Journal of the American College of Cardiology
© 2013 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 61, No. 21, 2013
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2012.11.080

STATE-OF-THE-ART PAPER

Platelet Function Tests in Clinical Cardiology

Unfulfilled Expectations

Diana A. Gorog, MD, PhD,* Valentin Fuster, MD, PhD†‡

London, United Kingdom; New York, New York; and Madrid, Spain

This review is a critical evaluation of publications in the past decade on the usefulness of platelet function tests (PFTs) in clinical cardiology, in aiding diagnosis, predicting risk, and monitoring therapy. The ideal PFT should: 1) detect baseline platelet hyperreactivity; 2) allow individualization of antiplatelet medication; 3) predict thrombotic risk; and 4) predict bleeding risk. The practicalities of clinical cardiology demand rapid, accurate, and reliable tests that are simple to operate at the bedside and available 24 h a day, 7 days a week. Point-of-care PFTs most widely evaluated clinically include PFA-100 and VerifyNow. None of these tests can reliably detect platelet hyperreactivity and thus identify a prothrombotic state. Identification of antiplatelet nonresponsiveness or hyporesponsiveness is highly test specific, and does not allow individualization of therapy. The power of PFTs in predicting thrombotic events for a given individual is variable and often modest, and alteration of antithrombotic treatment on the basis of the results of PFTs has not been shown to alter clinical outcome. PFTs in current mainstream use cannot reliably assess bleeding risk. These tests have been in use for over a decade, but the hopes raised by PFTs in clinical practice remain unfulfilled. Although physiologically relevant measurement of platelet function now is more important than ever, a critical reappraisal of available techniques in light of clinical requirements is needed. The use of native blood, global stimulus instead of individual agonists, contribution of thrombin generation by activated platelets to the test results, and establishment of a PFT therapeutic range for each antiplatelet drug should be considered and is discussed. (J Am Coll Cardiol 2013;61:2115–29) © 2013 by the American College of Cardiology Foundation

The interest in measuring platelet function in clinical cardiology stems from the overwhelming evidence that arterial thrombosis is the main cause of acute myocardial infarction (AMI) and major adverse clinical events (MACE) after percutaneous coronary intervention (PCI). The observation that individuals with platelet hyperreactivity are more prone to such thrombotic events raised hopes of identifying those at risk of acute coronary events who may benefit from additional antiplatelet medication. After 2 decades of aspirin monotherapy, the introduction of double and triple antiplatelet regimens has made the search for clinically applicable platelet function tests (PFT) ever more important. The expectation from an ideal PFT is to: 1) detect platelet hyperreactivity both in the normal population and in patients, allowing primary and secondary prevention; 2) detect individual variation in platelet reactivity in response to fixed doses of antiplatelet medication, thereby allowing individualization of therapy; 3) detect risk of future thrombosis; and 4) predict bleeding risk, in general and peri-PCI.

From the early 1960s, light transmittance platelet aggregometry (LTA) performed on citrated platelet-rich plasma contributed significantly to our understanding of the mechanism of platelet activation, aggregation, and the antiplatelet effect of medication (Fig. 1). Although LTA remains the gold standard, the practicalities of clinical medicine demand rapid, accurate, and reliable tests that are simple to operate at the bedside and are available 24 h a day, 7 days a week. From the late 1990s, emerging point-of-care PFTs such as PFA-100 (Siemens Medical, Munich, Germany) (1) and VerifyNow (Accumetrics, San Diego, California) (2,3) fulfilled (at least partly) the aforementioned criteria, allowing the clinical use of these tests. Others, such as multiple electrode platelet aggregometry, vasodilator-stimulated phosphoprotein (VASP) phosphorylation, Cone-and Plate(let) assay (IMPACT-R, DANED SA, Beersel, Belgium), and Plateletworks (Helena Laboratories, Beaumont, Texas), are variably limited by being labor intensive and time consuming, and require experience and expertise to perform and evaluate; therefore, they are more adapted to research than the point-of-care environment.

This review is a critical evaluation of publications in the past decade on the usefulness of PFTs in clinical cardiology in aiding diagnosis, predicting risk, and monitoring therapy.

From the *National Heart & Lung Institute, Imperial College, London, United Kingdom; †Zena and Michael A. Wiener Cardiovascular Institute, and the Marie-Josee and Henry R. Kravis Cardiovascular Health Center, the Mount Sinai School of Medicine, New York, New York; and the ‡Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain. Both authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received May 15, 2012; revised manuscript received November 20, 2012, accepted November 26, 2012.

JACC Vol. 61, No. 21, 2013
May 28, 2013:2115–29

Abbreviations and Acronyms

AA = arachidonic acid
ACS = acute coronary syndrome(s)
ADP = adenosine diphosphate
AMI = acute myocardial infarction
CYP = cytochrome P-450
DAPT = dual antiplatelet therapy
DES = drug-eluting stent(s)
HR = hazard ratio
HRPR = high residual platelet reactivity
LTA = light transmittance platelet aggregometry
MACE = major adverse cardiac event(s)
PCI = percutaneous coronary intervention
PFT = platelet function test
PRI = platelet reactivity index
PRU = $P2Y_{12}$ reactivity unit(s)
ST = stent thrombosis
TXB2 = thromboxane B2
VASP = vasodilator-stimulated phosphoprotein

Detecting Baseline Platelet Hyperreactivity (Prothrombotic Status) and Cardiac Risk

Platelet hyperreactivity is defined here as the enhanced response of platelets to agonists in subjects who are not taking antiplatelet medication. This definition is different from high residual platelet reactivity (HRPR) despite antiplatelet therapy, which is discussed later in the text.

There has been a continuous search for clinical markers that can predict the occurrence and risk of MACE. Although large studies showed significant correlation between elevated levels of various plasma markers (fibrinogen, von Willebrand factor, factor VIII) and the occurrence of ischemic events, because these markers are not specific for coronary disease and cannot be used to identify individuals at risk, they had no impact on everyday clinical practice. Measurement of spontaneous aggregability of platelets in response to shear stress (stirring) was the first test to detect platelet hyperreactivity. Among patients with prior AMI, those with spontaneous platelet aggregation had a 3- to 5-fold increase in MACE over a 5-year follow-up, compared with those without spontaneous aggregation (4). Platelet hyperreactivity was an independent risk factor for vascular occlusion in healthy volunteers (5) and was demonstrated in patients with acute coronary syndrome (ACS) (6), hyperlipidemia, hypertension, diabetes, rheumatoid arthritis, smoking, and acute exercise. Platelet hyperreactivity in healthy individuals was also detected by a platelet-counting technique, whole-blood flow cytometry, and by platelet aggregometry using submaximal concentrations of epinephrine (7,8). However, these laboratory tests lack standardization, definitions of clear-cut "normal" and "hyperreactive" ranges, and are not suitable for identifying individual patients at risk (Table 1).

Allowing Individualization of Antiplatelet Medication

In response to reports on "aspirin resistance," PFTs were used to detect and monitor sensitivity to antiplatelet medication. To reduce the large interindividual variability observed with PFTs, newer point-of-care tests substantially increased the concentration of agonist. Although platelet hyperreactivity can only be observed at very low agonist concentrations, point-of-care PFTs employ agonists at much higher concentrations. As such, interindividual variability in platelet response to antiplatelet medication is reduced considerably, but the ability to detect spontaneous platelet hyperreactivity in an individual subject is lost.

Although aspirin was the only effective antiplatelet agent available, monitoring was not required. Inhibition of cyclo-oxygenase-1 was regarded as the sole mechanism of the antiplatelet effect of aspirin. Because aspirin 75 mg daily inhibits thromboxane A2 formation by >90%, a dose of 75 to 150 mg daily was considered universally effective, and justified the "one dose for all" policy. Early reports of aspirin resistance, namely the reduced or absent inhibition of agonist-induced platelet aggregation, gave impetus to using point-of-care tests for monitoring therapy. Depending on the agonist and cutoff level selected, 5% to 60% of patients were termed "aspirin-resistant." Findings of resistance, despite nearly complete inhibition of arachidonic acid (AA)-induced aggregation, coupled with the observed dose dependence of resistance, suggested that aspirin may exert antiplatelet effects through non–cyclo-oxygenase-1 pathways (9). The antiplatelet effect of aspirin was limited in whole blood with normal calcium levels, and the administration of high-dose aspirin for 7 days (cumulative dose 960 mg) was associated with considerable recovery of AA-induced platelet aggregation (10). Although tests that employ AA-induced aggregation showed good agreement in the prevalence of aspirin resistance (0% to 6%) (9), this did not correlate well with non–AA-dependent assays (9), and furthermore, a comparison of 6 PFTs showed poor agreement between tests in detecting prevalence of aspirin resistance: 10.3% to 51.7% for aggregometry using adenosine diphosphate (ADP), 18.0% for whole-blood aggregometry, 59.5% for PFA-100, 6.7% for VerifyNow, and 22.9% for urinary thromboxane B2 (TXB2) concentration (11). Agreement was also poor when tests were compared with AA-induced LTA. One study found serum TXB2 a useful measure of aspirin nonresponsiveness (12), but another showed that urinary and serum TXB2 did not correlate with other PFTs (13,14). A growing list of publications demonstrated that PFA-100 was not capable of measuring aspirin resistance (15). These findings cast doubt on the reliability and usefulness of PFTs in correctly classifying patients as aspirin resistant, leading some to suggest that "the term 'aspirin resistance' based on inadequate knowledge of imperfect laboratory tests does a disservice to physicians and patients" (16).

