Platelet Function Testing in Patients with Coronary Artery Disease: Is the Who and the When any Clearer than the What and the What Then?

Running title: Rade; Platelet function testing and CAD

Jeffrey J. Rade, MD
University of Massachusetts Medical School, Worcester, MA

Address for Correspondence:
Jeffrey J. Rade, MD
Division of Cardiology
University of Massachusetts Medical School
55 Lake Avenue North
Worcester, MA 01655
Tel: 774-441-6310
Fax: 774-441-6303
E-mail: jeffrey.rade@umassmed.edu

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Antiplatelet therapy is one of the central pillars of treatment for patients with coronary artery disease. Aspirin and clopidogrel, the most widely used ADP-receptor antagonist, have each been shown to incrementally reduced the risk of recurrent adverse cardiovascular events by approximately 20%.1,2 These agents inhibit distinct but interrelated signaling pathways that mediate and potentiate platelet aggregation at sites of vascular injury. Exposure to subintimal collagen, von Willebrand factor and strong agonists such as thrombin stimulate platelets to generate thromboxane A₂ (TXA₂) through cyclooxygenase-1 (COX-1)-mediated metabolism of arachidonic acid and to release of ADP stored in dense granules. TXA₂ and ADP not only promote sustained activation of the platelet in which they are formed, but both are also released and amplify the thrombotic stimulus by activating adjacent quiescent platelets via binding to specific membrane receptors. Aspirin and clopidogrel synergistically suppress platelet activation and thrombosis by irreversibly binding to and inhibiting the COX-1 enzyme and the P₂Y₁₂ ADP receptor, respectively.

In the last decade, it has become clear that many patients taking aspirin and clopidogrel do not realize the full biologic effects of these agents and remain at increased risk for cardiovascular events. This apparent “non-responsiveness” or “resistance” has been revealed by a host of studies, utilizing a wide array of different types of platelet function and biochemical assays, indicating that high degrees of platelet reactivity persist despite use of these agents.3,4 The term high on-treatment platelet reactivity (HTPR) is a now frequently used to describe this phenomenon. Complicating the picture is the fact that TXA₂ and ADP are just two of a number of signaling pathways that mediate platelet activation and many platelet function assays do not specifically measure their effects in isolation (reviewed in reference 5). Thus, not all instances of HTPR are necessarily due to aspirin or clopidogrel resistance. Strictly defined, “resistance” is the

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failure of an agent to inhibit its biologic target. Studies utilizing assays that specifically measure COX-1 activity, such as arachidonic acid platelet aggregometry or serum TXB₂ formation, reveal that true aspirin resistance in compliant subjects is uncommon, with a prevalence of <1-5% in several large studies.⁶-⁸ In contrast, aspirin HTPR detected by assays measuring more global platelet function, such as the Platelet Function Analyzer-100 (PFA-100; Siemens HealthCare Diagnostics, Newark, DE) utilizing the collagen/epinephrine agonist cartridge, is significantly more common (prevalence 20-40%) and heavily influence by the degree of underlying platelet reactivity and level of von Willebrand factor in addition to platelet COX-1 activity.⁶-¹⁰

HTPR is also frequently observed following clopidogrel administration, with a prevalence of 10-45% depending on the assay used and patient population studied.⁴,¹¹ Unlike aspirin, a more substantial portion of this residual platelet reactivity is readily attributable to clopidogrel resistance owing to the inefficiency and inter-individual genetic variability in its bioactivation.⁴ Clopidogrel effect is best detected by assays that specifically measure P2Y₁₂ ADP receptor activation, such as vasodilator-stimulated phosphoprotein (VASP) phosphorylation and to a lesser extent, the VerifyNow P2Y₁₂ test (Accumetrics, San Diego, CA) and ADP platelet aggregometry.⁵,¹² Aspirin and clopidogrel HTPR frequently coexist in the same patient.¹³ This observation, along with studies correlating pre-and post-treatment platelet function studies testify to the importance of pretreatment platelet hyper-reactivity as a modifier of antiplatelet drug effect.¹⁴

Whether due to drug resistance, underlying platelet hyper-reactivity, or both, HTPR has been linked to increased risk of recurrent cardiovascular events. This has most convincingly been demonstrated in patients taking clopidogrel following recent acute coronary syndrome and/or percutaneous coronary intervention (PCI) with stent placement. A meta-analyses of twenty
studies comprising 9187 PCI patients observed clopidogrel HTPR to be present in a third of patients and associated with 3 to 4-fold increases in the risk of myocardial infarction, stent thrombosis and death.\textsuperscript{15} In a recent analysis of the landmark Gauging Responsiveness With a VerifyNow P2Y12 Assay: Impact on Thrombosis and Safety (GRAVITAS) trial, clopidogrel HTPR defined as \textgeq 208 P2Y12 reaction units occurred in half of all subjects and was associated with a \textgeq 5-fold increased risk of cardiovascular death, myocardial infarction and stent thrombosis at 60 days.\textsuperscript{16} Though less widely studied, aspirin HTPR has similarly been linked to increased cardiovascular risk. In a meta-analysis of 20 studies involving nearly 3000 subjects, aspirin non-responsiveness by a variety of assays occurred in 28\% and was associated with nearly a 4-fold increased risk of adverse cardiovascular events.\textsuperscript{9}

One open question has been whether measuring HTPR in patients with chronic stable coronary disease would have the same prognostic value as it does in a more acute patient population. There are several reasons to think that it may not. For one, a newly placed stent or ruptured plaque is inherently more thrombogenic than a fully endothelialized stent or a healed/quiescent plaque. Additionally, underlying platelet reactivity and the prevalence of aspirin and clopidogrel HTPR have both been observed to be greater during the acute phase of a cardiovascular illness than during the chronic phase.\textsuperscript{7,17,18} Two recent studies begin to examine this question.

