REVIEW

CME CREDIT

NEZAR FALLUJI, MD, MPH
Linda and Jack Gill Heart Institute, University of Kentucky, Lexington

STEVEN R. STEINHUBL, MD*
Linda and Jack Gill Heart Institute, University of Kentucky, Lexington

Variable response to antiplatelet therapy: What does it mean to clinicians?

ABSTRACT

Ex vivo tests of platelet function show that platelet function and the response to antiplatelet therapy vary markedly from person to person. But just how clinically significant are ex vivo measurements of platelet function, and will changes we make based on such information translate into improved outcomes for patients? The authors summarize what is known and not known about the impact and clinical significance of variable response to antiplatelet therapy.

KEY POINTS

The way a person responds to antiplatelet therapy is likely influenced by numerous genetic and environmental factors.

Some tests of platelet responsiveness have inherent limitations. For instance, measurements of urinary thromboxane metabolite are altered by systemic inflammatory conditions and by the degree of atherosclerosis, not just by the degree of platelet inhibition caused by aspirin.

The response to clopidogrel is influenced by which platelet function test is used, how the test is interpreted, and which anticoagulant is used in sampling.

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ANTIPLATELET THERAPY IS NOT A one-dose-fits-all endeavor. Platelet function and the response to antiplatelet therapy are known to vary markedly from person to person, and those who are considered "nonresponders" to antiplatelet therapy appear to be at higher risk of atherothrombotic events.

Antiplatelet resistance—or more precisely, the variable response to antiplatelet therapy—has been the focus of numerous investigations and reviews. While the *idea* of variable response is perhaps now more widely accepted by the medical community, its impact and significance are still far from well understood, and we do not yet have a treatment for it. We will attempt to shed some light on the evidence behind this concept, its potential impact, and the limitations of the current evidence.

A COMPLEX OF FACTORS

Plaque rupture or fissuring with the subsequent adhesion, activation, and aggregation of platelets is central in the pathogenesis of atherosclerosis-related vascular events.[1–5] Aspirin and the thienopyridines clopidogrel (Plavix) and ticlopidine (Ticlid) are potent inhibitors of platelet aggregation and are among the most effective therapies in managing the vascular complications of atherosclerosis and preventing such complications in patients with risk factors for atherosclerosis.

Platelet function

Circulating platelets do not normally encounter the collagen matrix that lies beneath vascular endothelium. But any break in the integrity of this vascular lining exposes

the platelets to collagen, which provides a surface for platelet adhesion and strongly stimulates platelet activation. This stimulation in turn activates signaling pathways that induce platelets to change their shape and to synthesize and secrete various molecules into the circulation, including thromboxane A2 and adenosine diphosphate (ADP). These two molecules in turn stimulate and activate neighboring platelets.

Activated platelets bind directly to the circulating coagulation protein fibrinogen via the abundant platelet integrin glycoprotein GPIIb/IIIa. Fibrinogen can simultaneously bind two GPIIb/IIIa receptors and can therefore function as a link between two platelets. This platelet-fibrinogen-platelet connection initiates the process of platelet aggregation. A cross-linked fibrin clot ultimately stabilizes the growing platelet aggregate.

In addition to collagen, ADP, and thromboxane A2, other agonists can activate platelets at sites of vascular injury. Tissue factor, expressed on all nonvascular cells, is exposed to circulating blood upon disruption of the endothelium. Tissue factor interacts with factor VIIa to promote local coagulation and ultimately the generation of thrombin, the most potent platelet agonist. Platelets facilitate this process by providing procoagulant phospholipids that accelerate thrombin generation. Consequently, platelet activation and fibrin deposition are intimately linked, maximizing the growth and strength of the hemostatic plug.[6,7]

The one-dose-fits-all approach to antiplatelet therapy is flawed

TESTS TO ASSESS PLATELET FUNCTION

In the early 20th century, the only methods to assess platelets were manual platelet counts and bleeding time. The complexity of platelet function, platelet sensitivity to manipulation, and difficulties in simulating hemostasis in vitro were major obstacles to developing sophisticated and reliable tests of platelet function.