The impressive reduction in MACE when thienopyridines were added to aspirin began the era of dual antiplatelet therapy (DAPT), with ticlopidine rapidly overtaken by clopidogrel, following the recognition of potentially fatal bone marrow suppression by ticlopidine. Studies reported

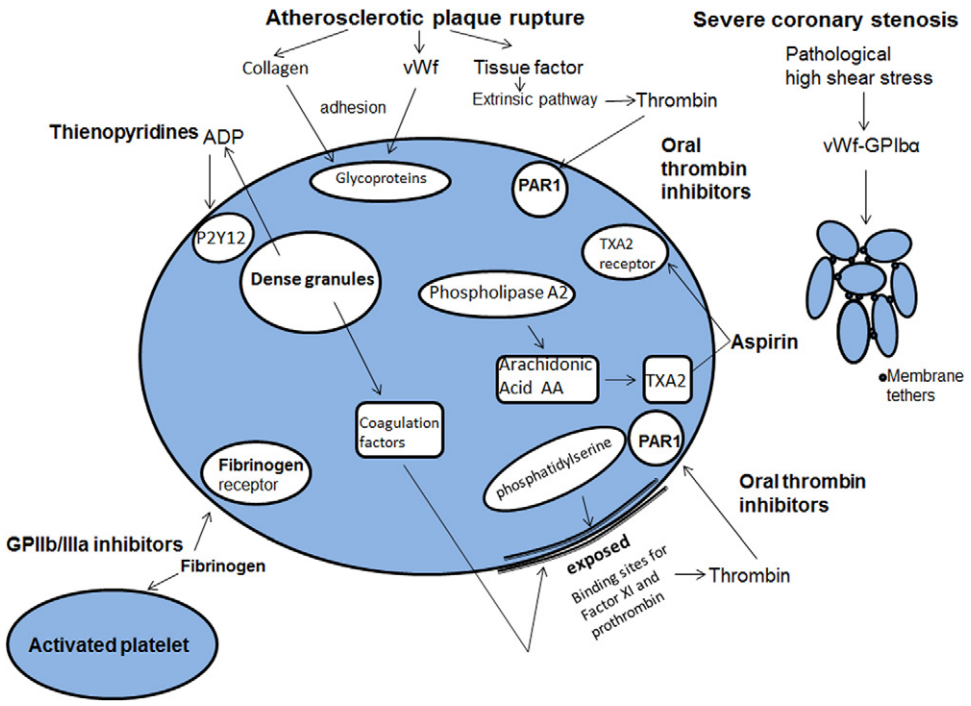

Figure 1 **Schematic Depicting Platelet Activation and Site of Action of Commonly Used Antithrombotic Agents**

The main pathways for platelet activation, including arachidonic acid (AA), adenosine diphosphate (ADP), von Willebrand factor (vWf), PAR-1, and surface glycoproteins (GP) Ib and IIb/IIIa, are depicted. These pathways have complex interactions, and the pathways in an individual may vary in the magnitude of their contribution to thrombosis based on the clinical setting. It is therefore very difficult to assess the overall platelet reactivity by using only a single pathway of platelet activation and measuring the response to a specific drug.

that 4% to 30% of patients exhibited "clopidogrel resistance" (17), some 5% to 6% exhibited DAPT resistance (18,19), and 1 study reported no subject to be unresponsive to both aspirin and clopidogrel, regardless of the assay used (20). Comparison of 3 PFTs (LTA, VASP, and VerifyNow) showed the prevalence of clopidogrel nonresponsiveness to be 13%, 39%, and 33%, respectively, with poor agreement between assays (11,21) although $P2Y_{12}$ receptor–specific tests, such as VASP and VerifyNow P2Y12, appear to correlate strongly with one another (22). The VASP-assay is considered the reference standard for assessing $P2Y_{12}$ antagonist therapy. Although some suggested the VASP assay to be the ideal PFT for monitoring clopidogrel responsiveness (23), in patients on clopidogrel, the VASP index did not correlate well with $P2Y_{12}$ receptor occupancy (24). The Cone-and-Plate(let) analyzer (IMPACT-R ADP test) was found to be unsuitable for monitoring the clopidogrel effect (25). When LTA, whole-blood aggregometry, PFA-100, and VerifyNow were compared, none could reliably distinguish between patients who had, or had not, ingested clopidogrel (26), although this study defined clopidogrel hyporesponsiveness as <50% inhibition of baseline platelet aggregation, based on contemporaneous American College of Cardiology/American Heart Association/Society Cardiovascular Angiography and Interventions guidelines, whereas on-treatment platelet reactivity is now regarded to be a better measure of thrombotic risk (27).

Thus, identification of clopidogrel nonresponsiveness is test specific, and additionally, clopidogrel's variable antiplatelet effect means that PFTs are also unsuitable for diagnosing drug noncompliance. Correlation between tests was, at best, modest; these assays are therefore not interchangeable (28). Whether individualization of clopidogrel treatment based on PFT results is necessary is questionable, given that clinical studies have shown clopidogrel benefit in trials where PFT was not performed. In the setting of PCI in the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events) trial (29) and STEMI in COMMIT (Clopidogrel and Metoprolol in Myocardial Infarction Trial) (30), clopidogrel significantly reduced MACE (30), without testing for or tailoring treatment to clopidogrel nonresponsiveness. Thus, whether individualization of clopidogrel treatment based on PFT results is necessary could be questioned, given that clinical studies have shown clopidogrel benefit in trials where PFT was not performed. However, whether the magnitude of benefit was greatest in those who were "responders" to clopidogrel is unknown, and if this were demonstrated to be true in future studies, it may support PFT-guided treatment. Furthermore, although VASP and multiple electrode platelet ag-

Table 1 PFTs in Clinical Cardiology

Test	Advantages	Limitations
Detecting platelet hyperreactivity	Platelet hyperreactivity associated with increase MACE in population studies	Platelet hyperreactivity not specific for cardiovascular disease and also detectable in many disease states High agonist concentrations in PFT reduce ability to detect spontaneous platelet hyperreactivity Lack of standardization between tests No clear-cut definition of normal and abnormal ranges
Detecting HRPR	PFTs identify reduced or absent inhibition of agonist-induced platelet aggregation (antiplatelet "resistance") HRPR on aspirin and clopidogrel widely studied with many PFTs High agonist concentrations in PFTs reduce individual variability in platelet response to antiplatelet medication	Incidence of "resistance" varies with agonist and cutoff level used Incidence and meaning of HRPR on newer antiplatelet agents (e.g., ticagrelor, prasugrel, thrombin antagonists) not widely studied Alteration in antiplatelet medication to reduce HRPR does not reduce cardiovascular events High agonist concentrations reduce ability to detect spontaneous platelet hyperreactivity Poor correlation between results of various PFTs Great variability in definition of resistance Unsuitable for diagnosing noncompliance At low plasma calcium levels in citrated blood in PFT, platelets do not generate thrombin; thus, current PFTs are unlikely to be suitable for monitoring thrombin antagonists
Predicting adverse cardiovascular events	Definite correlation between HRPR and risk of MACE and ST	No correlation between improved HRPR and reduction in events Large variation in level of risk reported between different clinical trials, different PFTs, different antiplatelet regimens and different clinical conditions Individualization of antiplatelet therapy based on PFTs results cannot be recommended Meaning of HRPR with more than 1 agonist unknown
Predicting bleeding risk	Enhanced platelet response associated with increased bleeding risk	No test accurately predicts bleeding risk for a given individual Thrombin is key player in hemostasis, but its contribution to hemostasis cannot be assessed in citrated blood used in PFT No clear-cut definition of level of platelet response that increases bleeding risk No evidence that alteration of antiplatelet therapy based on PFT results reduces bleeding

HRPR = high residual platelet reactivity; MACE = major adverse cardiac event(s); PFT = platelet function test; ST = stent thrombosis.

gregometry recorded a significant reduction of the number of clopidogrel-resistant patients after doubling the dosage of clopidogrel (31), this maneuver appears not to reduce the incidence of MACE in comparison with standard dosage (32,33).

Initially, small studies suggested that individualization of antiplatelet therapy based on the results of PFT may reduce MACE. In a randomized study of 162 patients, VASP-guided optimization of platelet reactivity in those demonstrating clopidogrel resistance (VASP index ≥50%), with repeated clopidogrel loading pre-PCI, reduced 30-day MACE, compared with conventional, non–PFT-tailored care (34). The same group showed that in 429 patients undergoing PCI, VASP-guided clopidogrel treatment in those with a VASP index ≥50%, reduced stent thrombosis (ST) (0.5% vs. 4.2%, $p < 0.01$) and MACE (0.5% vs. 8.9%, $p < 0.001$) compared with standard care (35). In 263 patients demonstrating resistance to aspirin, clopidogrel or both as detected by VerifyNow, randomized to receive tirofiban or placebo in addition to DAPT before PCI, tirofiban significantly reduced periprocedural AMI and adverse events at 30 days (36). In the much larger GRAVITAS (Gauging Responsiveness With a VerifyNow Assay–Impact on Thrombosis and Safety) trial, of 5,429 patients on DAPT undergoing PCI, 2,214 patients with HRPR on VerifyNow P2Y12 assay were randomized to clopidogrel 75 or 150 mg daily for 6 months. High-dose clopidogrel achieved a 22% reduction in HRPR, but failed to reduce cardiovascular death, AMI, or ST (32). These results do not support the application of VerifyNow P2Y12 assay to guide antiplatelet therapy (37).

The poor correlation between different PFTs is not necessarily a barrier to clinical adoption of any single test, because the tests assess different aspects of platelet function. PFTs that are most $P2Y_{12}$ receptor specific, such as VASP and VerifyNow P2Y12, correlate strongly with one another and with levels of clopidogrel's active metabolite (22). However, a weak correlation was observed between VASP and LTA ADP assay (28), perhaps because platelet aggregation induced by 2 μmol/l ADP was particularly sensitive to low levels of receptor blockade, whereas the VASP required ~60% receptor blockade to achieve substantial inhibition (38). There are many different PFTs available, and although VerifyNow is the dominant point-of-care PFT in clinical use, none stand out as superior to the others for guiding antiplatelet therapy, because large-scale clinical trials to address their clinical usefulness in tailoring therapy have not been performed.

