes. Interestingly, CYP2C19*2 was not shown to be associated with ST in this study. Subsequent studies have failed to yield significant association between PON1 and adverse clinical outcomes, whereas in all of those studies, the effects of CYP2C19 were confirmed. This raises the question of replication validity (the first study often suggests a stronger genetic effect than is found by subsequent studies), which frequently challenges genetic association studies. Thus, the potential therapeutic implications of PON1 remain unclear at the current time and warrant further investigation.

The prognostic performance of genetic testing for MACEs, ST, and bleeding is summarized in Table 3. Figure 3 plots posttest probability versus pretest probability given values for specificity and sensitivity of CYP2C19 loss-of-function and PON-1 genotype for the prediction of MACEs and ST. The positive predictive value of CYP2C19*2 loss-of-function alleles for MACE ranges from 8% to 21%, with the pooled positive predictive value of 9% to 10% reported in the meta-analyses. The positive predictive value for ST is smaller (2% to 3%) given the lower prevalence. The negative predictive value for MACE ranges from 80% to 92% and for ST from 98% to 99%. The corresponding positive likelihood ratio is <2.0 and negative likelihood ratio is >0.57, indicating a tiny to small impact on risk prediction. The AUC ranges from 0.47 to 0.57 for MACEs, indicating little discrimination. The AUC for ST is relatively higher (0.57–0.70), consistent with greater discrimination between CYP2C19*2 loss-of-function alleles and ST. The discrimination of CYP2C19*17 gain-of-function allele for bleeding is modest with an AUC of 0.51 to 0.60, and there is no discriminant capacity for predicting thrombotic outcomes (AUC <0.50). The prognostic performance of the ABCB1 genetic variant is inconsistent; a modest performance was observed only for MACEs in PLATO (AUC=0.59). The PON1 polymorphism was prognostic of ST in 1 study but not in another.

Taken together, these findings suggest that among patients treated with clopidogrel, carriage of loss-of-function CYP2C19 allele appears to be associated with a modest but significant risk of major adverse cardiovascular events, particularly ST. However, the prognostic performance of genetic testing is weak because of the low prevalence of adverse events.

Role of Platelet Function Test and Genotype in Drug-Drug Interaction

Evidence of a drug-drug interaction first emerged when pharmacodynamic studies revealed that concomitant administration of certain statins, calcium channel blockers, and proton pump inhibitors (PPIs) all CYP2C19 inhibitors, attenuates antplatelet effects of clopidogrel. Supportive evidence was provided by retrospective data from large observational studies demonstrating that the use of a PPI was associated with an increased risk of cardiovascular events for patients treated with clopidogrel. In response to these findings, the FDA updated the label of the drug to warn that the effectiveness of clopidogrel is reduced when administered in combination with omeprazole. However, post hoc analyses...
of prospective randomized clinical outcome studies have indicated that these drugs do not reduce the ability of clopidogrel to prevent thrombotic events.53–55 In TRITON, PPI use was not associated with an increased risk of adverse outcomes in either wild-type carriers or carriers of a loss-of-function CYP2C19 allele.55 Similar observations were made in the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI), in which PPI use was not associated with an increased risk of cardiovascular events or mortality in patients administered clopidogrel for recent MI, whatever the CYP2C19 genotype.56 The only randomized clinical trial (Clopidogrel and the Optimization of Gastrointestinal Events Trial [COGENT]) to address this issue demonstrated similar cardiovascular risk while gastrointestinal bleeding was reduced in subjects assigned to clopidogrel combined with omeprazole compared with clopi-
Is Antiplatelet Resistance Subject to Therapeutic Modification?

