

VIEWPOINT

Platelet Function Monitoring in Patients With Coronary Artery Disease

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Studies focused on patient responsiveness to antiplatelet therapies, particularly aspirin and clopidogrel, have increased in recent years. However, the relations of in vivo platelet function and adverse clinical events to results of ex vivo platelet function tests remain largely unknown. This article describes current methods of measuring platelet function in various clinical and research situations and their advantages and disadvantages, reviews evidence for antiplatelet response variability and resistance, discusses the potential pitfalls of monitoring platelet function, and demonstrates emerging data supporting the positive clinical and treatment implications of platelet function testing. (J Am Coll Cardiol 2007;50:1822-34) © 2007 by the American College of Cardiology Foundation

The rationale for a review article on platelet function monitoring in patients with coronary artery disease is the current intense controversy surrounding the clinical importance of non-responsiveness to antiplatelet therapy (1-5). Response variability and resistance to clopidogrel therapy were first reported in 2003 (6). Since then the number of studies focused on patient responsiveness to antiplatelet therapies has increased substantially and now is the subject of entire sessions at peer-reviewed international cardiovascular meetings (7-28). Recent studies have suggested a relation between high post-treatment platelet reactivity and clopidogrel nonresponsiveness in patients undergoing percutaneous revascularization and increased ischemic events including stent thrombosis (12,13,17-28). Variable respon-

siveness to clopidogrel has served as a rationale for the development of new P2Y₁₂ inhibitors with superior pharmacodynamic profiles (29). Similarly, nonresponsiveness to aspirin treatment and its relation to the occurrence of adverse clinical events are also subjects of considerable controversy in recent years (2-5,30-35). However, the determination of in vivo platelet function through ex vivo testing has significant limitations (36).

This paper discusses the differences among methods used to measure platelet function and activation, summarizes the evidence for antiplatelet response variability, and examines research studies reporting higher event rates in patients with nonresponsiveness to antiplatelet therapy by ex vivo testing.

Current Clinical Tests of Platelet Function

The answer to “which is the right test of platelet function?” can vary according to the purpose of testing. The first tests, developed and used to screen patients for bleeding disorders, have since been used to diagnose specific conditions, from the rare (Glanzmann thrombasthenia) to the common (storage-pool disease). More recently, platelet function measurements have been used to assess the effects of pro-hemostatic and antiplatelet therapies in clinical and research settings. Finally, monitoring of platelet function can be useful in the practice of transfusion medicine. In each of these situations, a different platelet function test might have particular applicability.

For patients with coronary artery disease (CAD), the potential utility of measuring platelet function includes monitoring antiplatelet therapy and predicting clinical outcomes. For example, although aspirin reduces the risk of

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thrombotic events in high-risk patients by approximately 25%, 10% to 20% of treated patients will have another thrombotic event during long-term follow-up (37). These patients might require additional platelet-directed therapy with clopidogrel or other agents. A key question relating to this issue is whether standardized laboratory tests assessing the platelet response to aspirin or clopidogrel can predict clinical “resistance.”

Methods of Measuring Platelet Function

Aggregation. Table 1 and Figures 1 and 2 summarize the principles and characteristics of the most commonly used platelet-function tests. The historical “gold standard” test is light transmittance aggregometry (turbidimetric) (LTA), which is based on the stimulation of platelet-platelet aggregation in platelet-rich plasma after stimulation with various agonists. Figure 3 shows the intersubject variability in platelet aggregation stimulated by commonly employed agonists (38). Light transmittance aggregometry has been the most widely used technique to monitor the effects of antiplatelet agents, including aspirin, clopidogrel, other P2Y₁₂ inhibitors, and platelet glycoprotein (GP) IIb/IIIa inhibitors (36). Potential disadvantages include the requirement for immediate processing, variable reproducibility, large required sample volumes, lengthy processing time, and expenses of the aggregometer and trained operators. Light transmittance aggregometry has also been the most widely investigated method to predict clinical outcomes (33). Impedance aggregometry is conceptually similar to LTA, but it uses whole blood instead of platelet-rich plasma and aggregation is measured by impedance, not light transmittance (39).

Receptor expression. The resting and stimulated expression of activation-dependent receptors can be quantified by flow cytometry with monoclonal antibodies (Fig. 1) (36). This technique has been particularly useful to assess pharmacologic effects. The most widely studied receptors include P-selectin and GP IIb/IIIa. Platelet-leukocyte aggregates also have been measured as a marker of platelet activation, and they have been proposed as a more stable measure of acute coronary syndromes (ACS) compared with P-selectin (40). Less information is available correlating measurement of receptor expression and clinical outcomes. Major limitations are the complexity of the technique, which requires an experienced laboratory staff, and the resultant high costs.

Intracellular signaling. The coupling of P2Y₁₂ to the inhibition of adenylate cyclase by an inhibitory G protein has been exploited to measure reactivity of the receptor in the presence of P2Y₁₂ inhibitors (Fig. 2) (41). Vasodilator-stimulated phosphoprotein (VASP) is phosphorylated by protein kinases that are activated by cyclic adenosine monophosphate. With flow cytometry and methods to make the platelet membrane permeable, it is possible to quantify the amount of phosphorylated VASP by monoclonal antibodies as a measure of unblocked P2Y₁₂ (41). Advantages include

the specificity for the P2Y₁₂ signaling pathway and the stability of the method compared with aggregometry.

Point-of-care assays. The VerifyNow method (Accumetrics, San Diego, California) uses arachidonic acid, adenosine diphosphate (ADP), or thrombin receptor-activating peptide (TRAP) to assess platelet responsiveness to aspirin, P2Y₁₂ inhibitors, or GP IIb/IIIa inhibitors, respectively (42). The technique measures platelet aggregation with fibrinogen-coated beads and has been used to predict outcomes in patients undergoing percutaneous coronary intervention (PCI). Advantages of the VerifyNow include its ease of use and correlation with turbidimetric aggregometry. In the thrombelastogram (TEG) PlateletMapping technique (Haemoscope Corporation, Niles, Illinois), the contribution of arachidonic acid-induced platelet aggregation and ADP-induced aggregation to the overall tensile strength of a platelet-fibrin clot can be quantified and correlated with turbidimetric aggregometry (23). The preparation of samples for thrombelastography is more complex than that for VerifyNow, but thrombelastography can provide coagulation measurements not possible with VerifyNow.

Platelet-released factors. The most widely measured factors are shown in Table 1 (36). Serum thromboxane B₂ and urinary 11-dehydro-thromboxane B₂ have been used to assess responsiveness to aspirin and to predict outcomes in aspirin-treated patients. Elevated soluble CD40 ligand and P-selectin have been observed in ACS, and at least 1 study evaluated the diagnostic utility of soluble P-selectin as a marker of myocardial infarction (MI) (43). All of the soluble markers are measured by immunoassays; limitations in their utility include their presence in extraplatelet sources.

Antiplatelet Response Variability or “Resistance”

Definition. Multiple signaling pathways mediate platelet activation and the occurrence of thrombotic events. Thus a treatment strategy directed against a single pathway cannot be expected to prevent the occurrence of all events (29). Because thrombosis results from multiple signaling pathways, treatment failure alone is not sufficient evidence of drug “resistance.” The optimal definition of “resistance” or nonresponsiveness to an antiplatelet agent might be evidence of persistent activity of the specific target of the antiplatelet agent (29). Because the active metabolite of clopidogrel irreversibly inhibits the P2Y₁₂ receptor by forming a covalent disulfide bond, there would be evidence of

Abbreviations and Acronyms

ACS	= acute coronary syndromes
CAD	= coronary artery disease
COX	= cyclooxygenase
GP	= glycoprotein
INR	= international normalized ratio
LTA	= light transmittance aggregometry
MACE	= major adverse cardiac events
MI	= myocardial infarction
PCI	= percutaneous coronary intervention
VASP	= vasodilator-stimulated phosphoprotein