Predicting Adverse Cardiovascular Events

There is overwhelming evidence of a correlation between reduced responsiveness to antiplatelet therapy (based on PFTs) and MACE (Table 2) (39–41). However, among reports, there is great variability in the definition of resistance, even between studies using the same technique. Some patients classified as nonresponders by one PFT were considered responders by another, and often, baseline differences were discounted. Although HRPR is frequent (30% to 40% for clopidogrel), the low frequency of MACE following PCI (0.5% to 2.5%) means that randomized studies need to have a sufficiently large sample size to demonstrate a relationship between PFT and events. Here, we focus on data obtained in patients tested with the most frequently used assays, PFA-100 and VerifyNow.

There is a wide range in the predictive power of PFTs for cardiovascular events. The PFA-100 showed no significant difference in MACE between aspirin responders and nonresponders (42–44). Low platelet responsiveness predicted a variable risk of coronary events, from hazard ratio (HR): 36 (45) to HR: 3 (46), a variation greater than that attributable simply to study population differences. In a head-to-head comparison in 1,069 PCI patients, the predictive power (area under the curve) of LTA, VerifyNow, and Plateletworks was modest (0.63, 0.62, and 0.61, respectively), whereas IMPACT-R and the PFA-100 assays were unable to discriminate between patients with and without MACE at 1 year (43). The PFA-100 does not appear useful for detection of clopidogrel effect (47). In 144 patients, VASP PRI detected all 21 low-risk MACE with a negative predictive value of 100% (48). However, in a small Swedish study, VASP assay results were not related to the occurrence of ST or AMI (49). Although studies have shown that low responders to ADP receptor antagonists, identified using VASP, have higher MACE events post-PCI (48,50–52), with a similar cut-point for clopidogrel and prasugrel (platelet reactivity index [PRI] >50%), others showed that VASP results failed to predict ST following drug-eluting stent (DES) implantation (53) and did not differ between patients with angiographically confirmed ST or AMI, and controls (49). In 222 patients undergoing PCI hyporesponsiveness to clopidogrel as detected with VerifyNow was able to predict 30-day MACE (area under the curve 0.649, $p = 0.032$) (54). In 683 post-PCI patients, VerifyNow P2Y12 assay was able to predict cardiovascular death/nonfatal AMI (HR: 2.55 and 3.36; $p = 0.034/0.004$, respectively) (55). In 1,789 ACS patients undergoing PCI, HRPR detected by LTA (ADP) occurred in 14% and was related to MACE (41). However, a recent study of 2,849 patients tested post-PCI with the VerifyNow P2Y12 assay showed no difference at 2 years in the occurrence of MACE between patients with and without HRPR (56). In 771 stable CAD patients, antiplatelet resistance, measured with specific or aggregation-based assays, added no incremental predictive value for MACE, over and above established risk factors (57). Of 1,226 Korean patients, in those undergoing PCI for AMI, HRPR with the VerifyNow P2Y12 assay was related to 1-year cardiovascular events, whereas in stable PCI patients, HRPR conferred no prognostic significance (58). In 186 patients receiving DES, there was no difference in the 1-year clinical event rate between those who were and were not classified as low responders to aspirin or to clopidogrel with VerifyNow (59). In 378 patients undergoing PCI, the results of VerifyNow P2Y12 had no prognostic value in predicting adverse cardiovascular events at 6 months (60).

In a recent meta-analysis of 6 studies involving >3,000 patients undergoing PCI, enhanced platelet reactivity detected by VerifyNow P2Y12 assay was associated with MACE, and the level of platelet reactivity was directly related to risk (39). The practical value of testing patients for "clopidogrel resistance" is best shown by the outcome of the GRAVITAS and ADAPT-DES (Assessment of Dual Antiplatelet Therapy with Drug-Eluting Stents) studies. In GRAVITAS, 2,796 patients were tested with VerifyNow P2Y12 post-PCI (61). Low on-treatment platelet reactivity (<208 $P2Y_{12}$ reactivity units [PRU]) was an independent predictor of reduced MACE at 60 days (HR: 0.23; 95% confidence interval: 0.05 to 0.98; $p = 0.047$) but only tended to be associated with improved outcome at 6 months. In >2,000 post-PCI patients with HRPR with VerifyNow (defined as ≥230 PRU), high-dose clopidogrel failed to reduce MACE, compared with standard-dose clopidogrel (32).

In ADAPT-DES, 8,575 patients undergoing PCI with DES were tested with VerifyNow (62). At 30 days, the rate of probable or definite ST was 0.46%, and was related to the level of platelet inhibition in response to ADP antagonists, but not related to the baseline level of platelet $P2Y_{12}$ response, aspirin, or overall platelet responsiveness after DAPT loading. The modest sensitivity, specificity, and poor prognostic utility of such testing, coupled with the low prevalence of events, indicated that testing for ADP antagonist responsiveness was unlikely to provide useful information to guide clinical decision making. These results indicate that for individuals, point-of-care PFTs provide no more information on MACE than C-reactive protein levels (56), hematocrit (63), mean platelet volume (64), or routine hematologic tests (65). However, 2 recent, albeit much smaller studies, showed promise for PFT. In the CILON-T (Efficacy of CILostazol ON Ischemic Complications After DES Implantation) trial, 716 patients undergoing PCI were analyzed with VerifyNow P2Y12 (PRU) and aspirin reactivity unit assays. At 6 months, neither PRU nor aspirin reactivity unit data on their own had predictive power, but the combined aspirin reactivity unit and PRU result was a significant predictor of MACE (HR: 6.34, $p = 0.021$) (66). In >1,000 ACS patients undergoing PCI, HRPR to more than 1 agonist (ADP, AA, and collagen) was strongly predictive of MACE (odds ratio: 4.7; $p < 0.0001$), whereas isolated platelet hyperreactivity to a single agonist had no

Table 2 Trials of PFT and Adverse Cardiovascular Events

First Author, Year (Ref. #) Trial Name	Test Used	Condition Evaluated	N	Trial Design	Intervention	Follow-Up	Outcome Assessed	Results
Brar et al., 2011 (39)	VerifyNow	Patients undergoing PCI on DAPT	3,059	Meta-analysis PRU ≥230 vs. PRU <230		At least 1 month	MACE (death, AMI, or ST)	15% vs.7 % ($p < 0.0001$)
Breet et al., 2010 (43)	LTA, VerifyNow, Plateletworks and IMPACT-R and PFA-100	Elective PCI on DAPT	1,069	Prospective observational study comparing HRPR vs. normal platelet reactivity		12 months	Death, AMI, ST, ischemic CVA	LTA: 12% vs. 6% ($p < 0.001$); VerifyNow 13% vs. 6% ($p < 0.001$); Plateletworks 13% vs. 6% ($p = 0.005$); IMPACT-R (p = NS) and PFA-100 (p = NS)
Marcucci et al., 2009 (55)	VerifyNow	ACS undergoing PCI with DES or BMS on DAPT	683	Prospective observational study comparing HRPR vs. normal platelet reactivity		12 months	CV death, AMI	17% vs. 12% ($p = 0.11$)
Campo et al., 2006 (40)	LTA and PFA-100	STEMI and stable CAD on DAPT	70 STEMI and 30 stable CAD	Prospective observational study comparing HRPR vs. normal platelet reactivity		12 months	Death, AMI, TVR	PFA-100 HR: 11, $p = 0.02$; LTA HR: 5.2 $p = 0.03$
Modica et al., 2009 (44)	PFA-100 and Aggregometry	AMI on DAPT	334	Prospective observational study comparing HRPR vs. normal platelet reactivity		44 months	Death, AMI, or stroke	p = NS
Gianetti et al., 2006 (45)	PFA-100	Stable or ACS patients undergoing PCI on DAPT	175	Prospective observational study assessing ASA or clopidogrel resistance		6 months	MACE (CV death, AMI, or TVR)	ASA resistance HR: 8.5, $p = 0.004$; clopidogrel resistance HR: 23, $p = 0.003$
Bonello et al., 2007 (48)	VASP	Stable or NSTEMI patients undergoing PCI on DAPT	144	Prospective observational study HRPR vs. normal platelet reactivity		6 months	MACE (CV death, AMI, ischemic stroke, or revascularization)	21% vs. 0% ($p < 0.01$)
Varenhorst et al., 2011 (49)	VASP and VerifyNow	ST or AMI in patients <6 months of prior PCI on DAPT	78	Retrospective case control			ST or AMI	VerifyNow PRU higher in ST patients than controls ($p = 0.001$); VASP: no difference (p = NS)
Bonello et al., 2011 (51), 2012 (52)	VASP	PCI for ACS receiving prasugrel as part of DAPT	310	Prospective observational study of HRPR vs. normal platelet reactivity		30 days 1 year	MACE (CV death, AMI, ST)	9% vs.0.4 % ($p < 0.001$) 22% vs. 3% ($p < 0.001$)
Ko et al., 2011 (54)	VerifyNow and MEA	Patients undergoing PCI on DAPT	222	Prospective observational study		30 days	MACE (CV death, AMI, stroke, or revascularization)	VerifyNow P2Y12 (but not MEA) predictive of periprocedural MI ($p = 0.02$) and 30-day MACE ($p = 0.03$)