**Platelet Function Test–Guided Treatment Strategy**

The growing body of evidence linking HPR on clopidogrel to risk of adverse events after PCI has led to investigations aimed at evaluating whether tailoring antiplatelet therapy according to results of platelet function tests will lead to improved clinical outcomes; ie, is platelet reactivity a predictor of treatment outcome? Several single-center nonrandomized studies have demonstrated that HPR on standard clopidogrel therapy can be lowered by using higher loading or maintenance doses of clopidogrel, adding cilostazol, switching to more potent alternative P2Y₁₂ receptor blockers such as prasugrel or ticagrelor, and adding eptiogrel or glycoprotein IIb/IIIa inhibitors. A significant reduction in periprocedural ischemic and 30-day MACEs without an increase in bleeding was observed in 2 studies using VASP-guided increased doses of clopidogrel. Of note, despite the use of up to 2400-mg doses of clopidogrel, 8% and 14% of patients remained resistant to clopidogrel. Similar reductions in post-PCI ischemic events without increasing bleeding were reported in 2 studies in which platelet treatment was intensified with VerifyNow- and LTA-guided treatment with glycoprotein IIb/IIIa inhibitors in poor responders to aspirin and/or clopidogrel.

As happens all too often, however, the results from large randomized multicenter prospective trials have failed to confirm the promising results seen in smaller studies. The recently published GRAVITAS study investigated the outcome of doubling the dose of clopidogrel in 2214 patients undergoing PCI who exhibited HPR (defined as ≥230 P2Y₁₂ reaction units) on VerifyNow assay 12 to 24 hours after PCI. Patients were randomized to either continuing with the usual 75-mg maintenance dose of clopidogrel or receiving an additional loading dose of 600 mg followed by a higher maintenance dose of 150 mg daily. At the 6-month follow-up, the composite end point of cardiovascular death, MI, and ST was identical in both groups (2.6%) and no significant difference in bleeding events. Several potential explanations have been advanced for the null results, including low event rate, inadequate power, low-risk patient population, suboptimal improvement in platelet reactivity, and time-dependent resolution of platelet reactivity in 40% of the control arm. However, the possibility that HPR after PCI is not modifiable by high-dose clopidogrel cannot be ruled out. A post-hoc analysis of the GRAVITAS trial suggested that achievement of on-clopidogrel reactivity <250 P2Y₁₂ reaction units at 12 to 24 hours after PCI or during follow-up was associated with a lower risk for cardiovascular events. These data need to be verified prospectively before any inferences relevant to clinical practice can be justified.
The nonrandomized comparison of patients with and without HPR who were treated with standard-dose clopidogrel in GRAVITAS yields additional insights into the predictive performance of VerifyNow. There was a numerically greater but statistically nonsignificant rate of the composite end point in patients with high HPR (2.3% versus 1.4%; hazard ratio, 1.68; 95% confidence interval, 0.76–3.72). However, the predictive performance was rather modest with a sensitivity of 76%, specificity of 35%, positive predictive value of 2% (reflecting low event rate), negative predictive value of 99%, and AUC of 0.59 (reflecting the modest strength of association).

Another large prospective randomized trial, TRIGGER-PCI, was recently halted because of futility after an interim blinded analysis 18 months after enrollment in 432 (250 of whom had reached the 6-month follow-up) yielded a lower-than-expected primary end-point event rate (2.3% versus the expected 7% rate). In TRIGGER-PCI, 2150 low-risk stable coronary artery disease patients with successfully implanted drug-eluting stents and ≥208 P2Y12 reaction units on VerifyNow (a lower cutoff than used in GRAVITAS and other studies) were planned to be randomized to receive either a 60-mg loading dose followed by a 10-mg daily dose of prasugrel or a 600-mg loading dose followed by a 75-mg daily dose of clopidogrel. The primary end point was MI or cardiovascular death at 6 months. The low event rate in TRIGGER-PCI has been attributed to the fact that low-risk patients were enrolled (very old or high-risk ACS patients and patients with previous stroke or unsuccessful or complicated PCI procedures were excluded) and in part to the higher use of second-generation drug-eluting stents, which are associated with lower rates of post-PCI ischemic or thrombotic events. Conceivably, the lower cutoff for HPR might also have altered the predictive performance of VerifyNow.