Table 1 Current Clinical Tests of Platelet Function

	Advantages	Drawbacks	Predicts Outcomes?	Able to Monitor		
				Aspirin	Thienopyridines	GP IIb/IIIa Inhibitors
Cessation of Blood Flow by Platelet Plug (for PFA, at High Shear)						
Bleeding time	In vivo; physiological	Nonspecific; not sensitive; scarring; high interoperator CV	No	No	No	No
PFA-100	Simple; rapid; small sample volume; no preparation; whole-blood assay	No instrument adjustment; depends on vWF, Hct	Yes	Yes	NR	NR
Platelet-Platelet Aggregation						
Platelet aggregometry: turbidimetric	Historical "gold standard"	Variable reproducibility; expensive; large sample volume; sample preparation; time-consuming	Yes	Yes*†	Yes	
Platelet aggregometry: impedance	Whole-blood assay	Expensive; large sample volume; sample preparation; time-consuming	Yes	Yes*	Yes†	Yes
VerifyNow	Simple; rapid; POC; small sample volume; no sample preparation; whole-blood assay	No instrument adjustment	Yes	Yes‡	Yes§	Yes
Plateletworks	Little sample preparation; whole-blood assay	Not well studied	No	Yes*	Yes†	Yes
Shear-Induced Platelet Adhesion						
Impact cone and plate(let) analyzer	Simple; rapid; POC; small sample volume; high shear; whole-blood assay	Not widely available	No	Yes*	Yes†	NR
Platelet Contribution to Clot Shear Elasticity						
Thrombelastogram	POC; whole-blood assay; platelet-fibrin clot formation and clot lysis data	Limited studies	Yes	Yes*	Yes†	Yes
Basis: Activation-Dependent Changes in Platelet Surface						
Platelet surface P-selectin, platelet surface activated GP IIb/IIIa, leukocyte-platelet aggregates	Low sample volume; whole-blood assay	Sample preparation; expensive; requires flow cytometer, experienced staff	Yes	Yes*	Yes†	Yes
Activation-Dependent Signaling						
VASP phosphorylation	Low sample volume; whole-blood assay; P2Y ₁₂ -dependent	Sample preparation; expensive; requires flow cytometer, experienced staff	Yes	No	Yes	No
Activation-Dependent Release From Platelets						
Platelet-derived microparticles	Low sample volume; whole-blood assay	Sample preparation; expensive; requires flow cytometer, experienced staff	No	No	No	No
Serum thromboxane B ₂	COX-1-dependent	Indirect; not platelet-specific	No	Yes	No	No
Urinary 11-dehydro thromboxane B ₂	COX-1-dependent	Indirect; not platelet-specific; depends on renal function	Yes	Yes	No	No
Plasma-soluble CD40 ligand	Most CD40 ligand is platelet-derived	Plasma separation can cause artifactual platelet activation	Yes	No	No	No
Plasma GP V	Platelet-specific	Plasma separation can cause artifactual platelet activation; reflects only thrombin-mediated platelet activation	No	No	No	No
α-granule constituents¶	Reflect platelet secretion	Plasma separation can cause artifactual platelet activation; endothelial cells also secrete P-selectin	No	No	No	No

Adapted from Michelson et al. (2). *With arachidonic acid. †With adenosine diphosphate. ‡With aspirin cartridge. §With P2Y₁₂ cartridge. ||With thrombin receptor-activating peptide (TRAP) cartridge. ¶Platelet factor 4, β-thromboglobulin, soluble P-selectin.
COX = cyclooxygenase; CV = coefficient of variation; GP = glycoprotein; Hct = hematocrit; NR = not recommended; PFA = Platelet Function Analyzer; POC = point of care; VASP = vasodilator-stimulated phosphoprotein; vWF = von Willebrand factor.

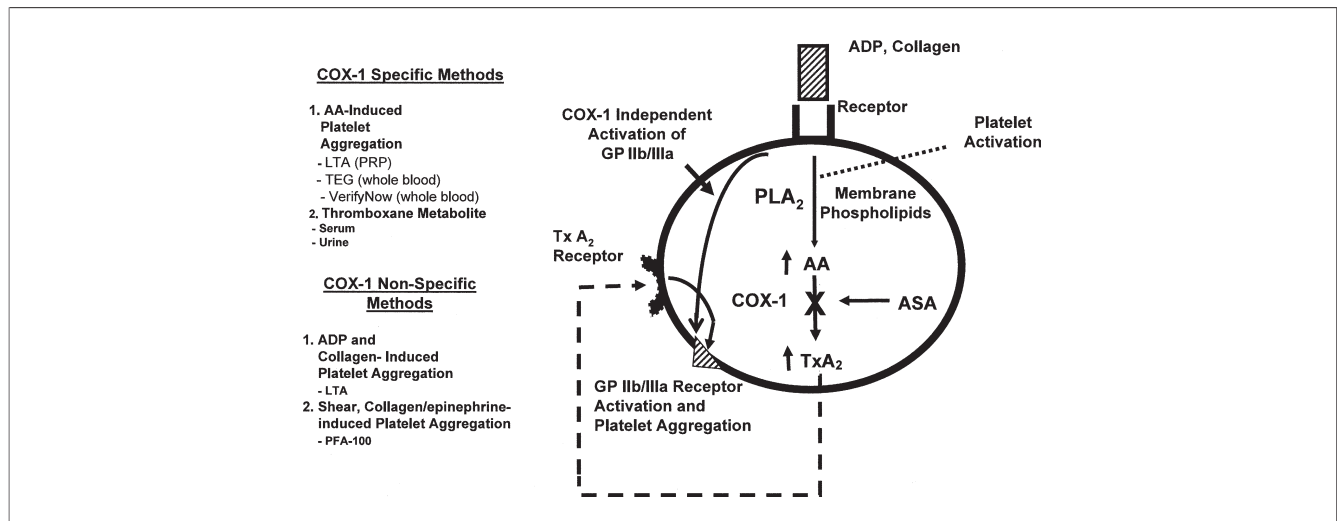


Figure 1 Laboratory Assessment of Aspirin Responsiveness

Arachidonic acid (AA) stimulation of platelet aggregation depends directly on cyclooxygenase (COX)-1 activity. In vivo production of thromboxane A₂ (TxA₂) is assessed by measurement of stable metabolites via enzyme-linked immunoassays. Adenosine diphosphate (ADP)- and collagen-stimulated aggregation are COX-1–nonspecific methods. Aggregation occurs through COX-1–independent and –dependent pathways after stimulation by the latter agonists. ASA = acetylsalicylic acid (aspirin); GP = glycoprotein; LTA = light transmittance aggregometry; PFA = platelet function analyzer; PLA₂ = phospholipase A₂; PRP = platelet-rich plasma; TEG = thrombelastography.

post-treatment P2Y₁₂ reactivity in nonresponsive patients. For aspirin, the identification of resistance would use a laboratory technique that detects residual activity of cyclooxygenase (COX)-1. Antiplatelet response variability and resistance and their proposed mechanisms have been described in previous publications (1–5,29).

Aspirin. Aspirin acetylates a serine moiety present in COX-1, resulting in irreversible inhibition (36). Laboratory

methods for assessing platelet responsiveness to aspirin can be categorized as COX-1–specific and COX-1–nonspecific (Fig. 1). The COX-1–specific methods include arachidonic acid-induced platelet aggregation measured by LTA, thrombelastography with the PlateletMapping assay, and enzyme-linked immunoassay determination of the stable metabolites of thromboxane A₂ in serum or in urine. The COX-1–nonspecific methods include ADP- or collagen-