Over the past several decades, many tests have been developed to assess the activation, secretory activities, and aggregatory responsiveness of platelets to various stimuli.[7] Among the most widely used tests are:

- **Urinary thromboxane B2**: A relatively simple, straightforward test that assesses the level of platelet activity by measuring the urinary excretion of thromboxane B2, a stable metabolite of thromboxane A2 metabolism.
- **Expression of P-selectin**: P-selectin is an intracellular adhesion molecule that moves to the plasma membrane surface when platelets are activated and degranulated. Assessing the surface expression of P-selectin through flow cytometry is technically demanding and requires special expertise.
- **Soluble P-selectin and soluble CD40L measurement**: Both of these intracellular molecules are expressed on the platelet surface during platelet activation. They are then cleaved and released into the circulation, where they can be measured as a marker of platelet activation. Tests such as these have a theoretical advantage in that they can measure in vivo platelet activation and they are less subject to artifactual increases caused by platelet activation ex vivo during blood draws and sample handling. However, neither of these has been used to measure responsiveness to antiplatelet therapies.
- **Light transmittance aggregometry**: This a widely used test that is still the gold standard. It is time-consuming and requires specialized training and experience. Platelet-rich plasma is prepared, and then platelet aggregation induced by an agonist such as collagen, arachidonic acid, or ADP is measured by quantifying the change in light transmittance through a test tube as aggregated platelets fall out of solution.
- **The VerifyNow rapid platelet function analyzer**: This system measures agonist-induced platelet agglutination in whole blood with fibrinogen-coated beads in an automated fashion.
- **The PFA-100 platelet function analyzer**: Automated system that draws whole blood at high shear over a collagen-epinephrine or collagen-ADP cartridge. Platelet function is determined by the time it takes for a clot to form and close a small orifice.
- **The single-platelet counting method (PlateletWorks)**: This test provides a standard complete blood cell count in addition to an assessment of platelet aggregation. Aggregation testing is based on performing a platelet count before and after intentional platelet

activation, using either collagen or ADP as the agonist. The procedure is simple, no special preparation is needed, and the results are available within minutes.

Many of these tests have been used in studies of variable response to antiplatelet therapy. However, as you will see in the discussions that follow, these tests have their limitations, and we still have much to learn about how to use the information these ex vivo measurements give us.

VARIABILITY IN RESPONSE TO ASPIRIN

Aspirin inhibits platelet aggregation by acetylating and therefore inhibiting platelet-derived prostaglandin H-synthase, also known as cyclo-oxygenase 1 (COX-1), which converts arachidonic acid into prostaglandin H2, a precursor of thromboxane A2. Thromboxane A2 is a vasoconstrictor and a stimulus for platelet aggregation.[8,9] Aspirin is rapidly absorbed from the stomach and has a half-life of 5 to 15 minutes in the circulation.

Aspirin is effective in both primary and secondary prevention of myocardial infarction (MI), stroke, and cardiovascular death and in the management of acute coronary syndrome and embolic stroke.[1–3,5] Small studies have suggested that 30 to 40 mg of aspirin daily would inhibit platelet aggregation and thromboxane A2 production as effectively as higher doses of aspirin.[9] Given that platelets are anucleic, the effect of aspirin lasts for the entire life span of the platelet, ie, 8 to 10 days.[8]

However, ex vivo tests of platelet function revealed that platelet response to aspirin varies from person to person. For example, Mehta at al[10] showed that a single 650-mg dose of aspirin produced only minimal platelet inhibition in 30% of patients with coronary artery disease. Other investigators[11–17] subsequently confirmed this interindividual variability in the ex vivo responsiveness to aspirin.

The clinical significance of this so-called nonresponsiveness or resistance has been investigated in a wide range of patients, with some studies suggesting that the variable response to aspirin may influence the outcome of patients at risk of atherothrombotic events. Grotemeyer et al[11] were among the first to show this correlation in a study of 180 patients with prior stroke. By measuring platelet reactivity 12 hours after an aspirin dose of 500 mg, they found that 33% of the patients had increased platelet activity, and they labeled these as nonresponders. After 2 years of follow-up, nonresponders were found to have a risk of thrombotic events (MI, recurrent stroke, or death) 10 times higher than that of prospectively identified aspirin-sensitive patients.[11]

In an another study of 100 patients with peripheral vascular disease who underwent elective percutaneous vascular angioplasty and were placed on 100 mg of daily aspirin, Mueller et al[18] reported a 40% incidence of aspirin resistance associated with an 87% increase in vascular reocclusion.