The results of the first trial to prospectively assess tailoring antiplatelet treatment to modify outcomes in ACS patients (Responsiveness to Clopidogrel and Stent-Related Events in Acute Coronary Syndrome [RECLOSE 2 ACS]) were recently published. In this open-label, single-center observational study, patients were identified by LTA as being poor responders to a 600-mg loading dose of clopidogrel. HPR was identified in 248 of 1789 patients (14% prevalence), much lower than the 41% reported in GRAVITAS trial, which was composed primarily of stable patients. At 2 years, the rate of the primary end point (a composite of cardiac death, MI, urgent revascularization, or stroke) was significantly higher in the HPR group than in the normal-response group (14.5% versus 8.7%; relative risk, 1.8; 95% confidence interval, 1.2–2.6); rates of cardiovascular mortality and ST were nearly doubled in the HPR group. However, doubling the maintenance dose of clopidogrel from 150 to 300 mg daily or switching to 500 to 1000 mg ticlopidine failed to mitigate the increased risk in HPR group.

Genotype Test–Guided Treatment Strategy

There is mixed evidence that modifying therapy in response to genetic testing might result in improved platelet aggregation in response to clopidogrel. Higher-dose clopidogrel therapy is effective in improving platelet aggregation responses to clopidogrel in CYP2C19*2 carriers. In the Clopidogrel and Response Variability Investigation Study 2 (CLOVIS-2) study, carriers of CYP2C19*2 displayed significantly lowered responses to clopidogrel, which were normalized by increasing the dose in heterozygous but not in homozygous carriers. However, preliminary results from the genetic substudy of GRAVITAS reported persistent HPR at 30 days in patients with 1 or 2 copies of loss-of-function CYP2C19*2 gene despite double-dose clopidogrel therapy. This finding challenges the recommendation of clopidogrel 150 mg as a potential option for poor metabolizers as indicated in the boxed warning label. In the Influence of Cilostazol-Based Triple Antiplatelet Therapy on Ischemic Complications After Drug-Eluting Stent Implantation (CILON-T) randomized controlled study, the addition of cilostazol significantly reduced HPR in carriers but not in noncarriers of the CYP2C19 loss-of-function allele.

In the Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel According to Cytochrome 2C19 Polymorphism (ACCELAM2C19) study, compared with high-maintenance-dose clopidogrel, adjunctive cilostazol significantly enhanced platelet inhibition and reduced the rate of HPR, especially in acute MI patients with CYP2C19 loss-of-function variants. In patients with HPR after PCI, prasugrel is more effective compared with high-dose clopidogrel in reducing platelet reactivity, particularly in CYP2C19*2 carriers. In the Escalating Clopidogrel by Involving a Genetic Strategy–Thrombolysis in Myocardial Infarction 56 (ELEVATE-TIMI 56) study evaluating patients with stable cardiovascular disease, tripling the maintenance dose of clopidogrel to 225 mg daily in CYP2C19*2 heterozygotes (carrying 1 loss-of-function allele) achieved levels of platelet reactivity similar to that seen with the standard 75-mg dose in noncarriers. In contrast, among CYP2C19*2 homozygotes (carrying 2 loss-of-function alleles), doses as high as 300 mg daily did not result in comparable degrees of platelet inhibition.

There are limited data regarding tailored therapy based on CYP2C19 carrier status and improvement in clinical outcomes. Several studies are currently underway to evaluate the potential clinical benefit associated with genotype-guided antiplatelet therapy. Given the weak to modest prognostic information provided by genetic testing for clopidogrel efficacy and safety, logistic challenges, cost, and lack of supportive randomized clinical trials, routine genetic testing cannot be recommended at the present time.

Taken together, the current body of evidence suggests that although HPR is a marker of risk, lingering questions remain regarding its status as a “modifiable” risk factor. Whether platelet function testing combined with genetic test–guided treatment might be a successful management strategy in
high-risk ACS patients is being investigated in ongoing randomized trials. Until the results of these large-scale trials of personalized antiplatelet therapy are available, the routine use of platelet function assays or genotype testing in the care of patients with cardiovascular disease remains unproven and therefore cannot be recommended at the present time.