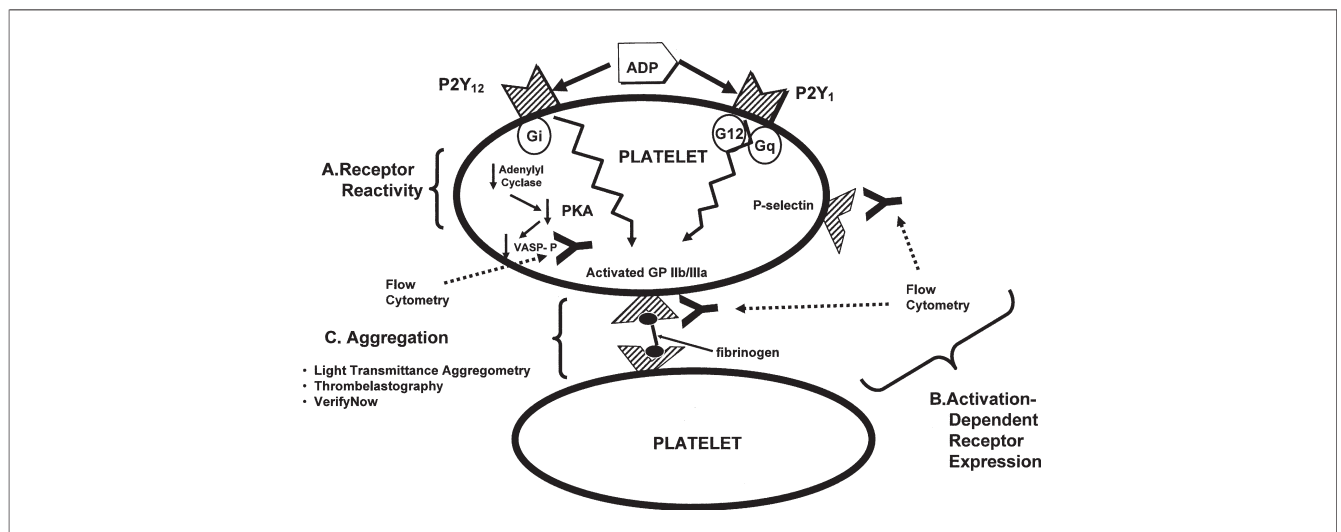
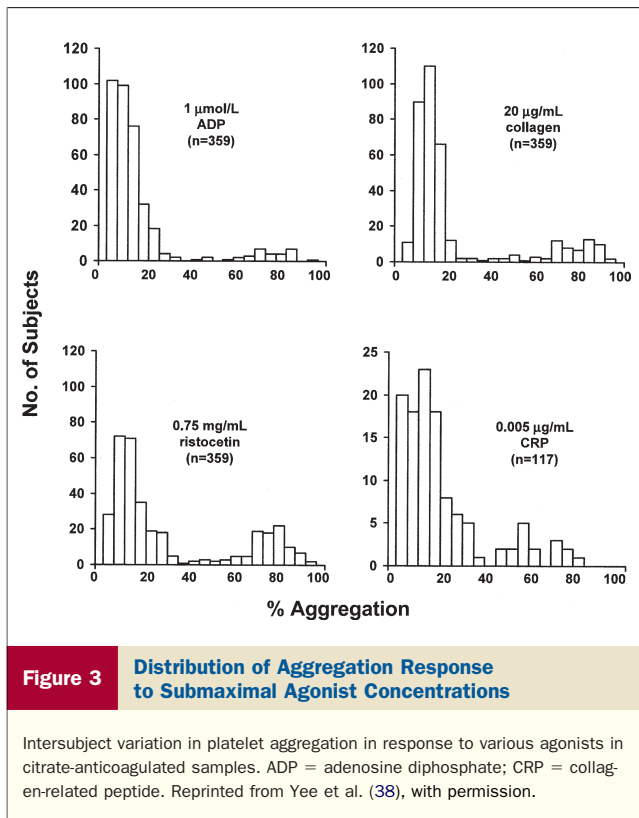


Figure 2 Laboratory Assessment of Clopidogrel Responsiveness

Adenosine diphosphate (ADP) stimulates 2 distinct receptors (P2Y₁ and P2Y₁₂) that are linked to specific signaling pathways (dashed arrows). Response can be measured by: **(A)** receptor reactivity; intracellular signaling downstream from the P2Y₁₂ receptor is measured by flow cytometry that assesses phosphorylation of vasodilator-stimulated phosphoprotein (VASP) with monoclonal antibodies (Y); the P2Y₁₂ is coupled by a G_i protein to adenylyl cyclase; PKA = protein kinase A; **(B)** activation-dependent receptor expression (active glycoprotein [GP] IIb/IIIa and P-selectin) identified by monoclonal antibodies (Y) with flow cytometry; **(C)** aggregation determined by light transmittance aggregometry (LTA), aggregation of platelets with fibrinogen-coated beads (VerifyNow), or measuring the contribution of platelet aggregation to total platelet–fibrin clot strength by thrombelastography.



induced platelet aggregation as determined by LTA or the Platelet Function Analyzer (PFA)-100 (Dade-Behring, Miami, Florida) (36).

Aspirin resistance is infrequent among patients undergoing elective PCI who are treated with 325 mg daily as assessed by arachidonic acid-induced platelet aggregation with LTA (44,45). In a recent randomized, double-blind, double-crossover investigation of aspirin dosing (81 to 325 mg) in patients with stable CAD, at any 1 dose, 0% to 6% were aspirin-resistant by COX-1-specific methods and 1% to 27% were resistant according to COX-1-nonspecific methods (46). Moreover, there was no consistency in the measurement of aspirin responsiveness between point-of-service assays in these patients receiving different doses of aspirin. Thus the incidence of aspirin resistance seems to be highly assay-dependent and is rare when determined by methods that directly indicate the activity of COX-1 (46,47). Treatment noncompliance can also affect the identification of aspirin “resistance” (44,45).

Aspirin resistance also might be associated with concomitant clopidogrel “resistance” (13,48). Patients identified as aspirin- and clopidogrel-resistant have exhibited high platelet reactivity to collagen in addition to ADP and arachidonic acid stimulation (13,49). The latter studies suggest a generalized high-platelet-reactivity phenotype that might be associated with an increased risk for ischemic events.

Clopidogrel. The detection of nonresponsiveness to clopidogrel has been most widely based on LTA studies using

ADP as an agonist (6,8-24,26,36). Flow cytometric measurement of activation-dependent receptor expression after ADP stimulation, point-of-care assays, and VASP phosphorylation have also been used to detect nonresponsiveness (Table 1, Fig. 2) (6,20-23,25-28).

In our early investigations of the antiplatelet effect of clopidogrel and aspirin therapy in patients undergoing coronary stenting we observed overall modest inhibition of 5 μmol/l ADP-induced aggregation and large variability in the response (6,50). We defined resistance as an absolute change in aggregation (ΔA) $\leq 10\%$. Thus, in our definition we did not normalize for baseline aggregation as has been done by other investigators (Table 2). Müller et al. (8) defined nonresponsiveness as a relative change in aggregation $< 10\%$. In the first study by our group reporting clopidogrel resistance, patients undergoing PCI received a 300-mg loading dose of clopidogrel, and platelet function was measured serially after stenting (6). This study reported a normal distribution of ΔA , and some patients had no demonstrable antiplatelet effect by LTA measurements over the next 24 h (Fig. 4) (6). The representation of the response profile as depicted in Figure 4 precisely describes the antiplatelet effects in an entire study population (50) and has been subsequently used by other investigators to provide further evidence of clopidogrel response variability (51).

The response to clopidogrel has been most studied in patients undergoing PCI (Table 2), and numerous studies have reported wide variations in response to therapy and rates of nonresponse of 5% to 44% (6-19). Measurement of VASP phosphorylation has also shown high residual reactivity of the P2Y₁₂ receptor in selected patients treated with clopidogrel (52). Differences in the prevalence of resistance between studies might be related to differences in dosing, differences in definitions (e.g., measurement of relative change in aggregation versus absolute change in aggregation, measurement of maximum versus late aggregation), laboratory methods, or insufficient time allowed before blood sampling to detect a maximum effect (Table 2). Clopidogrel response variability has multiple proposed etiologies and has been reviewed by the authors in prior publications (1,2,29).

Pitfalls in Monitoring Platelet Function

A basic problem with attempting to measure platelet function or drug-related inhibition, let alone correlating it with clinical outcomes, is that no single test encompasses the complexity of platelet biology and function (Fig. 5) (29,36). After plaque rupture or endothelial denudation, platelets adhere to von Willebrand factor and collagen and then are exposed to tissue factor-generated thrombin. Platelet signaling pathways become activated, with transmission of information through G-protein-coupled pathways to the cytoskeleton, leading to platelet secretion. Aggregation is initiated through binding of fibrinogen and other ligands to the activated GP IIb/IIIa receptor. Platelets also secrete