In a case-control study of 488 patients from the Heart Outcome Prevention Evaluation (HOPE) trial, Eikelboom et al[19] used urinary thromboxane levels as a surrogate of platelet activation and reported that patients in the highest quartile of 11-dTXB2 levels had a rate of thrombotic events 1.8 times higher than that of age-matched and sex-matched controls.[19] In a more recent study of 151 patients undergoing elective percutaneous coronary intervention (PCI), Chen et al[20] reported that those with inadequate response to aspirin, assessed by point-of-care testing with the Ultegra VerifyNow system, had an incidence of peri-PCI MI that was 2.9 times higher than in those with an adequate response.[20]

Studies show rates of aspirin resistance from 0.5% to 75%; such a range is not clinically useful

LIMITATIONS OF THE STUDIES

Looking at all the available data, one could conclude that the prevalence of aspirin resistance is as high as 75%[19] and as low as 0.5%[21] in a relatively similar group of patients. Such a wide range of reported prevalence is clearly not useful from a clinical or epidemiologic standpoint. Furthermore, even though the above studies suggest that we are able to measure clinically important outcomes based on the ex vivo responsiveness of platelets to aspirin, such a conclusion has flaws on closer inspection.

A common theme in the above investigations is that they assessed platelet function in

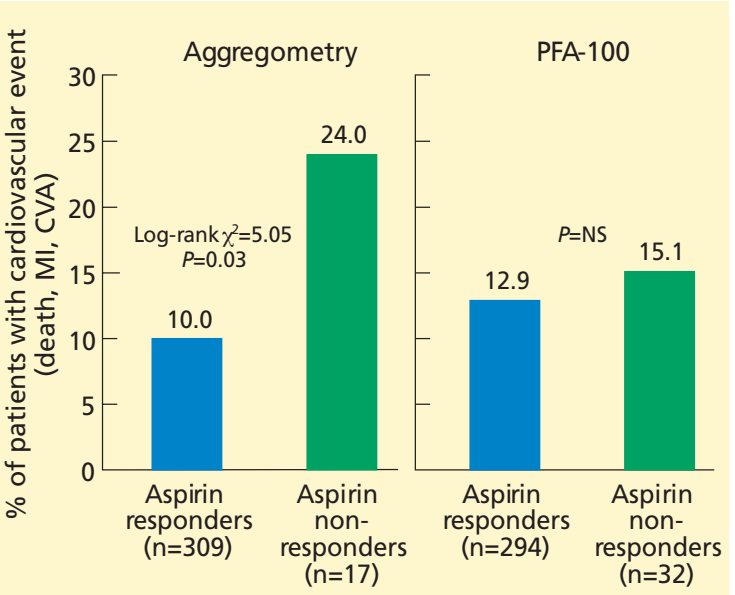

FIGURE 1. In a study of 326 patients undergoing cardiac catheterization, aggregometry identified 5.2% as nonresponders, whereas the PFA-100 platelet function analyzer identified 9.8%. Not only did the prevalence of nonresponders vary depending on the test used, but nonresponsiveness on one test had no correlation with nonresponsiveness on the other test in terms of clinical outcomes after a mean follow-up of 1.9 years. (MI = myocardial infarction, CVA = cerebrovascular accident)

ADAPTED FROM DATA FROM GUM PA, KOTTKE-MARCHANT K, POGGIO ED, ET AL. PROFILE AND PREVALENCE OF ASPIRIN RESISTANCE IN PATIENTS WITH CARDIOVASCULAR DISEASE. AM J CARDIOL 2001; 88:230–235; AND FROM 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; 41:961–965.

patients *already taking aspirin* and thus are not a true assessment of platelet responsiveness to aspirin. What has actually been measured is the patient's underlying platelet function plus the effect of aspirin. However, there is marked variability in platelet function among individuals even before they start aspirin therapy, and this variability itself may correlate with the risk of future thrombotic events.[22–24]

The study by Gum et al[25] points out another limitation in interpreting the results of platelet function tests when investigating antiplatelet resistance: the prevalence of nonresponders varied depending on which test was used, and aspirin nonresponsiveness identified by one test had no correlation with nonresponsiveness as determined by the other test (**FIGURE 1**).[24–26] Furthermore, in a follow-up study,[26] the investigators noted that the use of aggregometry to assess platelet responsiveness to aspirin correlated with clinical outcomes, whereas measuring platelet responsiveness with PFA-100 in the same patients did not have the same correlation (**FIGURE 1**). Despite this discrepancy, many investigators continue to use both techniques. In addition, patients in these studies are identified as "aspirin resistant," although this term means something completely different if it is measured by one technique vs another.