**What Is the Optimal Test/Treatment Strategy to Guide Patient Care?**

Routine use of these tests should ideally be predicated on whether they fulfill key requirements of a clinically useful screening tool and treatment response marker: (1) The test must be practical in the clinical setting; (2) the test must be prognostic, ie, accurately characterize low- and high-risk patients; (3) the test must be predictive, ie, lead to treatment strategies that improve outcome; and (4) the process should be cost-effective. Practical clinical use is supported by the availability of an established point-of-care assay for platelet function testing, and point-of-care tests for genetic tests have been recently introduced, although none are approved for marketing. Risk prognostication is modest and highly variable for thrombotic ischemic outcomes and weak to none for bleeding complications. The biological and methodological variability of platelet function tests and low prevalence of high-risk genotype and outcome events further compound the already weak to modest performance of these tests. Evidentiary support for improved outcomes or cost-effectiveness is currently lacking. Thus, from a rigorous evidence-based perspective, there is insufficient justification for routine use of either platelet function or genetic testing in clinical practice. However, from a purely pragmatic perspective, such testing might be a consideration in high-risk patients in whom the information is likely to alter management and/or result in improved outcomes. Clinical judgment is required to assess clinical risk and variability in patients considered to be at increased risk. While acknowledging the uncertainties in the evidence, the current American College of Cardiology/AHA guidelines recognize the potential role of these tests in clinical practice and provide optional recommendations for platelet function testing and genetic testing (both Class IIb, Level of Evidence C), provided that the results may alter management.

Although there are no high-quality prospective randomized studies to guide our clinical decision making, based on the limited amount of available evidence, there are several courses of action available to the thoughtful clinician.

**Maintain Status Quo**

In absence of definitive evidence regarding whether it is best to increase the dose of clopidogrel or to switch to another agent in loss-of-function CYP2C19 carriers (poor and intermediate metabolizers) and persistent high on-treatment reactivity, an obvious course of action is to continue treating patients as usual without regard to CYP2C19 genotype or platelet reactivity. This is currently the most widely used approach in most patient care settings.

**Use an Alternative Antiplatelet Treatment Strategy**

Potential treatment options include agents that are not dependent (or less dependent) on CYP2C19 pathway for activation, eg, currently available agents such as prasugrel, ticagrelor, ticlopidine, dipyridamole, and cilostazol or those that are likely to become available in the future such as elinogrel. Among these agents, prasugrel and ticagrelor have been shown to have a favorable benefit-to-risk ratio compared with clopidogrel in high-risk ACS patients undergoing stenting. The downside with this strategy is that it will expose 70% of patients with normal CYP2C19*1/*1 alleles who are unlikely to benefit and potentially likely to be harmed by increased bleeding associated with prasugrel or ticagrelor. A recent study reported insufficient platelet inhibition even with prasugrel in 25% patients with ACS. Furthermore, prasugrel has limited approved indications and is contraindicated in certain patient populations at high risk for bleeding, and there is some uncertainty regarding increased malignancy rates. All of these factors, plus the fact that clopidogrel will come off patent in the very near future, make the option of prescribing prasugrel or ticagrelor to all patients unwise at this time.

**Use Genotype Information to Stratify Risk and to Inform Treatment Decisions**

This approach is best justified for patients considered to be at high risk for recurrent events on clopidogrel or in patients undergoing high-risk stenting in whom an acute or subacute thrombotic event might be catastrophic. With this approach, CYP2C19*2 homozygotes (poor metabolizers) who constitute 2% to 4% in the population would be considered candidates for another agent, eg, prasugrel or ticagrelor. For CYP2C19*2 heterozygotes (intermediate metabolizers), who constitute approximately one third of the population, the treatment decision regarding alternative antiplatelet agents should be based on clinical predictors (eg, diabetes mellitus, disease burden, prior MI, renal function), procedural risk factors, and/or platelet reactivity. The implementation of this approach in clinical practice is challenged by the undesirable logistic and cost considerations of genetic testing and its modest prognostic and predictive performance.