Table 2 Clopidogrel Response Variability/Resistance Studies

Study (Ref. #)	n	Patients	Clopidogrel Dose (mg), Load/qd	Definition of Clopidogrel Resistance	Time	Incidence
Gurbel <i>et al.</i> (6)	92	PCI	300/75	≤10% absolute change in ADP-LTA	24 h	31%–35%
Jaremo <i>et al.</i> (7)	18	PCI	300/75	ADP-induced fibrinogen binding <40% of baseline	24 h	28%
Müller <i>et al.</i> (8)	119	PCI	600/75	5 and 20 μmol/l ADP-induced aggregation, <10% relative change	4 h	5%–11%
Mobley <i>et al.</i> (9)	50	PCI	300/75	1 μmol/l ADP-induced aggregation, TEG and Ichor PW, <10% absolute inhibition	Pre and post	30%
Lepantalo <i>et al.</i> (10)	50	PCI	300/75	5 μmol/l ADP-induced aggregation and PFA-100, 10% inhibition and 170 s	2.5 h	40%
Angiolillo <i>et al.</i> (11)	48	PCI	300/75	6 μmol/l ADP-induced aggregation, <40% relative inhibition	10 min, 4 h, 24 h	44%
Matetzky <i>et al.</i> (12)	60	PCI	300/75	5 μmol/l ADP-induced aggregation and CPA, 4th quartile inhibition	Daily for 5 days	25%
Lev <i>et al.</i> (13)	150	PCI	300	5 μmol/l ADP-induced aggregation, <10% absolute change	20–24 h	24%
Angiolillo <i>et al.</i> (14)	52	Diabetics nondiabetics/ CAD and PCI	300	<10% relative inhibition	24 h	38%/8%
Gurbel <i>et al.</i> (15)	190	PCI	300 or 600/75	5 and 20 μmol/l ADP-induced aggregation, <10% absolute inhibition	24 h	28%–32%/8%
Dziewierz <i>et al.</i> (16)	31	CAD	300	20 μmol/l ADP-induced aggregation, <10% absolute change	24 h	23%
Geisler <i>et al.</i> (17)	379	PCI	600	20 μmol/l ADP-induced aggregation, <30% absolute inhibition	6 h	6%
Buonamici <i>et al.</i> (19)	804	PCI	600	10 μmol/l ADP-induced aggregation, >70% post-treatment aggregation	12–18 h	13%

ADP = adenosine diphosphate; CAD = coronary artery disease; CPA = cone and platelet analyzer; LTA = light transmittance aggregometry; PCI = percutaneous coronary intervention; PFA-100 = Platelet Function Analyzer-100; PW = Plateletworks; TEG = thrombelastography.

microparticles that play a multifaceted role in inflammation and tissue-factor release processes from leukocytes. Finally, coagulation factors are assembled on the surface of activated platelets and are inhibited by endogenous anticoagulant pathways. No single test of platelet function can capture all of these distinct yet integrated biological events. The bleeding time might be considered a test of platelet aggregate formation and primary hemostasis, but this test varies greatly among individuals, is not specific or sensitive for drug- or disease-related effects, and is highly subject to technical and operator influences (53).

Choice of anticoagulant. Citrate, a widely used anticoagulant for collecting blood samples, was introduced in 1902 as a way to identify the importance of ionized calcium in the coagulation–protein reaction system. The use of citrate anticoagulation, however, can sequester calcium, confounding the ability to assess interindividual pharmacodynamic responses and variability in platelet aggregation. Figure 3 shows intersubject variability in response to ADP, collagen, ristocetin, and collagen-reactive peptide agonists (38). At least some of this heterogeneity reflects differences in citrate concentrations among samples (due to differing volumes collected), but how much reflects the anticoagulant versus

the individual is unknown. Expression of P-selectin on the platelet surface is likewise increased by citrate anticoagulation of samples versus other anticoagulants (54).

One study compared the effects of citrate and a nonchelating anticoagulant, D-phenylalanyl-prolyl-arginine chloromethyl ketone (PPACK), on ex vivo platelet aggregation testing within a multicenter study of the GP IIb/IIIa inhibitor eptifibatide in patients with ACS (55). At all time points measured after eptifibatide administration, inhibition of platelet aggregation in response to ADP stimulation was greater for citrate- versus PPACK-anticoagulated samples. Labarthe *et al.* (56) measured clopidogrel responsiveness in 12 patients and 20 control subjects with both citrate- and PPACK-anticoagulated samples. They noted that clopidogrel responsiveness might be more accurately reflected when using PPACK as the anticoagulant. They also suggested that measurement of final aggregation, which includes platelet disaggregation, might be a better measurement of clopidogrel nonresponsiveness than measurement of maximum ADP-induced aggregation. In contrast, in a recent study of 100 patients undergoing PCI with stenting who received either a 300- or 600-mg loading dose of clopidogrel, final aggregation and maximum aggregation were

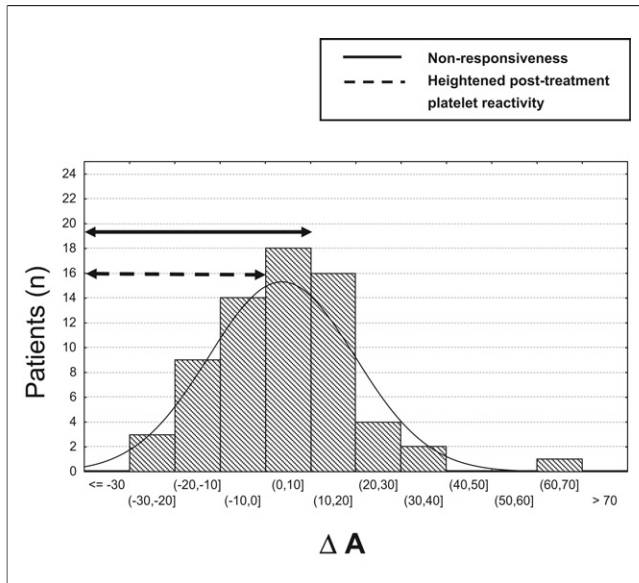


Figure 4 Response Variability to Clopidogrel

Presentation of platelet function data as a histogram of the absolute change in aggregation (ΔA) in response to 5 $\mu\text{mol/l}$ adenosine diphosphate (ADP) stimulation at 2 h after treatment with 300-mg clopidogrel loading dose. The ΔA is defined as baseline aggregation (%) minus the post-stent aggregation (%) and is divided into deciles. Nonresponsiveness or resistance is defined as $\Delta A \leq 10\%$. Nonresponsiveness is present in those patients below the **solid arrow**. Heightened post-treatment reactivity is present in those patients below the **dashed arrow**. The **curve** represents a normal distribution of data (Statistica software, Tulsa, Oklahoma). Adapted from Gurbel et al. (50), with permission.

affected to the same degree in nonresponsive patients (57). Thus, these measurements in the latter study seemed essentially equivalent in determining the prevalence of nonresponsiveness.

Choice of agonist. The choice and concentration of agonist also have major implications for platelet function

measurement. When assessing platelet response to aspirin, for example, the focus should be primarily on inhibition of aspirin's primary target, the COX-1 pathway; thus arachidonic acid is the most appropriate agonist. If the agonist stimulates multiple pathways leading to platelet activation, then the "response" to aspirin might be underestimated compared with methods using arachidonic acid. For clopidogrel, this construct translates to measuring ADP-induced responses. Because ADP interacts with at least 2 receptors on the platelet surface, P2Y₁₂ and P2Y₁, platelet function tests will determine the combined biological effects of ADP on these receptors. Assessment of clopidogrel's effects on P2Y₁₂ might be most accurately determined by measures of intracellular signaling events downstream from the receptor (e.g., quantification of VASP phosphorylation) (41).

Effect of thrombin and platelet count. Studies with nonchelating anticoagulants have delineated the importance of thrombin's activation of platelets in clotting, particularly through the G-coupled protease-activated receptor (PAR)-1 pathway. When whole blood is anticoagulated with corn trypsin inhibitor and tissue factor is added, platelet activation (reflected by osteonectin secretion) generally precedes thrombin generation (58). In fact, this reaction begins with <1% of the amount of thrombin ultimately produced. Thus platelets are particularly sensitive to thrombin-mediated activation, and thrombin generation provides the receptor sites for factors Xa, Va, VIIIa, and IXa, which leads to the expression of most of the thrombin in the reaction system.