Continued on the next page

Table 2 Continued

First Author, Year (Ref. #) Trial Name	Test Used	Condition Evaluated	N	Trial Design	Intervention	Follow-Up	Outcome Assessed	Results
Reny et al., 2012 (57) ADRIE	PFA-100 C/Epi and VASP-PRI	Stable CAD on DAPT	771	Prospective observational study comparing HRPR to at least 1 antiplatelet agent vs. normal platelet reactivity		3 years	MACE (CV death, AMI, UA, revascularization, ischemic limb, or ischemic CVA)	16% vs. 15% ($p = 0.71$)
Ahn et al., 2012 (58)	VerifyNow	Undergoing PCI for AMI or stable CAD on DAPT	1,226	Prospective observational study comparing HRPR vs. normal platelet reactivity		1 year	MACE (CV death, AMI, ST)	For AMI group: 9% vs. 0.4% ($p < 0.001$) For non-AMI group: 7% vs. 8% ($p = 0.193$)
Yu et al., 2012 (59)	VerifyNow	Undergoing PCI with DES on DAPT	186	Prospective observational study comparing HRPR vs. normal platelet reactivity		1 year	MACE (CV death, AMI, ST, and stroke)	6% vs. 13% ($p = 0.99$)
Paulu et al., 2012 (60)	VerifyNow	Undergoing PCI on DAPT	378	Prospective observational study comparing HRPR vs. normal platelet reactivity		6 months	MACE (death, AMI, stroke)	No difference in platelet reactivity between patients with and without events; OR: 1.0 ($p = 0.9$)
Park et al., 2011 (56)	VerifyNow	Undergoing PCI with DES on DAPT	2,849	Prospective observational study comparing HRPR vs. normal platelet reactivity		2.2 years	MACE (death, AMI, ST, stroke)	2.8% vs. 2.4% ($p = 0.18$)
Kirtane et al., 2012 (69) ADAPT-DES	VerifyNow	Undergoing PCI with DES on DAPT	8,575	Prospective observational study comparing HRPR vs. normal platelet reactivity		30 days	ST	HRPR associated with ST, especially in ACS patients ($p = 0.0001$) Substantial overlap in PFT results between patients who had and who had not had ST
Lee 2011 (66) CILON-T	VerifyNow	Undergoing PCI with DES on DAPT	716	Prospective observational study comparing HRPR vs. normal platelet reactivity		6 months	MACE (CV death, AMI, stroke)	HRPR (combined ASA + clopidogrel resistance) predictive of MACE (HR: 6.3, $p = 0.02$) Neither ASA nor clopidogrel resistance alone predicted MACE
Marcucci et al., 2010 (67)	LTA	ACS patients undergoing PCI on DAPT	1,108	Prospective observational study comparing HRPR vs. normal platelet reactivity		12 months	CV death and AMI	HRPR to more than 1 agonist predicted AMI and death ($p < 0.0001$) Isolated platelet hyperreactivity to only 1 agonist had no predictive value
Mangiacapra et al., 2012 (68) ARMYDA-PROVE	VerifyNow	Elective PCI on DAPT	732	Prospective observational study comparing HRPR vs. normal platelet reactivity		30 days	Ischemic events (death, AMI, or TVR) and bleeding events	15% vs. 8% ($p = 0.005$)

Continued on the next page

Table 2 Continued

First Author, Year (Ref. #) Trial Name	Test Used	Condition Evaluated	N	Trial Design	Intervention	Follow-Up	Outcome Assessed	Results
Trenk et al., 2012 (115) TRIGGER-PCI	VerifyNow	Stable CAD undergoing PCI with DES and HRPR on DAPT	212	Prospective randomized trial	Prasugrel vs. clopidogrel	6 months	CV death or AMI	0% vs. 1% (prasugrel vs. clopidogrel) p = NS Greater platelet inhibition with prasugrel vs. clopidogrel
Price et al., 2011 (32) GRAVITAS	VerifyNow	Patients with HRPR within 12-24h of PCI with DES on DAPT	2,214	Prospective, randomized double blind	High-dose (150 mg) vs. low-dose (75 mg) clopidogrel	6 months	MACE (CV death, AMI, ST)	2.3% vs. 2.3% (p = 0.97)
Bonello et al., 2008 (34)	VASP	Patients with HRPR receiving DES on DAPT	162	Prospective, randomized open-label	Clopidogrel dose adjusted to obtain VASP <50% vs. standard care	1 month	MACE (CV death, ACS, ST, revascularization)	0% vs. 10% (p = 0.007)
Bonello et al., 2009 (35)	VASP	Patients with HRPR receiving DES on DAPT	429	Prospective, randomized, open-label	Clopidogrel dose adjusted to obtain VASP <50% vs. standard care	1 month	ST	0.5% vs. 4.2% (p < 0.01)
Vaglimigli et al., 2009 (36) 3T/2R	VerifyNow	Elective PCI; resistant to ASA, clopidogrel, or both; on DAPT	263	Prospective, randomized, double-blind	Tirofiban or placebo, in addition to DAPT	1 month	Troponin elevation 3× ULN	20% vs. 35% (p = 0.009)
Parodi et al., 2011 (41)	LTA	ACS undergoing PCI on DAPT	1,789	Prospective open-label interventional trial	Patients with HRPR had clopidogrel dose increased or switched to ticlopidine	2 years	CV death, AMI, urgent revascularization, or stroke	15% vs. 9% (p = 0.003)

ACS = acute coronary syndrome(s); AMI = acute myocardial infarction; ASA = acetyl salicylic acid; CAD = coronary artery disease; CV = cardiovascular; CVA = cerebrovascular accident; DAPT = dual antiplatelet therapy; DES = drug-eluting stent; HR = hazard ratio; LTA = light transmittance aggregometry; MEA = multiple electrode platelet aggregometry; NSTEMI = non–ST-segment elevation myocardial infarction; OR = odds ratio; PCI = percutaneous coronary intervention; PRU = platelet reactivity unit(s); STEMI = ST-segment elevation myocardial infarction; TVR = target vessel revascularization; UA = unstable angina; ULN = upper limit of normal; VASP = vasodilator-stimulated phosphoprotein; other abbreviations as in Table 1.

predictive value (67). These studies highlight the common shortcoming of all point-of-care PFTs of using different agonists in separate cartridges to assess different platelet receptor antagonists. In order to monitor dual or triple antiplatelet therapy and perhaps the novel oral thrombin antagonists, platelet response to 4 agonists (ADP, AA, collagen, and thrombin) should be measured simultaneously. Whether the increased costs of measuring response to multiple stimuli will be justified by the benefit of improved risk assessment, remains to be seen.

Recently, cut values have been proposed for on-treatment platelet reactivity for PFTs that predict MACE predominantly in the setting of PCI, based on receiver-operating characteristic curve analysis (27). Several studies have defined the cut-point for risk, when using the VerifyNow (PRU above 208 to 235) (39,68), or VASP (50% to 53%) (48,50,51). The level of on-treatment platelet reactivity is proposed to be a better measure of thrombotic risk than responsiveness to clopidogrel. HRPR was defined as: 1) PRI >50% by VASP-P analysis; 2) >235 to 240 PRU using VerifyNow; 3) >46% maximal 5-μmol/l ADP-induced aggregation; and 4) >468 arbitrary aggregation units/min in response to ADP by Multiplate analyzer (Roche, Basel, Switzerland). The net reclassification index, a measure of incremental prognostic value (see the following text), using the VerifyNow P2Y12 assay in the meta-analysis of post-PCI patients, was 23% (39), and in ADAPT-DES, the net reclassification index was 28.7% for PRU >208, leading to reclassification of almost one-third of patients (69). However, there are no large-scale clinical studies demonstrating that adjustment of antiplatelet therapy based on any of these improves clinical outcome.

Influence of Genetic Variability on PFT

Clopidogrel is transformed into its active metabolite by cytochrome P-450 (CYP) enzymes. Genetic polymorphism exists for CYP2C19 expression. Depending on ethnicity, 30% to 55% of persons harbor a loss of function of the CYP2C19 allele (*CYP2C19*2*). Compared with noncarriers, *CYP2C19*2* carriers treated with clopidogrel had lower levels of active clopidogrel metabolite; HRPR as assessed using LTA (70) and VASP (71); and a higher rate of MACE, including ST (70). HRPR, detected by VASP and VerifyNow, could be overcome by tripling the maintenance dose of clopidogrel to 225 mg daily in *CYP2C19*2* heterozygotes, but not in homozygotes (72), although repeated boluses of clopidogrel 600 mg did overcome the HRPR in the majority of patients (73). Prasugrel appears more effective than high-dose clopidogrel in overcoming HRPR in *CYP2C19*2* carriers (74). In the GIFT (Genotype Information and Functional Testing) study, the genetic substudy of GRAVITAS, blood samples were tested for 40 polymorphisms, and these correlated with the results of on-treatment VerifyNow P2Y12 assay. HRPR was increased 11-fold in homozygotes and increased 62% in heterozygotes for the *CYP2C19*2* gene, compared with noncarriers. The study did not find any association of the *CYP2C19*17* gain-of-function gene with reduced on-treatment platelet reactivity (75).

Polymorphism also exists in paraoxonase 1 (PON1), a crucial enzyme responsible for clopidogrel bioactivation (76), but studies have failed to confirm an association between PON1 polymorphism and on-clopidogrel platelet reactivity using VerifyNow (75,77–80).

The results of meta-analyses are conflicting. A report on 9 studies in 9,685 patients undergoing PCI (81) and another involving 10 studies in 11,959 patients (82), both concluded that reduced-function CYP2C19 alleles expose patients treated with clopidogrel to increased risk of cardiovascular events. However, 2 recent meta-analyses (not confined to stented patients) showed that although there was an association between CYP2C19 genotype and clopidogrel responsiveness, genotype was not associated with cardiovascular events, with the possible exception of ST in the subgroup of patients undergoing PCI, where ST was associated with CYP2C19 loss-of-function alleles (83,84).