In this issue of Circulation, Reny and colleagues report the findings of the Antiplatelet Drug Resistance and Ischemic Events (ADRIE) study.\textsuperscript{19} In the ADRIE study, 771 subjects with stable coronary artery disease chronically treated with aspirin, clopidogrel, or both were subjected to a battery of platelet function studies detecting the presence of aspirin and/or clopidogrel HTPR. The subjects were followed for 3 years with the primary outcome being a
composite endpoint of acute coronary syndrome, revascularization, stroke/transient ischemic attack, acute limb ischemia or cardiovascular death. Laudable characteristics of the multicenter study design were the exclusion of subjects who were within 1 month of an acute ischemic event or revascularization procedure and the serial assessment of HTPR using assays that were specific for platelet COX-1 activity (arachidonic acid platelet aggregometry and serum TXA₂ formation) and P2Y12 ADP receptor activity (VASP phosphorylation) in addition to tests of more global platelet function (collagen and ADP platelet aggregometry, PFA-100 using the collagen/epinephrine agonists cartridge). The presence of HTPR was defined for each assay based on values obtained from the literature. The composite endpoint occurred in 16% of subjects and by multivariate analysis was associated with the traditional risk factors of hypertension, smoking, advanced age and elevated LDL. There was neither an association between outcome and presence of HTPR, as determined by any of the platelet function assays, nor did inclusion of assay data in the multivariate modeling improve its predictive value. Though the study was adequately powered, the prevalence of HTPR was unusually high for assays measuring aspirin effect. For example, 73% of subjects were classified as having aspirin HTPR based on serum TXA₂ formation ≥12 ng/mL. The cutoff for serum TXA₂ formation was obtained from the literature and may have not been appropriate for the laboratory methodology used in this study. By arachidonic acid-induced platelet aggregation ≥20%, 30% of subjects were defined as having aspirin HTPR. This is also higher than expected, though it appears that some subjects not taking aspirin were included. More convincing, however, were the data for assays assessing clopidogrel HTPR or global platelet reactivity.

In a second relevant study, Pettersen and colleagues report in the latest issue of the Journal of the American Heart Association the results of the Aspirin Non-responsiveness and
Clopidogrel in Clinical Endpoint Trial (ASCET). In ASCET, 1001 subjects with angiographically-documented but stable coronary artery disease on aspirin monotherapy were randomized to continue on aspirin or switch to clopidogrel for a minimum period of 2 years. At the time of randomization, serum TXA$_2$ formation was measured and aspirin HTPR assessed by PFA-100 using the collagen/epinephrine agonist cartridge. The primary endpoint, a composite of acute coronary syndrome, non-hemorrhagic stroke or death, occurred in 106 (11%) subjects. Serum TXA$_2$ formation was low (mean 3 ng/mL, range 0-21 ng/mL), indicating a high degree of platelet COX-1 inhibition in nearly all subjects. Despite this, 26% of subjects had HTPR as defined by the PFA-100 assay using an accepted cutoff value. There was no significant difference in the primary endpoint between subjects with and without HTPR (13% versus 10%, p=0.3) or based on treatment with aspirin or clopidogrel (13% versus 8%, p=0.2). The major limitation, acknowledged by the authors, was that the study was underpowered due to a significantly lower number of adverse events than was predicted. Thus, it is difficult to make any firm conclusions regarding the utility of using the PFA-100 to risk stratify patients with stable coronary artery disease. These data, however, provide more evidence that this assay is measuring something beyond aspirin-mediated inhibition of platelet COX-1 and thus should not be used to guide aspirin therapy.

In summary, ADRIЕ and ASCET are two relatively large studies investigating the utility of performing platelet function testing in patients with chronic stable coronary artery disease for the purpose of risk stratification. While each had limitations, their collective message suggests that this utility is likely to be low, especially in an unselected patient population. While this should be somewhat reassuring to clinicians, a more definitive answer awaits further investigation. More pressing, however, is the need for answers to unresolved questions.
surrounding platelet function testing in acute patient populations. While relatively more is known about the relationship between the presence of HTPR and adverse outcome in the acute setting, ambiguity still exists regarding what assay or assays best identify risk and, more importantly, what treatment or treatments will effectively modify that risk.

**Conflict of Interest Disclosures:** Dr. Rade is party to a licensing agreement between Siemens Healthcare Diagnostics, manufacturer of the PFA-100, and Johns Hopkins University.

**References:**