Platelet counts also can influence the clotting time and aggregation measured in platelet-rich plasma. In a study of healthy volunteers and individuals receiving chemotherapy, platelet counts as low as 50,000 mm^3 influenced clotting times in a system using corn trypsin inhibitor-anticoagulated samples and tissue factor agonist (59). The same effect was observed in

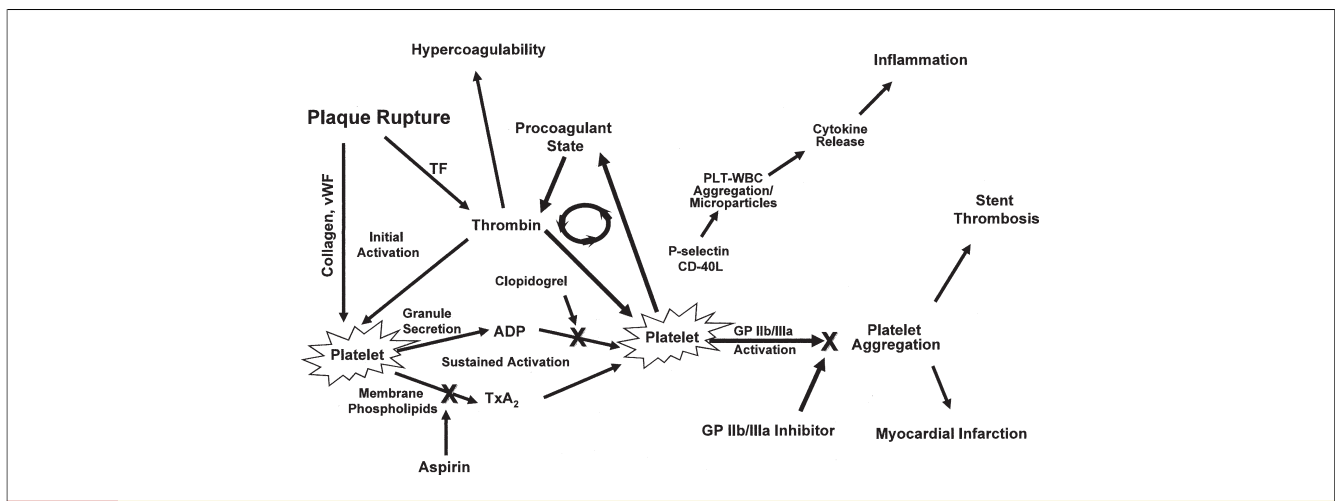


Figure 5 Relation of Platelets, Plaque Rupture, Thrombus Formation, and Adverse Clinical Outcomes

Platelets mediate an array of in vivo processes leading to adverse events. Initial activation is stimulated by key primary agonists (collagen, von Willebrand factor [vWF], thrombin). The activated platelet surface is the site of coagulation-factor generation, leading to amplification of thrombin generation. Release of key secondary agonists (adenosine diphosphate [ADP], TxA₂) upon activation amplifies aggregation. PLT = platelet; TF = tissue factor; WBC = white blood cell; other abbreviations as in Figure 1.

measurement of total thrombin generation, at platelet counts $<100,000 \text{ mm}^3$. Furthermore, if the number of platelets in the system was increased, clotting times and thrombin generation returned to normal ranges. Adjusting the platelet count in platelet-rich plasma with autologous platelet-poor plasma has been recommended for platelet aggregation studies. However, a recent study suggested that adjusting platelet counts before aggregation might affect the final platelet aggregation measurements by causing artifactual inhibition (60).

Aspirin treatment does not attenuate thrombin generation substantially in whole blood. In fact, samples from some individuals taking aspirin have shown heightened thrombin generation when stimulated with tissue factor (61). In contrast, when sampling blood escaping from a microvascular wound in the forearm, thrombin generation was suppressed after administration of aspirin (62). The same individuals showed no suppression of thrombin generation in whole blood when tissue factor was added. Thus aspirin seems to affect alternative and/or complementary pathways to the tissue-factor-mediated process after microvascular trauma.

Glycoprotein IIb/IIIa inhibitors also have been tested in the corn trypsin inhibitor-tissue factor model of coagulation. At lower concentrations of the reagents, abciximab and eptifibatid depress thrombin generation (61). At high concentrations, however, this effect is undetectable; it is masked by the overall kinetics of the reaction system. Similar findings have been reported when performing the high-concentration experiment in the presence of prostaglandin E1 (61). Thus the ability to detect drug-related antiplatelet activity depends greatly on the intensity of stimulation used in *in vitro* models.

No single test can encompass or reflect all aspects of platelet biology. Moreover, the choice of anticoagulant for sampling and agonist can influence test results. Inherent heterogeneity in platelet performance even under normal circumstances creates challenges when attempting to measure treatment responses. Measurement tools and definitions for normal/abnormal responsiveness to platelet-directed therapy must be carefully constructed to reflect receptor signaling and pathway specificity. Population-based and mechanistic studies, performed in parallel with trials, registries, and/or research networks assessing clinical outcomes, are required to answer several basic questions, advance the field, and foster patient-specific therapy.

Correlating Measures of Interindividual Variability in Platelet Function With Clinical Outcomes

Studies attempting to correlate measures of platelet function—and its attenuation with drug therapies—with clinical outcomes have often focused on a receptor-signaling pathway or reaction (activation or aggregation). Most commonly, platelet function has been measured by the degree of

aggregation in response to a specific concentration of agonist, but platelet-fibrin interactions also have been measured, as have platelet activation via measures of platelet and soluble P-selectin, urinary thromboxane metabolites, and other markers. These measures have been used primarily to assess relationships between platelet function and stent thrombosis or major adverse cardiac events (MACE).

The number of patients studied within investigations designed to link *ex vivo* response variability to clinical resistance is small overall, and there has been an absence of serial measurements (12,13,17–28,30–35). Although some studies support a link between laboratory-based and clinical “resistance,” none has been definitive. In addition and more importantly, no published study has examined whether tailoring therapy according to laboratory findings of aspirin or clopidogrel resistance can improve clinical outcomes.

The International Society on Thrombosis and Haemostasis Working Group on Aspirin Resistance (63) has recently stated that a clinically meaningful definition of resistance should be based on data linking therapy-dependent laboratory tests to clinical outcomes. The group also noted that the correct treatment, if any, of antiplatelet-therapy resistance is unknown, given that no study has addressed the clinical effectiveness of altering therapy specifically on the basis of laboratory findings of resistance. Other than within clinical trials, which the group encouraged, it is currently not appropriate to test patients for aspirin or clopidogrel resistance or to change therapy on the basis of such testing.

A clinical trial designed to investigate antiplatelet resistance would require sufficient power to answer 2 key questions: 1) which simple, inexpensive, and rapid test of platelet function (or combination of tests) best predicts clinical outcomes of antiplatelet therapy for specific individuals with specific indications for treatment; and 2) are individual outcomes improved when treatment is changed in response to the test(s) results? A basic objective is to develop absolute or relative thresholds (upper and lower) for test results, similar to the practice of tailoring warfarin therapy according to a standardized international normalized ratio (INR). The landscape for developing an equivalent testing strategy for platelet-directed therapy is considerably more complex with the concomitant use of several drugs with different mechanisms of action and multiple testing platforms.

Clinical trials also must investigate, in patients with known resistance and/or drug failure, the potential benefit of dose adjustment or supplementary treatments with different pharmacodynamic profiles. For example, if a patient with CAD suffers an event while already taking aspirin, it might be more effective to increase the dose of aspirin, add a thienopyridine agent for additional secondary prevention, or both rather than simply substituting drugs.

Relation of platelet reactivity to outcomes of aspirin therapy. Early studies (30,31) suggested a relation between high platelet reactivity among patients receiving aspirin and

an increased risk of vascular events (Table 3). In patients enrolled in the HOPE (Heart Outcome Prevention Evaluation) trial, Eikelboom et al. (32) showed a relation between high urinary 11-dehydro thromboxane B₂ levels, a measure of platelet thromboxane A₂ generation, and the risk for subsequent ischemic events. Chen et al. (35) showed a relation between high platelet reactivity measured by Ultegra Rapid Platelet Function Assay-ASA (Accumetrics) and periprocedural myonecrosis in patients undergoing PCI.

With LTA, Gum et al. (33,34) assessed the relation between aspirin resistance and the composite outcome of death, MI, or stroke over a mean 1.8 years of follow-up in 326 patients with stable CAD taking 325 mg of aspirin for ≥1 week before enrollment. In all, 5.2% of patients were considered aspirin-resistant (≥70% mean aggregation in response to 10 μmol/l ADP and ≥20% aggregation after incubation with 0.5 mmol/l arachidonic acid). During follow-up, 24% of aspirin-resistant patients had an event versus 10% of nonresistant patients (p = 0.03), but the relationships between aspirin resistance (as a categorical or continuous variable) and the component clinical events were not statistically significant. After adjustment for several risk factors, aspirin resistance was an independent predictor of long-term adverse events.

With the PFA-100 analyzer to measure aspirin-mediated platelet inhibition, several studies have reported increased event rates in patients with a profile of aspirin resistance (64,65). This method has several limitations, however, including poor correlation to other measures of platelet performance and dependence on von Willebrand factor level and activity and platelet count. The PFA-100 method also uses collagen and epinephrine as agonists, neither of which is specific for COX-1 activity, the target of aspirin. A major limitation of all published studies of aspirin resistance is a lack of serial platelet function measurements, particularly because the degree of aspirin resistance can fluctuate over time and can be affected by aspirin dose (46,66).