Predicting Bleeding Risk

Because there is a fine line between platelet inhibition and bleeding risk, these 2 factors must be carefully balanced, and any test that could assist the clinician would be highly desirable. Although aspirin is a mild platelet inhibitor, its advantage is the relatively low incidence of bleeding. Clopidogrel reduces AMI but at a cost of increased bleeding (85). In 17,383 patients undergoing PCI, triple therapy (glycoprotein IIb/IIIa receptor antagonist, aspirin, clopidogrel, and/or cilostazol) was associated with an 80% increase in blood transfusions and an 8-fold increase in thrombocytopenia, compared with aspirin monotherapy (86). In patients undergoing PCI, enhanced clopidogrel responsiveness carried an increased risk of major bleeding (87–89). Campo et al. (90) tested 300 patients post-PCI using VerifyNow, and showed that on-treatment platelet reactivity was predictive of bleeding events, and they identified a safety range for platelet reactivity, outside of which thrombotic or bleeding risk is increased. In 2,500 patients undergoing PCI, bleeding was twice as common in those with enhanced platelet response to ADP than in normal respondents (88). In some 500 ACS patients, clopidogrel hyperresponder status as measured with LTA and VASP was associated with major bleeding events over 30 days (91,92). Furthermore, very low VerifyNow PRU values, within the bleeding range, have been documented in clopidogrel-resistant patients switched to ticagrelor (93). VASP index was measured in 301 ACS patients post-PCI, treated with prasugrel. A cut value of PRI <16% was predictive of bleeding events at 1 year (52). The POPular (Do Point-of-Care Platelet Function Assays Predict Clinical Outcomes in Clopidogrel Pretreated Patients Undergoing Elective PCI) study assessed the value of clopidogrel-induced platelet inhibition in predicting bleed-

ing risk in the setting of PCI, using 5 different tests (LTA, VerifyNow, Plateletworks, IMPACT-R, and PFA-100). None of the tests predicted bleeding complications (10).

In patients undergoing PCI (n = 2,533), ADP-induced platelet aggregation was assessed by VerifyNow P2Y12 assay after 600-mg clopidogrel loading. The primary efficacy endpoint was the 30-day incidence of definite or probable ST, and the primary safety endpoint was the occurrence of major bleeding. Analyses of cutoff values derived from receiver-operating characteristic curves revealed the existence of a "therapeutic window" for $P2Y_{12}$ receptor inhibition, within which sufficient antiplatelet effect could be achieved without increased bleeding (94). The safety threshold of PFTs could be determined with the skin bleeding time test. In a recent study, bleeding episodes in patients on DAPT were significantly associated with the platelet response to ADP 2μmol/l, but not in response to 4 or 8 μmol/l (95).

In addition to double and triple antiplatelet medication, novel oral thrombin antagonists and platelet thrombin receptor inhibitors will soon be added to the present armamentarium. The need to identify patients at risk of bleeding is much greater for oral thrombin inhibitors than for aspirin/clopidogrel treatment. Although it is claimed that the predictable pharmacokinetics makes monitoring of thrombin inhibition dispensable, this is unlikely to be the case. Because the local thrombin-generating capacity of platelets shows high individual variation (96), fixed-dose thrombin antagonist treatment would mandate monitoring to reduce bleeding. A PFT providing both the optimal range of platelet reactivity necessary to prevent thrombotic events, as well as the range to avoid excessive bleeding, would therefore be highly desirable (97).

Possibilities to Improve Clinical Usefulness of PFTs

Because PFTs are not particularly useful in everyday clinical practice, a critical reappraisal of these tests is needed. We propose the following to potentially improve the clinical relevance and usefulness of PFTs.

1. Use of native, instead of citrate-anticoagulated blood:
   All PFTs presently in clinical use employ citrate-anticoagulated blood at very low plasma calcium ion concentration. Such an unphysiological environment distorts the response of platelets to various agonists (17). The difference in citrate concentrations (3.2% or 3.8%) has significant influence on the results (15,98). Other anticoagulants (heparin, thrombin inhibitors) are also unsuitable because these either interfere with the reactivity of platelets or exclude the possibility of measuring the generation or effect of thrombin.

2. Use of a global stimulus:
   Most PFTs in clinical use are aggregation based. It has long been assumed that soluble agonists, generated by platelets, initiate platelet aggregation and thrombus growth. Assessment of thrombotic status on the basis of platelet aggregation response to only 1 or 2 agonists ignores the complexity of the mechanism of arterial thrombus formation. Arterial thrombosis occurs at pathological high shear stresses ($>10{,}000\ s^{-1}$), which create rapid and strong bonds between platelets without prior activation (99,100). The release of soluble agonists (thromboxane, ADP, thrombin) occurs secondary to shear-induced platelet activation (101). Although the IMPACT-R and PFA-100 are generally regarded as "high shear–induced PFTs," the shear rate in IMPACT-R is only $1{,}800\ s^{-1}$(therefore, this test measures platelet adhesion but not aggregation) and in PFA-100 is generously estimated at 5,000 to $6{,}000\ s^{-1}$. Platelet interaction with von Willebrand factor, thrombin generation by shear-activated platelets, and shedding of microparticles by shear-activated platelets (a major contributor to thrombin generation) occur only at shear rates exceeding $10{,}000\ s^{-1}$, and aggregates thus formed are unstable until the shear rate is $\geq 20{,}000\ s^{-1}$ (may reach $250{,}000\ s^{-1}$), such as that in the apex of a >70% arterial stenosis (102,103).
3. Involvement of platelet-dependent thrombin generation:
   Current PFTs do not take into account the procoagulant activity of platelets, namely the significant contribution of platelets to coagulation through local thrombin generation. Thrombin is the most potent platelet agonist and, through fibrin formation, stabilizes the unstable platelet aggregates into a firm thrombus, capable of causing lasting arterial occlusion. Aspirin dose-dependently reduces thrombin generation with consequent attenuation of thrombin-mediated coagulant reactions (104). By inhibiting the $P2Y_{12}$ receptor, clopidogrel inhibits platelet-dependent thrombin generation (105). Further, current point-of-care PFTs most frequently employ citrate-anticoagulated blood, and at the very low plasma calcium levels therein, platelets do not generate thrombin (17), and consequently, the effect of thrombin on platelets is also attenuated (106).
4. Clear definition of safety/efficacy thresholds and evaluation of clinical usefulness:
   In order to incorporate PFTs into routine use, PFTs should have clearly defined normal ranges indicating both the range for efficacy and the threshold for safety, in various clinical settings. To be used for screening, any PFT, like other biomarkers, should be evaluated for clinical utility in terms of accuracy in identifying patients at risk, the strength of its association with outcome, its ability to discriminate between individuals at different risk levels (C-value), and the incremental value it adds in prognostic information over standard risk factors (107). Cut values should be based on receiver-operating characteristic curve analysis, but this is not enough (108,109). The incremental value of PFT, over and above conventional risk scoring, should be evaluated in terms of its

discriminatory and reclassification (net reclassification index) value (107,109). Validation of PFT usefulness in large prospective studies is required to confirm any proof-of-concept findings. PFT should be undertaken not only when practicable, but when it can discriminate sensitively between high- and low-risk patients, can lead to alterations in treatment that improve outcomes and is cost effective (109). None of the PFTs discussed here have been fully evaluated and validated to meet requirements for adoption for screening, according to the criteria for novel biomarker evaluation recommended by the American Heart Association (109).

5. Quality control:
Quality assurance is as important for PFTs as for other conventional laboratory-based tests (110) and is particularly pertinent, given the discrepancy in results between studies using the same PFT. Reproducibility is a prerequisite for successful use of a biomarker (109). Internal quality control is required to assess the accuracy and reproducibility of a test on an ongoing basis, and external quality assessment to check agreement between one laboratory's results with those of others. Error is most often pre-analytical (variable blood draw, collection, blood/anticoagulant ratio, transportation, and storage) but may also be analytical (calibration, reagent issues, methodological or instrument problems), post-analytical, and interpretative. Barriers to adoption of standardized quality assurance for PFTs include: 1) the need for frequent blood samples, both from many normal donors and affected patients, which maybe practically, clinically, and ethically challenging; 2) time-delay issues, due to transportation, or with tests employing non-anticoagulated blood; and 3) clinicians, because many near-patient PFTs are performed by clinicians with little or no training in quality control, which may be acceptable for some tests, such as the VerifyNow, where the methodology is so simple as to render the chance of erroneous result extremely unlikely, whereas complex test procedures, such as whole-blood aggregometry and LTA, have a much higher potential for analytical error (110,111). There is no ideal external quality assurance, although modified forms for PFTs have been proposed, and uptake should be encouraged (110,112).

Future Directions and Clinical Considerations

PFTs have the most to offer in detecting those who are at highest risk of future cardiovascular events. Importantly, PFT results will never be the sole determinant, but 1 of multiple contributors to cardiovascular risk. Many studies using PFTs have evaluated these tests in stable cardiovascular disease, where event rates are low. In order to assess the predictive benefit of these tests and to assess whether individualization of antiplatelet therapy based on the results of PFT will alter clinical outcome, large randomized clinical trials should be performed in the highest-risk groups (such as ACS patients with high GRACE [Global Registry of Acute Coronary Events] risk scores) so that these are adequately powered to detect outcomes. Because of the low incidence of MACE and bleeding complications following PCI, assessment of the utility of PFTs requires evaluation in very large and costly clinical trials, to which there are numerous barriers. Funding such trials is enormously expensive. Drug manufacturers may have little interest in detecting persistent on-treatment platelet hyperreactivity. Device manufacturers, however, are unlikely to be able to fund trials of sufficient magnitude. Furthermore, choice of PFT(s), timing of testing, and the ever-expanding armamentarium of antithrombotic medications coming online are going to make the ideal trial nearly impossible to perform.

The optimal timing of PFT is important, especially in ACS. Baseline assessment is often not possible before initiation of treatment, because many, especially those with ST-segment elevation myocardial infarction, will have received DAPT and often a thrombin inhibitor by the time of presentation. Despite testing just before hospital discharge, administration of earlier anticoagulants may still distort PFT results. Assessing patients at 6 weeks follow-up to guide therapy, after the acute inflammatory response has settled, has the obvious limitation that the first 4 to 6 weeks after an ACS is actually the highest thrombotic risk period. Whether a single result or whether serial testing is required for ongoing therapy is also unclear.