Finally, some tests used to assess platelet responsiveness have inherent limitations. For instance, measurements of urinary thromboxane metabolite are altered by systemic inflammatory conditions and by the degree of atherosclerosis, and not just by the degree of platelet inhibition caused by aspirin.[27–30] **TABLE 1** lists some of the possible mechanisms behind the variability in responsiveness to antiplatelet therapy.[31–43]

VARIABILITY IN RESPONSE TO ADP-RECEPTOR BLOCKERS

ADP binds platelets primarily through two G-protein receptors, P2Y1 and P2Y12. The stimulation of the P2Y1 receptor results in conformational changes in the platelets and induces transient and weak platelet activation. Activation of the P2Y12 receptor leads to sustained platelet aggregation and mediates thromboxane A2 production, platelet alpha-granule release, and the expression of P-selectin on the activated platelets, in addition to thrombus growth and stability.[44,45]

The thienopyridine clopidogrel is currently the most widely used ADP-receptor blocker. It is a pro-drug that requires oxidation by the hepatic cytochrome P450 enzyme to generate its active metabolite. Clopidogrel-induced platelet inhibition is both dose-dependent and time-dependent: a much faster onset of action but a similar final level of ADP-induced platelet inhibition has been reported with 600 mg of clopidogrel vs 300 mg.[46,47] In the CREDO trial (Clopidogrel for the Reduction of Events During Observation),[48] pretreatment with 300 mg of clopidogrel for at least 15 hours was needed to achieve the benefit of clopidogrel.[48]

As with aspirin resistance, the concept of clopidogrel resistance stems from investigations that revealed failure of clopidogrel therapy to inhibit ex vivo platelet function as assessed by a given test. Interindividual variability in clopidogrel-induced platelet inhibition has been consistently demonstrated.[49–51] In a study of 544 patients, Serebruany et al[51] showed that the response to clopidogrel was a normal bell-shaped distribution, consistent with polyenvironmental and polygenetic influences on responsiveness.[51] Hochholzer et al[52] confirmed this wide range of variability in response to clopidogrel in a study of 1,001 patients.

These studies clearly confirm that the ex vivo response to clopidogrel can vary substantially from patient to patient, but whether this variability has clinical implications is not known.

Muller et al[53] classified patients as nonresponders and semiresponders to clopidogrel (defined arbitrarily as a < 10% reduction and a 10%–29% reduction in platelet aggregation, respectively) in their study of 105 aspirin-treated patients with stable coronary artery disease who underwent elective PCI. Clopidogrel's inhibition of platelet aggregation was determined by the response to 5 μmol/L and to 20 μmol/L of ADP-induced aggregation 4 hours after treatment with 600 mg of clopidogrel. The reported prevalence of patients who were nonresponders and semiresponders varied depending on the ADP dose used to induce aggregation.[53] Of interest, 2 of the 105 patients studied had acute stent thrombosis and both patients were among those identified as clopidogrel nonresponders.

This correlation between thrombotic events and clopidogrel responsiveness as measured by platelet function tests was also supported by Matetzky et al,[54] who evaluated 60 patients with acute ST-elevation MI who were undergoing PCI. Patients in the lowest quartile of responsiveness to clopidogrel had a significantly higher risk of recurrent cardiovascular events.[54] Interestingly, another method of platelet function testing used in this study—the DiaMed Impact cone and platelet analyzer (DiaMed Israel Ltd)—did not identify clinically important differences in platelet inhibition.

TABLE 1

Possible mechanisms for the variability in response to antiplatelet therapy

Decreased bioavailability
Noncompliance[30]
Drug-drug interaction: for instance, concomitant use of a nonsteroidal anti-inflammatory drug that may inhibit aspirin-mediated COX-1 acetylation and attenuates the antithrombotic benefits of aspirin,[31] or concomitant use of cytochrome P450 3A4 inhibitors with clopidogrel[32]
Under-dosing[33]

Increased platelet function
Increased platelet COX-2 activity[34]
Accelerated platelet turnover[35]
Alternative pathways of platelet activation, such as catecholamine-induced platelet activation[36]

Genetic factors
Single nucleotide polymorphisms in genes coding for multiple platelet proteins (eg, receptor, enzymes)[37–39]

Clinical factors
Smoking[40]
Exercise[41,42]
Inflammation[43]