Relation of clopidogrel responsiveness/high post-treatment platelet reactivity to clinical outcomes. Published studies investigating the association between platelet reactivity to ADP (indicative of clopidogrel responsiveness) and the occurrence of ischemic events have involved patients undergoing PCI, with most employing LTA measurement of platelet function (Table 4) (12,13,17-24,26). These gener-

ally small populations have reported links between clopidogrel-induced platelet inhibition, post-treatment platelet reactivity, periprocedural myonecrosis, stent thrombosis, and recurrent ischemic events.

Matetzky et al. (12) examined 60 consecutive patients with MI undergoing primary PCI with stenting, all of whom received clopidogrel, aspirin, and eptifibatide. Ten consecutive patients undergoing primary angioplasty without stenting and given no clopidogrel were the control subjects. Platelet aggregation was determined by LTA in response to 5 μmol/l ADP and 10 μmol/l epinephrine and by cone and plate(let) analysis expressed as the percent surface coverage by platelets and average size of surface-bound aggregates. When treated patients were grouped into quartiles by percent reduction in ADP-induced aggregation at day 6 versus baseline (before clopidogrel administration), the responses varied from a mean aggregation of 103% of the baseline value in the first quartile (considered clopidogrel-resistant) to only 33% of the baseline value in the fourth quartile (p < 0.01 across groups). This variability persisted for epinephrine-induced aggregation and aggregate size measurements. Over 6-month follow-up, 7 of the 8 major cardiac events occurred in the clopidogrel-resistant group; 40% of the first-quartile patients had another ischemic event. No correlation between platelet inhibition measured by the cone and plate(let) device and clinical outcomes was reported.

In the CLEAR-PLATELETS (Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets) study, ADP-induced aggregation was measured serially over 18 to 24 h in 120 patients undergoing stenting treated with 300 or 600 mg of clopidogrel. Fifty percent of these patients were randomized to treatment with eptifibatide (21). Patients with MI according to creatine kinase-MB release had significantly greater mean platelet reactivity to ADP compared with patients without MI (Fig. 6), suggesting a threshold of mean platelet aggregation that might be used as a reference point for future studies.

The relation of platelet reactivity and stent thrombosis has also been investigated with most studies reporting that high post-treatment platelet activity as determined by various methods is associated with stent thrombosis (19,25-28). However, the reported measures varied widely in patients with and without thrombosis. Further studies lend strong support to the association between high post-

Table 3 Relation of Aspirin Responsiveness to Clinical Outcomes

Study (Ref. #)	n	Patients	Method	Results
Mueller et al. (31)	100	PVD	Platelet aggregation (whole blood)	87% increase in reocclusion
Eikelboom et al. (32)	976	High-risk CAD	Urinary 11-dehydro TxB ₂	Increase in MI/stroke/death with increased TxB ₂
Gum et al. (33,34)	325	Stable CAD	Light transmittance aggregometry	3.12-fold increase in MI/stroke/death
Chen et al. (35)	151	PCI	RPFA	2.9-fold increase in myocardial necrosis
Grottemeyer et al. (30)	180	Post-CVA	Platelet aggregates	10-fold increase in vascular events

CAD = coronary artery disease; CVA = cerebrovascular accident; MI = myocardial infarction; PCI = percutaneous coronary intervention; PVD = peripheral vascular disease; RPFA = rapid platelet function analyzer; Tx = thromboxane.

Table 4 Relation of Platelet Reactivity to ADP/Clopidogrel Nonresponsiveness to Clinical Outcomes

Study (Ref. #)	n	Results	Clinical Relevance
Matetzky et al. (12)	60	Clopidogrel nonresponsiveness; STEMI patients loaded with clopidogrel	Ischemic events (6 months)
Geisler et al. (17)	379	↑ Pre-procedural platelet aggregation, CAD patients loaded with clopidogrel	Ischemic events (3 months)
Gurbel et al. (20)	192	↑ Post-procedural platelet aggregation, patients loaded with clopidogrel at time of elective PCI	Ischemic events (6 months)
Gurbel et al. (21,22)	120	↑ Periprocedural mean platelet aggregation; patients loaded with clopidogrel at time of elective PCI ± eptifibatide	In-hospital post-PCI myonecrosis, inflammation marker release
Bliden et al. (23)	100	↑ Pre-procedural platelet aggregation; patients on maintenance clopidogrel therapy pre-elective PCI	Ischemic events (12 months)
Cuisset et al. (24)	106	↑ Pre-procedural platelet aggregation; NSTEMI ACS patients treated with clopidogrel pre-PCI	Ischemic events (1 month)
Lev et al. (13)	120	Clopidogrel and aspirin nonresponsiveness; patients loaded with clopidogrel immediately after elective PCI	In-hospital post-PCI myonecrosis
Hochholzer et al. (18)	802	↑ Pre-procedural platelet aggregation; patients treated with clopidogrel pre-elective PCI	Ischemic events (1 month)
Barragan et al. (25)	36	↑ P2Y ₁₂ reactivity ratio by VASP-P measurement	Stent thrombosis
Gurbel et al. (26)	120	↑ P2Y ₁₂ reactivity ratio by VASP-P measurement, platelet aggregation, and ADP-stimulated GP IIb/IIIa expression	Stent thrombosis
Ajzenberg et al. (27)	49	↑ Shear-induced platelet aggregation	Stent thrombosis
Wong et al. (28)	264	↓ Platelet inhibition by VerifyNow P2Y ₁₂ assay	Stent thrombosis
Buonamici et al. (19)	804	↑ Post-treatment platelet aggregation	Stent thrombosis

ACS = acute coronary syndrome; CAD = coronary artery disease; GP = glycoprotein; MACE = major adverse cardiovascular events; NSTEMI = non-ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; STEMI = ST-segment elevation myocardial infarction; VASP-P = phosphorylated vasodilator-stimulated phosphoprotein.

treatment platelet reactivity to ADP and the occurrence of post-stenting ischemic events. Hochholzer et al. (18) studied 802 patients undergoing stenting treated with 600 mg clopidogrel and found that patients with post-treatment platelet aggregation above the median level had a 6.7-fold increase in the risk of 30-day MACE. In another study of 804 patients undergoing stenting, Buonamici et al. (19) demonstrated that stent thrombosis was more prevalent in

patients with post-treatment platelet aggregation $\geq 70\%$ in response to 10 $\mu\text{mol/l}$ ADP stimulation.

Relation of platelet reactivity to outcomes of GP IIb/IIIa inhibitor therapy. The GOLD (AU-Assessing Ultegra) study reported a relation between inhibition of platelet function and MACE. The study included 500 patients undergoing PCI and used the VerifyNow GP IIb/IIIa assay (67). In all, 25% of patients did not achieve $\geq 95\%$ platelet inhibition 10 min after administration of a GP IIb/IIIa inhibitor. These patients had a significantly higher incidence of MACE (14.4% vs. 6.4% for patients with $\geq 95\%$ inhibition; $p = 0.006$). In multivariate analysis, platelet inhibition $\geq 95\%$ 10 min after GP IIb/IIIa inhibitor administration was independently associated with a 50% lower risk of MACE.