It also unclear how PFT results might influence prescribing. In the acute setting, dosages can certainly be increased, which, with many agents, can improve PFT results, but there are also many newer agents that reduce MACE in high-risk groups, such as ticagrelor and prasugrel, irrespective of PFT. In aspirin-resistant patients identified by LTA, ticagrelor achieved greater inhibition of platelet aggregation than clopidogrel (113). Prasugrel is more effective than clopidogrel in reducing platelet reactivity in healthy subjects, as measured with aggregometry (114); in patients with HRPR on clopidogrel assessed with VerifyNow (74,115); and in stable CAD patients as measured by LTA; and prasugrel lowers the proportion of pharmacodynamic non-responders compared with clopidogrel (116). Ticagrelor produces a significantly higher platelet inhibition compared with prasugrel, as measured with VerifyNow (93). However, the clinical utility of switching drugs on the basis of PFT results in reducing adverse events could not be demonstrated in stable CAD patients, perhaps due to the low event rate after PCI with contemporary DES in this setting (115). Whether it is clinically more (cost) effective to limit prasugrel and ticagrelor to patients with HRPR, or whether it is more efficacious to administer these drugs for all licensed indications without performing PFT, remains to be established.

Novel agents to prevent coronary thrombosis, targeting thrombin-mediated pathways, are forthcoming. These include direct Xa inhibitors (apixaban, rivaroxaban, and dar-

JACC Vol. 61, No. 21, 2013
May 28, 2013:2115–29

exaban), direct thrombin inhibitors (dabigatran), and PAR-1 antagonists (vorapaxar and atopaxar). Although substantial review of these agents is beyond the scope of this review, rivaroxaban appears to show the most promise in ACS, reducing MACE without unacceptable increase in bleeding (117). Atopaxar, evaluated only in a relatively small trial, did not increase bleeding and shows a possible signal for ischemic event reduction (118). Vorapaxar inhibits thrombin receptor activating peptide (TRAP)-mediated platelet aggregation as measured with LTA, in a dose- and time-dependent manner (119). The effect of thrombin on platelet aggregation may be measured with thromboelastography by assessing the rapidity of fibrin–platelet clot formation, a marker of thrombin activity, and clot strength, reflecting thrombin-induced fibrin–platelet interaction (120,121). The role of PFT in assessing response to these agents and guiding therapy remains to be established, but results may be difficult to interpret due to the relative contribution of thrombin generation by activated platelets to the test results.

Conclusions

Despite having been in use for more than a decade, PFTs have failed to realize the hopes they generated for clinical practice. Identification of those at risk, who most require antiplatelet medication, is still an urgent task. Unfortunately, PFTs in current clinical use have very limited ability to detect platelet hyperreactivity, a prothrombotic condition in healthy subjects. Identification of antiplatelet nonresponsiveness is highly test specific, and does not allow individualization of antiplatelet therapy or identification of noncompliance. The usefulness of PFTs in predicting MACE after PCI is variable at best and often modest. Whether PFTs have the ability to predict adverse cardiac events is contentious. Although HRPR has been shown by some studies to be associated with an increased risk of adverse cardiac events, many studies show that PFTs, especially point-of-care tests, cannot reliably discriminate between patients with and without a primary endpoint during follow-up. PFTs may be useful at a population level, but not for the individual. Large clinical trials have not been performed that demonstrate that altering treatment, on the basis of PFT results, leads to an improvement in clinical outcomes. None of the PFTs currently meet the criteria for screening as a novel biomarker according to American Heart Association criteria. Because the increased effectiveness of novel antiplatelet drugs comes at a price of greater bleeding, identification of such risk would be of paramount importance. However, PFTs provide very limited prognostic information on bleeding, and their value in this setting is not established. Although finding a physiologically relevant and clinically applicable PFT is more important than ever, a critical reappraisal of present techniques in light of clinical requirements is needed. Radical changes—such as the use of native, instead of anticoagulated, blood; global stimulus, instead of several different agonists; involvement of platelet-dependent thrombin generation in the test result; and identifying a "safety window" for each antiplatelet drug, with discriminatory and reclassification value of PFT established in large-scale clinical trials—would be highly desirable.

Reprint requests and correspondence: Prof. Diana A. Gorog, Cardiology Department, E&N Herts NHS Trust, AL7 4HQ London, United Kingdom. E-mail: d.gorog@imperial.ac.uk.

REFERENCES

1. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in vitro platelet function analyzer—PFA-100. Semin Thromb Hemost 1995;21 Suppl 2:106–12.
2. Smith JW, Steinhubl SR, Lincoff AM, et al. Rapid platelet-function assay: an automated and quantitative cartridge-based method. Circulation 1999;99:620–5.
3. Matzdorff A. Platelet function tests and flow cytometry to monitor antiplatelet therapy. Semin Thromb Hemost 2005;31:393–9.
4. Trip MD, Cats VM, van Capelle FJ, Vreeken J. Platelet hyperreactivity and prognosis in survivors of myocardial infarction. N Engl J Med 1990;322:1549–54.
5. Breddin HK, Lippold R, Bittner M, Kirchmaier CM, Krzywanek HJ, Michaelis J. Spontaneous platelet aggregation as a predictive risk factor for vascular occlusions in healthy volunteers? Results of the HAPARG study. Haemostatic parameters as risk factors in healthy volunteers. Atherosclerosis 1999;144:211–9.
6. Eto K, Takeshita S, Ochiai M, Ozaki Y, Sato T, Isshiki T. Platelet aggregation in acute coronary syndromes: use of a new aggregometer with laser light scattering to assess platelet aggregability. Cardiovasc Res 1998;40:223–9.
7. Yee DL, Bergeron AL, Sun CW, Dong JF, Bray PF. Platelet hyperreactivity generalizes to multiple forms of stimulation. J Thromb Haemost 2006;4:2043–50.
8. Yee DL, Sun CW, Bergeron AL, Dong JF, Bray PF. Aggregometry detects platelet hyperreactivity in healthy individuals. Blood 2005;106:2723–9.
9. Gurbel PA, Bliden KP, DiChiara J, et al. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. Circulation 2007;115:3156–64.
10. Perneby C, Wallen NH, Rooney C, Fitzgerald D, Hjemdahl P. Dose- and time-dependent antiplatelet effects of aspirin. Thromb Haemost 2006;95:652–8.
11. Lordkipanidze M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur Heart J 2007;28:1702–8.
12. Kidson-Gerber G, Weaver J, Gemmell R, Prasan AM, Chong BH. Serum thromboxane B2 compared to five other platelet function tests for the evaluation of aspirin effect in stable cardiovascular disease. Heart Lung Circ 2010;19:234–42.
13. Grove EL, Hvas AM, Johnsen HL, et al. A comparison of platelet function tests and thromboxane metabolites to evaluate aspirin response in healthy individuals and patients with coronary artery disease. Thromb Haemost 2010;103:1245–53.
14. Frelinger AL, Li Y, Linden MD, et al. Aspirin 'resistance': role of pre-existent platelet reactivity and correlation between tests. J Thromb Haemost 2008;6:2035–44.
15. Harrison P. The influence of citrate concentrations on PFA-100 closure times, platelet hyper-reactivity and aspirin monitoring. Thromb Res 2010;126:e137–8.
16. Mehta JL, Mohandas B. Aspirin resistance: fact or fiction? A point of view. World J Cardiol 2010;2:280–8.
17. Gorog DA, Sweeny JM, Fuster V. Antiplatelet drug 'resistance'. Part 2: laboratory resistance to antiplatelet drugs—fact or artifact? Nat Rev Cardiol 2009;6:365–73.
18. Gori AM, Marcucci R, Migliorini A, et al. Incidence and clinical impact of dual nonresponsiveness to aspirin and clopidogrel in patients with drug-eluting stents. J Am Coll Cardiol 2008;52:734–9.