Platelet function varies markedly among patients, even before starting aspirin

As with aspirin resistance, the prevalence of clopidogrel resistance, based on the published data, varies widely, ranging from 5% to as high as 40%. Such a wide range of reported prevalence among similar patient populations can partly be explained by the mechanisms pointed out in TABLE 1. Furthermore, the variability in response to clopidogrel that was noted in the above-mentioned investigation was confounded by important limitations. The assessment of platelet function in patients already taking clopidogrel is not a true assessment of just the effect of clopidogrel, but rather a combined measurement of the patient's inherent platelet function plus the effect of clopidogrel, and sometimes even the effect of aspirin.

In addition, the absorption and metabolism of clopidogrel may explain some of the noticed variability in platelet response to clopidogrel.[46,47,52,55–57] A polymorphism of P2Y12 receptors has been identified, and while the impact of this polymorphism on platelet response to clopidogrel has not been clearly defined, it may still play a role in explaining some of the noted variability in the antiplatelet effect of clopidogrel.[23,58]

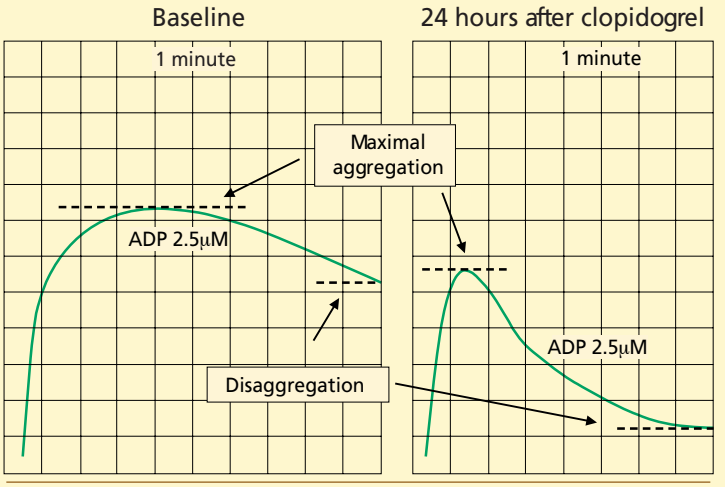

FIGURE 2. Aggregation in response to adenosine diphosphate (ADP) at baseline and 24 hours after 75 mg of clopidogrel. While the maximal aggregatory response to ADP is not significantly reduced by clopidogrel, the stabilization of aggregation (more reflective of P2Y12 receptors, the target of the active metabolite of clopidogrel) is significantly reduced.

ADAPTED WITH MODIFICATIONS FROM LABARTHE B, THEROUX P, ANGIOL 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; 46:638–645.

Lastly, but perhaps most importantly, the response to clopidogrel is influenced by which platelet function test is used, how the test is interpreted, and which anticoagulant is used in sampling. Labarthe et al[59] showed that a much lower rate of nonresponse to clopidogrel is noted when the assessment of platelet responsiveness to ADP focused instead on the stabilization of aggregation (partially reflective of the P2Y12 receptor activity that clopidogrel inhibits) rather than on the immediate ADP-induced platelet aggregation (partially reflective of the P2Y1 receptor which clopidogrel does not inhibit). This assessment combined with the use of antithrombins (rather than citrate) as preservatives reduced the nonresponder rate from 36% to 6%.[59]

ISSUES THAT STILL NEED ANSWERS

Clearly, since platelet responsiveness to antiplatelet therapy varies widely from person to person, and since evidence is mounting that patients who are nonresponders may be at increased risk of future atherothrombotic events despite chronic antiplatelet therapy, a one-dose-fits-all approach to antiplatelet therapy is flawed.

As with antihypertensive therapy, the response to antiplatelet therapy is likely influenced by multiple genetic and environmental factors. Ex vivo platelet responsiveness testing has been proposed as a way to determine a patient's level of response to antiplatelet therapy. But we are still unsure of the clinical significance of measuring platelet function ex vivo, and most importantly, we lack proof that changes in therapy based on ex vivo measurements can improve outcomes, so we are not yet ready for widespread clinical implementation of platelet responsiveness testing.

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ADDRESS: *Steven R. Steinhubl, MD, 900 South Limestone Street, 326 Charles T. Wethington Building, Lexington, KY 40536-0200; e-mail Steinhubl@uky.edu.*