Evidence for a threshold of post-treatment platelet reactivity associated with long-term ischemic events. There is preliminary evidence for a potential threshold of platelet reactivity as measured by LTA after ADP stimulation that is associated with an increased risk of post-discharge ischemic events after PCI. In the PREPARE POST-STENTING (Platelet Reactivity in Patients and Recurrent Events Post-Stenting) study, the first investigation linking high post-treatment platelet reactivity to ADP and the occurrence of post-discharge ischemic events; a threshold of approximately 50% periprocedural platelet aggregation in response to 20 $\mu\text{mol/l}$ ADP was associated with the occurrence of ischemic events during 6-month follow-up (20). Similarly, in another study, approximately 40% platelet aggregation in response to 20 $\mu\text{mol/l}$ ADP was associated

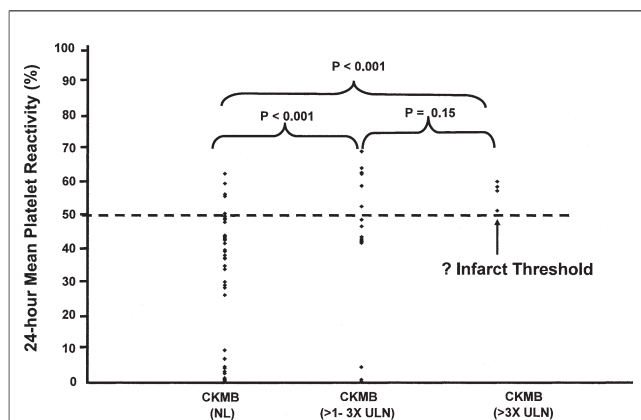


Figure 6 Evidence of a Possible Threshold for Ischemic Events After Elective Stenting

Relation between creatine kinase-MB (CKMB) release and mean platelet aggregation in response to 5 $\mu\text{mol/l}$ adenosine diphosphate measured serially over 18 to 24 h in patients undergoing stenting in the CLEAR PLATELETS study. Adapted from Gurbel et al. (21) with permission. NL = normal limit; ULN = upper limit of normal.

with the occurrence of stent thrombosis (23). In a third study by the same group, a threshold of approximately 40% pre-procedural platelet aggregation in response to 5 $\mu\text{mol/l}$ ADP among patients receiving long-term clopidogrel and aspirin therapy before undergoing stenting was associated with the occurrence of ischemic events over the ensuing 12 months (26) (Fig. 7). The latter study suggests the potential utility of a pre-PCI platelet reactivity measurement as a potential marker of long-term ischemic events. The timing and method of measuring platelet function that best correlates with patient outcomes is under investigation.

The aforementioned studies might provide a “testable” level of post-treatment platelet reactivity in future studies, similar to the INR ranges established for warfarin therapy. Our data suggest that adequate protection against ischemic events with aspirin and clopidogrel therapy might be achieved by low to moderate levels of post-treatment platelet reactivity in the majority of patients. These findings have implications on bleeding risks as well during dual antiplatelet therapy that might accompany markedly low levels of post-treatment platelet reactivity. Currently, the relation of bleeding to levels of ADP-induced aggregation is unknown. Future studies will be required to determine whether there is a therapeutic window for oral antiplatelet agents akin to the INR used for warfarin therapy.

Limitations of Focusing on Platelet Function Alone to Predict Risk

Most of the studies summarized in the preceding text measured platelet function in isolation. None of the studies assessed platelet–fibrin interactions, the kinetics of thrombin generation, or measurements of platelet–fibrin clot strength, which could play important roles in predicting ischemic events. A recent study compared the ability of thrombelastography measurements (maximum platelet–fibrin clot strength and time to initial platelet–fibrin clot formation) and platelet reactivity to ADP measured by LTA to predict ischemic events 6 months after PCI (20). Of 193 patients studied, 32% of those in the highest quartile of ADP-induced aggregation had an ischemic event by 6 months versus 10% of patients in the lowest quartile ($p = 0.02$). When stratified by platelet–fibrin clot strength, 58% of patients in the highest quartile had an ischemic event by 6 months versus only 2% of patients in the lowest quartile ($p < 0.001$) (20). Methods that measure coagulation and platelet interactions might prove to be better predictive tools than isolated measurements of platelet function.

Conclusions

The measurement of platelet function and response to pharmacological antagonists is inherently complex and confounded by several methodological factors, making it difficult to correlate results with clinical outcomes and, in turn, to use the results to guide therapy. A major limitation that has hampered both knowledge acquisition and translation to

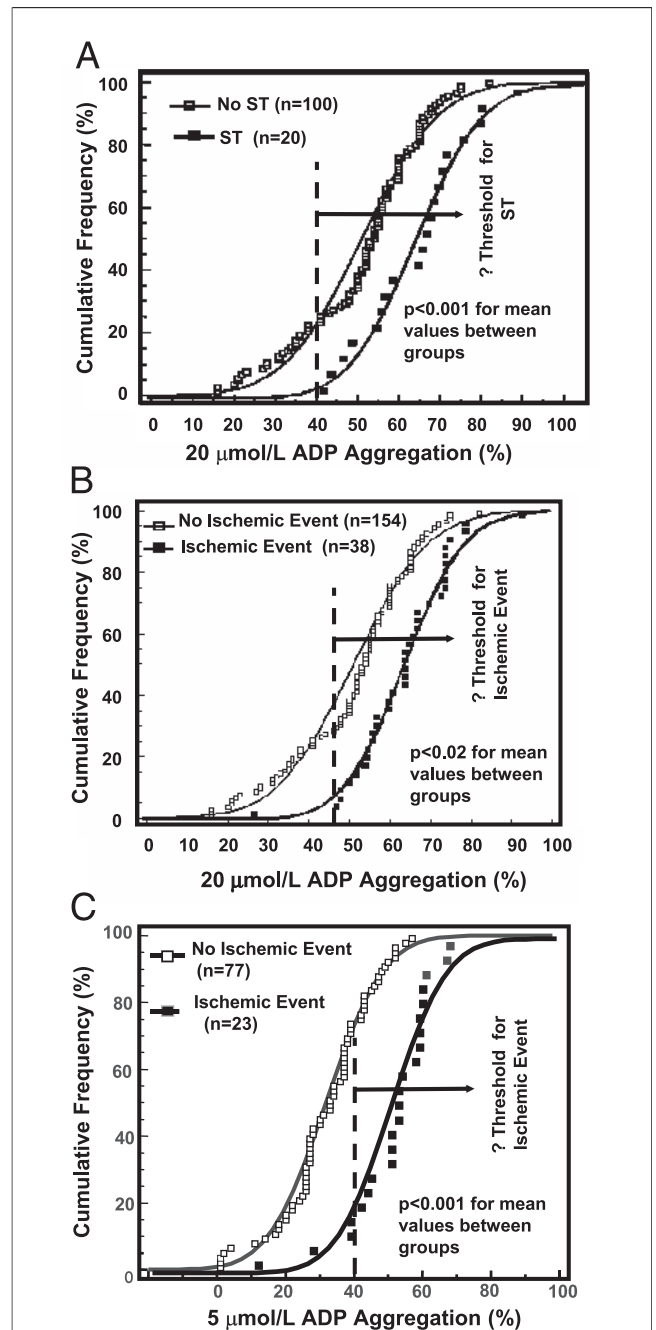


Figure 7 Cumulative Frequency Distributions of ADP-Induced Platelet Aggregation in Relation to Clinical Outcomes

(A) Distributions measured in patients with and without stent thrombosis. Data from Gurbel et al. (26). (B) Distributions measured in patients with and without ischemic events within 6 months of elective coronary-artery stenting; after percutaneous coronary intervention (PCI) measurement. Data from Gurbel et al. (20). (C) Distributions measured in patients with and without ischemic events within 1 year treated with clopidogrel therapy before elective coronary artery stenting; before PCI measurement. Data from Bliden et al. (23). ADP = adenosine diphosphate; ST = stent thrombosis.

the clinical arena is the absence of established investigative platforms and trials of sufficient size to either support or refute hypothesis-generating observations from small stud-

ies. The clinical trials community, working closely with platelet biologists, clinician-scientists, and the pharmaceutical and device industries, must formulate an agenda, building on the strengths of collaboration to address an area of unmet clinical need.