19. Saraf S, Christopoulos C, Salha IB, Stott DJ, Gorog DA. Impaired endogenous thrombolysis in acute coronary syndrome patients predicts cardiovascular death and nonfatal myocardial infarction. J Am Coll Cardiol 2010;55:2107–15.
20. Lordkipanidze M, Diodati JG, Schampaert E, Palisaitis DA, Pharand C. Prevalence of unresponsiveness to aspirin and/or clopidogrel in patients with stable coronary heart disease. Am J Cardiol 2009; 104:1189–93.
21. Cuisset T, Frere C, Poyet R, et al. Clopidogrel response: head-to-head comparison of different platelet assays to identify clopidogrel non responder patients after coronary stenting. Arch Cardiovasc Dis 2010;103:39–45.
22. Varenhorst C, James S, Erlinge D, et al. Assessment of P2Y(12) inhibition with the point-of-care device VerifyNow P2Y12 in patients treated with prasugrel or clopidogrel coadministered with aspirin. Am Heart J 2009;157:562 e1–9.
23. Bouman HJ, Parlak E, van Werkum JW, et al. Which platelet function test is suitable to monitor clopidogrel responsiveness? A pharmacokinetic analysis on the active metabolite of clopidogrel. J Thromb Haemost 2010;8:482–8.
24. Bal Dit Sollier C, Berge N, Boval B, Dubar M, Drouet L. Differential sensitivity and kinetics of response of different ex vivo tests monitoring functional variability of platelet response to clopidogrel. Thromb Haemost 2010;104:571–81.
25. van Werkum JW, Bouman HJ, Breet NJ, ten Berg JM, Hackeng CM. The Cone-and-Plate(let) analyzer is not suitable to monitor clopidogrel therapy: a comparison with the flowcytometric VASP assay and optical aggregometry. Thromb Res 2010;126:44–9.
26. Lordkipanidze M, Pharand C, Nguyen TA, Schampaert E, Palisaitis DA, Diodati JG. Comparison of four tests to assess inhibition of platelet function by clopidogrel in stable coronary artery disease patients. Eur Heart J 2008;29:2877–85.
27. Bonello L, Tantry US, Marcucci R, et al. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. J Am Coll Cardiol 2010;56:919–33.
28. Gaglia MA, Torguson R, Pakala R, et al. Correlation between light transmission aggregometry, VerifyNow P2Y12, and VASP-P platelet reactivity assays following percutaneous coronary intervention. J Interv Cardiol 2011;24:529–34.
29. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 2001;345:494–502.
30. Chen ZM, Jiang LX, Chen YP, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. Lancet 2005;366:1607–21.
31. Aleil B, Jacquemin L, De Poli F, et al. Clopidogrel 150 mg/day to overcome low responsiveness in patients undergoing elective percutaneous coronary intervention: results from the VASP-02 (Vasodilator-Stimulated Phosphoprotein-02) randomized study. J Am Coll Cardiol Intv 2008;1:631–8.
32. Price MJ, Berger PB, Teirstein PS, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA 2011;305:1097–105.
33. Mehta SR, Bassand JP, Chrolavicius S, et al. Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. N Engl J Med 2010;363:930–42.
34. Bonello L, Camoin-Jau L, Arques S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. J Am Coll Cardiol 2008;51:1404–11.
35. Bonello L, Camoin-Jau L, Armero S, et al. Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. Am J Cardiol 2009;103:5–10.
36. Valgimigli M, Campo G, de Cesare N, et al. Intensifying platelet inhibition with tirofiban in poor responders to aspirin, clopidogrel, or both agents undergoing elective coronary intervention: results from the double-blind, prospective, randomized Tailoring Treatment with Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel study. Circulation 2009;119:3215–22.
37. Lischke S, Schneider DJ. Recent developments in the use of antiplatelet agents to prevent cardiovascular events. Future Cardiol 2011;7:403–13.
38. Judge HM, Buckland RJ, Sugidachi A, Jakubowski JA, Storey RF. Relationship between degree of $P2Y_{12}$ receptor blockade and inhibition of $P2Y_{12}$-mediated platelet function. Thromb Haemost 2010; 103:1210–7.
39. Brar SS, ten Berg J, Marcucci R, et al. Impact of platelet reactivity on clinical outcomes after percutaneous coronary intervention. A collaborative meta-analysis of individual participant data. J Am Coll Cardiol 2011;58:1945–54.
40. Campo G, Valgimigli M, Gemmati D, et al. Value of platelet reactivity in predicting response to treatment and clinical outcome in patients undergoing primary coronary intervention: insights into the STRATEGY Study. J Am Coll Cardiol 2006;48:2178–85.
41. Parodi G, Marcucci R, Valenti R, et al. High residual platelet reactivity after clopidogrel loading and long-term cardiovascular events among patients with acute coronary syndromes undergoing PCI. JAMA 2011;306:1215–23.
42. Atiemo AD, Ng'Alla LS, Vaidya D, Williams MS. Abnormal PFA-100 closure time is associated with increased platelet aggregation in patients presenting with chest pain. J Thromb Thrombolysis 2008;25:173–8.
43. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA 2010;303:754–62.
44. Modica A, Karlsson F, Mooe T. The impact of platelet function or C-reactive protein, on cardiovascular events after an acute myocardial infarction. Thromb J 2009;7:12.
45. Gianetti J, Parri MS, Sbrana S, et al. Platelet activation predicts recurrent ischemic events after percutaneous coronary angioplasty: a 6 months prospective study. Thromb Res 2006;118:487–93.
46. Hobikoglu GF, Norgaz T, Aksu H, et al. The effect of acetylsalicylic acid resistance on prognosis of patients who have developed acute coronary syndrome during acetylsalicylic acid therapy. Can J Cardiol 2007;23:201–6.
47. Mueller T, Dieplinger B, Poelz W, Haltmayer M. Utility of the PFA-100 instrument and the novel multiplate analyzer for the assessment of aspirin and clopidogrel effects on platelet function in patients with cardiovascular disease. Clin Appl Thromb Hemost 2009;15:652–9.
48. Bonello L, Paganelli F, Arpin-Bornet M, et al. Vasodilator-stimulated phosphoprotein phosphorylation analysis prior to percutaneous coronary intervention for exclusion of postprocedural major adverse cardiovascular events. J Thromb Haemost 2007;5:1630–6.
49. Varenhorst C, Koul S, Erlinge D, et al. Relationship between clopidogrel-induced platelet $P2Y_{12}$ inhibition and stent thrombosis or myocardial infarction after percutaneous coronary intervention: a case-control study. Am Heart J 2011;162:363–71.
50. Frere C, Cuisset T, Quilici J, et al. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. Thromb Haemost 2007;98:838–43.
51. Bonello L, Pansieri M, Mancini J, et al. High on-treatment platelet reactivity after prasugrel loading dose and cardiovascular events after percutaneous coronary intervention in acute coronary syndromes. J Am Coll Cardiol 2011;58:467–73.
52. Bonello L, Mancini J, Pansieri M, et al. Relationship between post-treatment platelet reactivity and ischemic and bleeding events at 1-year follow-up in patients receiving prasugrel. J Thromb Haemost 2012;10:1999–2005.
53. Pinto Slottow TL, Bonello L, Gavini R, et al. Prevalence of aspirin and clopidogrel resistance among patients with and without drug-eluting stent thrombosis. Am J Cardiol 2009;104:525–30.
54. Ko YG, Suh JW, Kim BH, et al. Comparison of 2 point-of-care platelet function tests, VerifyNow Assay and Multiple Electrode Platelet Aggregometry, for predicting early clinical outcomes in patients undergoing percutaneous coronary intervention. Am Heart J 2011;161:383–90.
55. Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. Circulation 2009;119:237–42.