**Use Platelet Function Information to Stratify Risk and to Inform Treatment Decisions**

The advocates of this approach argue that platelet reactivity is “closer” to the final phenotype (ie, cardiovascular outcomes) and takes into account environmental and genetic factors that may influence platelet function. Although some of these tests are available as point-of-care assays, they are limited by biological and methodological variability and lack of stan-
dardized criterion for HPR. In addition, the prognostic performance is rather modest, is outcome and indication specific, and is dependent on time of assessment poststenting. The dynamic nature of platelet reactivity renders the results difficult to interpret during acute periods and would likely need to be repeated over time, unlike genotype, which is stable throughout a person’s lifetime. This would make platelet function testing less desirable for risk stratification. Studies aimed at evaluating platelet reactivity-guided tailored antiplatelet approaches have failed to yield favorable results. Whether a combination of platelet function– and genotype-guided therapy (eg, dose initially according to genotype and then follow response by means of platelet function testing) may be superior to either method alone is currently being investigated.

Conclusions

On the basis of the totality of evidence, the value of routine platelet function testing and genotyping in all patients remains unproven; therefore, they cannot be recommended at this time. Neither genotyping nor platelet function testing is a strong discriminator of subsequent clinical outcomes, highlighting the complexity of platelet biology and function, the multifactorial nature of cardiovascular risk, and the challenges underlying risk stratification and treatment decisions. Further trials need to be conducted to help formulate the best and most cost-effective testing strategy to ensure optimal antiplatelet treatment strategy in appropriate patients and clinical indications. Accordingly, until the evidence of benefit exceeding harm with these testing strategies becomes available, physicians should be free to exercise clinical judgment and to use the current data as they deem appropriate on a case-by-case basis.

Disclosures

None.

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interaction between proton pump inhibitors and clopidogrel. CMAJ. 2009; 180:713–718.


Antiplatelet agents are administered to percutaneous coronary intervention patients to directly prevent catastrophic events. Conclusive evidence exists that high on-treatment platelet reactivity is associated with heightened thrombotic risk. Kaul et al argue against routine platelet function testing and genotyping; we agree with them, and we stated, “the evidence is strong enough now to recommend genotyping and phenotyping in the high-risk PCI patient.” They focus on the poor prognostic value of a pulmonary function test. The low positive predictive value of a platelet function testing results from thrombotic event occurrence being influenced by numerous factors. Any 1 factor (clinical, biological, or genetic) will never satisfy the predictive criteria demanded by these authors. Therefore, future algorithms that include a pulmonary function test and genetic testing with other factors, similar to a Thrombolysis in Myocardial Infarction (TIMI) or Global Registry of Acute Coronary Events (GRACE) risk score, will optimally identify thrombosis-prone patients and guide decisions for alternative therapies. It may be impossible now to conduct any prospective personalized antiplatelet therapy trial where there is randomization to an inferior pharmacodynamic regimen once high on-treatment platelet reactivity is identified.

In the Testing Platelet Reactivity in Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy with Prasugrel (TRIGGER-PCI) trial, many patients were not randomized after knowledge of high on-treatment platelet reactivity carriage. Prognostic indicators (platelet reactivity and genotype) should be interpreted in the context of the clinical presentation, not as stand-alone tests. As with clinical risk factors, they are meant to help physicians assess risk—not to diagnose which patients will have events. Discounting the utility of prognostic indicators on the basis of poor diagnostic performance results from inappropriate interpretation of the information provided. Finally, tremendous clinical value usually results from a confirmed and not just assumed therapeutic effect in the high risk patient.
The Role of Platelet Reactivity and Genotype Testing in the Prevention of Atherothrombotic Cardiovascular Events Remains Unproven
Vamsi Krishna, George A. Diamond and Sanjay Kaul

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