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REFERENCES

- Gurbel PA, Tantry US. Drug insight: clopidogrel nonresponsiveness. *Nat Clin Pract Cardiovasc Med* 2006;3:387-95.
- Michelson AD, Frelinger AL, Furman MI. Resistance to antiplatelet drugs. *Eur Heart J* 2006;8 Suppl G:G53-8.
- Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. *Arterioscler Thromb Vasc Biol* 2004;24:1980-7.
- Patrono C, Rocca B. Drug insight: aspirin resistance—fact or fashion? *Nat Clin Pract Cardiovasc Med* 2007;4:42-50.
- Wang TH, Bhatt DL, Topol EJ. Aspirin and clopidogrel resistance: an emerging clinical entity. *Eur Heart J* 2006;27:647-54.
- Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;107:2908-13.
- Jaremo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med* 2002;252:233-8.
- Müller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemostasis* 2003;89:783-7.
- Mobley JE, Bresee SJ, Wortham DC, Craft RM, Snider CC, Carroll RC. Frequency of nonresponse antiplatelet activity of clopidogrel during pretreatment for cardiac catheterization. *Am J Cardiol* 2004;93:456-8.
- Lepantalo A, Virtanen KS, Heikkilä J, Wartiovaara U, Lassila R. Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary interventions. *Eur Heart J* 2004;25:4768-83.
- Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Identification of low responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. *Thromb Res* 2005;115:101-8.
- Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004;109:3171-5.
- Lev EI, Patel RT, Maresh KJ, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. *J Am Coll Cardiol* 2006;47:27-33.
- Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005;54:2430-5.
- Gurbel PA, Bliden KP, Hayes KM, Yoho JA, Herzog WR, Tantry US. The relation of dosing to clopidogrel responsiveness and the incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. *J Am Coll Cardiol* 2005;45:1392-6.
- Dziewierz A, Dudek D, Heba G, Rakowski T, Mielecki W, Dubiel JS. Inter-individual variability in response to clopidogrel in patients with coronary artery disease. *Kardiologia Pol* 2005;62:108-17.
- Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006;27:2420-5.
- Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006;48:1742-50.
- Buonamici P, Marcucci R, Migliorini A, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J Am Coll Cardiol* 2007;49:2312-7.
- 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.
- Gurbel PA, Bliden KP, Zaman KA, Yoho JA, Hayes KM, Tantry US. Clopidogrel loading with eptifibatid to arrest the reactivity of platelets: results of the CLEAR PLATELETS study. *Circulation* 2005;111:1153-9.
- Gurbel PA, Bliden KP, Tantry US. Effect of clopidogrel with and without eptifibatid on tumor necrosis factor-alpha and C-reactive protein release after elective stenting: results from the CLEAR PLATELETS 1b study. *J Am Coll Cardiol* 2006;48:2186-91.
- Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol* 2007;49:657-66.
- Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J Thromb Haemostasis* 2006;4:542-9.
- Barragan P, Bouvier JL, Roquebert PO, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. *Catheter Cardiovasc Interv* 2003;59:295-302.
- Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST study. *J Am Coll Cardiol* 2005;46:1827-32.
- Ajzenberg N, Aubry P, Huisse MG, et al. Enhanced shear-induced platelet aggregation in patients who experience subacute stent thrombosis: a case-control study. *J Am Coll Cardiol* 2005;45:1753-6.
- Wong G, Price M, Valencia R, Lee S, Gollapudi R, Teirstein PS. Measurement of clopidogrel inhibition with a point-of-care assay identifies patients at risk for stent thrombosis after percutaneous coronary intervention. Presented at: Transcatheter Cardiovascular Therapeutics; October 22-27, 2006; Washington, DC.
- Gurbel PA, Tantry US. Clopidogrel resistance? *Thromb Res* 2007;120:311-21.
- Grottemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post-stroke patients. *Thromb Res* 1993;71:397-403.
- Mueller MR, Salat A, Stangl P, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb Haemostasis* 1997;78:1003-7.
- Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 2002;105:1650-5.
- Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 2001;88:230-5.
- Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003;41:961-5.
- Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. *J Am Coll Cardiol* 2004;43:1122-6.
- Michelson AD. Platelet function testing in cardiovascular diseases. *Circulation* 2004;110:e489-93.
- Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Br Med J* 2002;324:71-86.
- Yee DL, Sun CW, Bergeron AL, Dong JF, Bray PF. Aggregometry detects platelet hyperreactivity in healthy individuals. *Blood* 2005;106:2723-9.
- Ingerman-Wojenski C, Smith JB, Silver MJ. Evaluation of electrical aggregometry: comparison with optical aggregometry, secretion of ATP, and accumulation of radiolabeled platelets. *J Lab Clin Med* 1983;101:44-52.

40. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 2001;104:1533-7.
41. Geiger J, Brich J, Honig-Liedl P, et al. Specific impairment of human platelet P2Y(AC) ADP receptor-mediated signaling by the antiplatelet drug clopidogrel. *Arterioscler Thromb Vasc Biol* 1999;19:2007-11.
42. Smith JW, Steinhubl SR, Lincoff AM, et al. Rapid platelet-function assay: an automated and quantitative cartridge-based method. *Circulation* 1999;99:620-5.
43. Gurbel PA, Kereiakes DJ, D'Alesandro MR, Bahr RD, O'Connor CM, Serebruany VL. Role of soluble and platelet-bound P-selectin in discriminating cardiac from noncardiac chest pain at presentation in the emergency department. *Am Heart J* 2000;139:320-8.
44. Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. *J Am Coll Cardiol* 2005;46:1705-9.
45. Schwartz KA, Schwartz DE, Ghosheh K, Reeves MJ, Barber K, DeFranco A. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. *Am J Cardiol* 2005;95:973-5.
46. 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.
47. Pulcinelli FM, Riondino S, Celestini A, et al. Persistent production of platelet thromboxane A2 in patients chronically treated with aspirin. *J Thromb Haemost* 2005;3:2784-9.
48. Templin C, Schaefer A, Stumme B, Drexler H, von Depka M. Combined aspirin and clopidogrel resistance associated with recurrent coronary stent thrombosis. *Clin Res Cardiol* 2006;95:122-6.
49. DiChiara J, Bliden KP, Tantry US, et al. Platelet aspirin responsiveness measured by VerifyNow: correlation with multiple methods and identification of a high platelet reactivity phenotype. *Platelets* 2007;18:414-23.
50. Gurbel PA, Bliden KP. A new method of representing drug-induced platelet inhibition: better description of time course, response variability, non-response, and heightened activity. *Platelets* 2003;14:481-3.
51. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005;45:246-51.
52. Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J Thromb Haemost* 2005;3:85-92.
53. Lind SE. The bleeding time. In: Michelson AD, editor. *Platelets*. 2nd edition. San Diego, CA: Elsevier/Academic Press, 2007:485-93.
54. Schneider DJ, Tracy PB, Mann KG, Sobel BE. Differential effects of anticoagulants on the activation of platelets ex vivo. *Circulation* 1997;96:2877-83.
55. Tardiff BE, Jennings LK, Harrington RA, et al. Pharmacodynamics and pharmacokinetics of eptifibatid in patients with acute coronary syndromes: prospective analysis from PURSUIT. *Circulation* 2001;104:399-405.
56. Labarthe B, Theroux P, Angioi M, Ghitescu M. Matching the evaluation of the clinical efficacy of clopidogrel to platelet function tests relevant to the biological properties of the drug. *J Am Coll Cardiol* 2005;46:638-45.
57. Gurbel PA, Bliden KP, Etherington A, Tantry US. Assessment of clopidogrel responsiveness: measurements of maximum platelet aggregation, final aggregation and their correlation with vasodilator-stimulated phosphoprotein in resistant patients. *Thromb Res* 2007 Mar 30; [Epub ahead of print].
58. Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. *Blood* 2002;100:148-52.
59. Butenas S, Branda RF, van't Veer C, Cawthorn KM, Mann KG. Platelets and phospholipids in tissue factor-initiated thrombin generation. *Thromb Haemost* 2001;86:660-7.
60. Cattaneo M, Lecchi A, Zighetti ML, Lussana F. Platelet aggregation studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize platelet count. *Haematologica* 2007;92:694-7.
61. Butenas S, Cawthorn KM, van't Veer C, DiLorenzo ME, Lock JB, Mann KG. Antiplatelet agents in tissue factor-induced blood coagulation. *Blood* 2001;97:2314-22.
62. Undas A, Brummel K, Musial J, Mann KG, Szczeklik A. Blood coagulation at the site of microvascular injury: effects of low-dose aspirin. *Blood* 2001;98:2423-31.
63. Michelson AD, Cattaneo M, Eikelboom JW, et al. Aspirin resistance: position paper of the Working Group on Aspirin Resistance. *J Thromb Haemost* 2005;3:1309-11.
64. Marcucci R, Panicia R, Antonucci E, et al. Usefulness of aspirin resistance after percutaneous coronary intervention for acute myocardial infarction in predicting one-year major adverse coronary events. *Am J Cardiol* 2006;98:1156-9.
65. Poulsen TS, Jorgensen B, Korsholm L, et al. Prevalence of aspirin resistance in patients with an evolving acute myocardial infarction. *Thromb Res* 2007;119:555-62.
66. Helgason CM, Bolin KM, Hoff JA, et al. Development of aspirin resistance in persons with previous ischemic stroke. *Stroke* 1994;25:2331-6.
67. Steinhubl SR, Talley JD, Braden GA, et al. Point-of-care measured platelet inhibition correlates with a reduced risk of an adverse cardiac event after percutaneous coronary intervention: results of the GOLD (AU-Assessing Ultegra) multicenter study. *Circulation* 2001;103:2572-8.