56. Park DW, Lee SW, Yun SC, et al. A point-of-care platelet function assay and C-reactive protein for prediction of major cardiovascular events after drug-eluting stent implantation. J Am Coll Cardiol 2011;58:2630–9.
57. Reny JL, Berdague P, Poncet A, et al. Antiplatelet drug response status does not predict recurrent ischemic events in stable cardiovascular patients: results of the Antiplatelet Drug Resistances and Ischemic Events study. Circulation 2012;125:3201–10.
58. Ahn SG, Lee SH, Yoon JH, et al. Different prognostic significance of high on-treatment platelet reactivity as assessed by the VerifyNow P2Y12 assay after coronary stenting in patients with and without acute myocardial infarction. J Am Coll Cardiol Intv 2012;5:259–67.
59. Yu LH, Kim MH, Zhang HZ, et al. Impact of platelet function test on platelet responsiveness and clinical outcome after coronary stent implantation: platelet responsiveness and clinical outcome. Korean Circ J 2012;42:382–9.
60. Paulu P, Osmancik P, Tousek P, et al. Lack of association between clopidogrel responsiveness tested using point-of-care assay and prognosis of patients with coronary artery disease. J Thromb Thrombolysis 2012 Oct 6 [E-pub ahead of print].
61. Price MJ, Angiolillo DJ, Teirstein PS, et al. Platelet reactivity and cardiovascular outcomes after percutaneous coronary intervention: a time-dependent analysis of the Gauging Responsiveness with a VerifyNow P2Y12 assay: Impact on Thrombosis and Safety (GRAVITAS) trial. Circulation 2011;124:1132–7.
62. Stone G. ADAPT-DES: a large-scale, prospective, multicenter registry examining the relationship of platelet responsiveness and stent thrombosis after DES implantation. Paper presented at: TCT 2011: Transcatheter Cardiovascular Therapeutics 23rd Annual Scientific Symposium; October 27, 2011; San Francisco, CA.
63. Greenberg G, Assali A, Vaknin-Assa H, et al. Hematocrit level as a marker of outcome in ST-segment elevation myocardial infarction. Am J Cardiol 2010;105:435–40.
64. Goncalves SC, Labinaz M, Le May M, et al. Usefulness of mean platelet volume as a biomarker for long-term outcomes after percutaneous coronary intervention. Am J Cardiol 2011;107:204–9.
65. Turner SJ, Ketch TR, Gandhi SK, Sane DC. Routine hematologic clinical tests as prognostic markers in patients with acute coronary syndromes. Am Heart J 2008;155:806–16.
66. Lee SP, Park KW, Shin DH, et al. Efficacy of predicting thrombotic events with combination of dual point-of-care testing (POCT) after drug-eluting stent implantation for coronary heart disease: results from the CILON-T randomized trial POCT substudy. J Atheroscler Thromb 2011;18:914–23.
67. Marcucci R, Gori AM, Paniccia R, et al. High on-treatment platelet reactivity by more than one agonist predicts 12-month follow-up cardiovascular death and non-fatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting. Thromb Haemost 2010;104:279–86.
68. Mangiacapra F, Patti G, Barbato E, et al. A therapeutic window for platelet reactivity for patients undergoing elective percutaneous coronary intervention: results of the ARMYDA-PROVE (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity for Outcome Validation Effort) study. J Am Coll Cardiol Intv 2012;5:281–9.
69. Kirtane AJ, Parise H, Witzenbichler B, et al. Does platelet function testing add significant incremental risk stratification to unselected patients undergoing DES implantation? The ADAPT-DES study. [Abstract]. J Am Coll Cardiol 2012;59 Suppl 1:E292.
70. Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med 2009;360:354–62.
71. Cuisset T, Quilici J, Cohen W, et al. Usefulness of high clopidogrel maintenance dose according to CYP2C19 genotypes in clopidogrel low responders undergoing coronary stenting for non ST elevation acute coronary syndrome. Am J Cardiol 2011;108:760–5.
72. Mega JL, Hochholzer W, Frelinger AL 3rd, et al. Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. JAMA 2011;306:2221–8.
73. Bonello L, Armero S, Ait Mokhtar O, et al. Clopidogrel loading dose adjustment according to platelet reactivity monitoring in patients carrying the 2C19*2 loss of function polymorphism. J Am Coll Cardiol 2010;56:1630–6.
74. Alexopoulos D, Dimitropoulos G, Davlouros P, et al. Prasugrel overcomes high on-clopidogrel platelet reactivity post-stenting more effectively than high-dose (150-mg) clopidogrel: the importance of CYP2C19*2 genotyping. J Am Coll Cardiol Intv 2011;4:403–10.
75. Price MJ, Carson AR, Murray SS, et al. First pharmacogenomic analysis using whole exome sequencing to identify novel genetic determinants of clopidogrel response variability: results of the Genotype Information and Functional Testing (Gift) exome study. J Am Coll Cardiol 2012;59 Suppl:E9.
76. Bouman HJ, Schomig E, van Werkum JW, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. Nat Med 2011;17:110–6.
77. Hulot JS, Collet JP, Cayla G, et al. CYP2C19 but not PON1 genetic variants influence clopidogrel pharmacokinetics, pharmacodynamics, and clinical efficacy in post-myocardial infarction patients. Circ Cardiovasc Interv 2011;4:422–8.
78. Lewis JP, Fisch AS, Ryan K, et al. Paraoxonase 1 (PON1) gene variants are not associated with clopidogrel response. Clin Pharmacol Ther 2011;90:568–74.
79. Sibbing D, Koch W, Massberg S, et al. No association of paraoxonase-1 Q192R genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting. Eur Heart J 2011;32:1605–13.
80. Snoep JD. Unraveling the pharmacogenetics of clopidogrel: the paraoxonase-1 controversy. Circ Cardiovasc Interv 2011;4:401–4.
81. Mega JL, Simon T, Collet JP, et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. JAMA 2010;304:1821–30.
82. Hulot JS, Collet JP, Silvain J, et al. Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. J Am Coll Cardiol 2010;56:134–43.
83. Holmes MV, Perel P, Shah T, Hingorani AD, Casas JP. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. JAMA 2011;306:2704–14.
84. Bauer T, Bouman HJ, van Werkum JW, Ford NF, ten Berg JM, Taubert D. Impact of CYP2C19 variant genotypes on clinical efficacy of antiplatelet treatment with clopidogrel: systematic review and meta-analysis. BMJ 2011;343:d4588.
85. Tsai TT, Ho PM, Xu S, et al. Increased risk of bleeding in patients on clopidogrel therapy after drug-eluting stents implantation: insights from the HMO Research Network-Stent Registry (HMORN-stent). Circ Cardiovasc Interv 2010;3:230–5.
86. Geeganage C, Wilcox R, Bath PM. Triple antiplatelet therapy for preventing vascular events: a systematic review and meta-analysis. BMC Med 2010;8:36.
87. Tsukahara K, Kimura K, Morita S, et al. Impact of high-responsiveness to dual antiplatelet therapy on bleeding complications in patients receiving drug-eluting stents. Circ J 2010;74:679–85.
88. Sibbing D, Schulz S, Braun S, et al. Antiplatelet effects of clopidogrel and bleeding in patients undergoing coronary stent placement. J Thromb Haemost 2010;8:250–6.
89. Patti G, Pasceri V, Vizzi V, Ricottini E, Di Sciascio G. Usefulness of platelet response to clopidogrel by point-of-care testing to predict bleeding outcomes in patients undergoing percutaneous coronary intervention (from the Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty-Bleeding Study). Am J Cardiol 2011;107:995–1000.
90. Campo G, Parrinello G, Ferraresi P, et al. Prospective evaluation of on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention relationship with gene polymorphisms and clinical outcome. J Am Coll Cardiol 2011;57:2474–83.
91. Cuisset T, Cayla G, Frere C, et al. Predictive value of post-treatment platelet reactivity for occurrence of post-discharge bleeding after non-ST elevation acute coronary syndrome. Shifting from antiplatelet resistance to bleeding risk assessment? EuroIntervention 2009;5:325–9.
92. Cuisset T, Quilici J, Loosveld M, et al. Comparison between initial and chronic response to clopidogrel therapy after coronary stenting for acute coronary syndrome and influence on clinical outcomes. Am Heart J 2012;164:327–33.
93. Alexopoulos D, Galati A, Xanthopoulou I, et al. Ticagrelor versus prasugrel in acute coronary syndrome patients with high on-

clopidogrel platelet reactivity following percutaneous coronary intervention: a pharmacodynamic study. J Am Coll Cardiol 2012;60:193–9.
94. Sibbing D, Steinhubl SR, Schulz S, Schomig A, Kastrati A. Platelet aggregation and its association with stent thrombosis and bleeding in clopidogrel-treated patients: initial evidence of a therapeutic window. J Am Coll Cardiol 2010;56:317–8.
95. Altman R, Rivas AJ, Gonzalez CD. Bleeding tendency in dual antiplatelet therapy with aspirin/clopidogrel: rescue of the template bleeding time in a single-center prospective study. Thromb J 2012; 10:3.
96. Monroe DM, Hoffman M, Roberts HR. Platelets and thrombin generation. Arterioscler Thromb Vasc Biol 2002;22:1381–9.
97. Ferreiro JL, Sibbing D, Angiolillo DJ. Platelet function testing and risk of bleeding complications. Thromb Haemost 2010;103: 1128–35.
98. Nazarian SM, Thompson JB, Gluckman TJ, et al. Clinical and laboratory factors associated with shear-dependent platelet hyperreactivity in patients on chronic aspirin therapy. Thromb Res 2010; 126:379–83.
99. Maxwell MJ, Westein E, Nesbitt WS, Giuliano S, Dopheide SM, Jackson SP. Identification of a 2-stage platelet aggregation process mediating shear-dependent thrombus formation. Blood 2007;109: 566–76.
100. Bark DL Jr., Ku DN. Wall shear over high degree stenoses pertinent to atherothrombosis. J Biomech 2010;43:2970–7.
101. Nesbitt WS, Westein E, Tovar-Lopez FJ, et al. A shear gradient-dependent platelet aggregation mechanism drives thrombus formation. Nat Med 2009;15:665–73.
102. Ruggeri ZM, Orje JN, Habermann R, Federici AB, Reininger AJ. Activation-independent platelet adhesion and aggregation under elevated shear stress. Blood 2006;108:1903–10.
103. Reininger AJ, Heijnen HF, Schumann H, Specht HM, Schramm W, Ruggeri ZM. Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. Blood 2006;107:3537–45.
104. Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. Blood 2007;109:2285–92.
105. van der Meijden PE, Feijge MA, Giesen PL, Huijberts M, van Raak LP, Heemskerk JW. Platelet $P2Y_{12}$ receptors enhance signalling towards procoagulant activity and thrombin generation. A study with healthy subjects and patients at thrombotic risk. Thromb Haemost 2005;93:1128–36.
106. Kalb ML, Potura L, Scharbert G, Kozek-Langenecker SA. The effect of ex vivo anticoagulants on whole blood platelet aggregation. Platelets 2009;20:7–11.
107. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–72; discussion 207–12.
108. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928–35.
109. Hlatky MA, Greenland P, Arnett DK, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation 2009;119:2408–16.
110. Favaloro EJ. Internal quality control and external quality assurance of platelet function tests. Semin Thromb Hemost 2009;35:139–49.
111. Cattaneo M, Hayward CP, Moffat KA, Pugliano MT, Liu Y, Michelson AD. Results of a worldwide survey on the assessment of platelet function by light transmission aggregometry: a report from the platelet physiology subcommittee of the SSC of the ISTH. J Thromb Haemost 2009;7:1029.
112. Favaloro EJ, Bonar R. External quality assurance for the PFA-100(R). J Thromb Haemost 2011;9:878–80.
113. Gurbel PA, Bliden KP, Butler K, et al. Response to ticagrelor in clopidogrel nonresponders and responders and effect of switching therapies: the RESPOND study. Circulation 2010;121:1188–99.
114. Brandt JT, Payne CD, Wiviott SD, et al. A comparison of prasugrel and clopidogrel loading doses on platelet function: magnitude of platelet inhibition is related to active metabolite formation. Am Heart J 2007;153:66 e9–16.
115. Trenk D, Stone GW, Gawaz M, et al. A randomized trial of prasugrel versus clopidogrel in patients with high platelet reactivity on clopidogrel after elective percutaneous coronary intervention with implantation of drug-eluting stents: results of the TRIGGER-PCI (Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel) study. J Am Coll Cardiol 2012;59:2159–64.
116. Jernberg T, Payne CD, Winters KJ, et al. Prasugrel achieves greater inhibition of platelet aggregation and a lower rate of non-responders compared with clopidogrel in aspirin-treated patients with stable coronary artery disease. Eur Heart J 2006;27:1166–73.
117. Mega JL, Braunwald E, Wiviott SD, et al. Rivaroxaban in patients with a recent acute coronary syndrome. N Engl J Med 2012;366: 9–19.
118. O'Donoghue ML, Bhatt DL, Wiviott SD, et al. Safety and tolerability of atopaxar in the treatment of patients with acute coronary syndromes: the lessons from antagonizing the cellular effects of Thrombin-Acute Coronary Syndromes trial. Circulation 2011;123: 1843–53.
119. Becker RC, Moliterno DJ, Jennings LK, et al. Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: a randomised, double-blind, placebo-controlled phase II study. Lancet 2009;373:919–28.
120. Gurbel PA, Bliden KP, Guyer K, Aggarwal N, Tantry US. Delayed thrombin-induced platelet-fibrin clot generation by clopidogrel: a new dose-related effect demonstrated by thrombelastography in patients undergoing coronary artery stenting. Thromb Res 2007;119: 563–70.
121. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING study. J Am Coll Cardiol 2005;46:1820–6.

Key Words: aggregation ■ PCI ■ platelets ■ thrombosis.