Assessing the Current Role of Platelet Function Testing

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ABSTRACT

In vitro platelet function tests are commonly applied in research and offer justification for using antiplatelet therapy. However, studies assessing the ability of standardized platelet function tests to predict patients' clinical response to aspirin or clopidogrel have generated contradictory results. At this time, there is no standardized definition for resistance to antiplatelet therapy, and the appropriate treatment of patients who are hyporesponsive to these agents is not known. Although such tests have a role in research, their place in guiding therapy remains to be established, and prospective trials are urgently needed. The ideal platelet function test for clinical practice would be rapid, easy-to-use, inexpensive, and reliable.

Key words: catheterization/diagnostic interventional <cardiac, acute coronary syndromes <ischemic heart disease, platelets, thrombosis/hypercoagulable states

Introduction

Tests of platelet function have been used in multiple ways in both clinical and research settings.[1,2] They were first employed to screen patients for bleeding disorders, and then to predict the risks of procedural bleeding and thrombosis. More recently, with the advent of more targeted prohemostatic and antiplatelet therapies, platelet function tests have been used to assess medication effectiveness, both during drug development and in clinical situations.

For patients with cardiovascular disease (CVD), the major applications of platelet function tests include the prediction of clinical outcomes and the monitoring of antiplatelet therapy. The key question relating to these issues is whether standardized laboratory tests of platelet response to aspirin (ASA) or clopidogrel therapy are predictive of the patient's clinical response to these agents. This article discusses the currently available tests of platelet function, their advantages and drawbacks, and their relationship to clinical outcomes of antiplatelet therapy. Current guidelines for platelet function testing and suggestions for additional research are also discussed.

Current Platelet Function Tests

Table 1 details some of the characteristics of the most commonly used platelet function tests.[3,4] The historical "gold standard" is turbidometric platelet aggregometry, which measures platelet coaggregation in platelet-rich plasma (Figure 1).[5] Samples are exposed to an agonist, such as adenosine diphosphate (ADP) or arachidonic acid, and the increase in light transmittance resulting from platelet–platelet aggregation is measured. Its advantages are that it can be used to monitor ASA, thienopyridines, and platelet glycoprotein (GP) IIb/IIIa inhibitor therapy.[3] Its disadvantages include the large sample volumes required, long processing times, and complex sample preparation. Impedance aggregometry (Figure 2) is conceptually similar to turbidometric platelet aggregometry, but, rather than light transmission, it measures the increase in electrical impedance across two precious metal wires that results from platelet coaggregation in response to an agonist.[5] It has the same disadvantages as turbidometric platelet aggregometry, but it uses whole blood instead of platelet-rich plasma.[3]

Other aggregation tests include the VerifyNow test (Figure 3) (Acumetrics, San Diego, Calif., USA), a simple,

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TABLE 1: Current clinical tests of platelet function

Test	Advantages	Drawbacks	Able to monitor: ASA	Thieno-Pyridines	GP IIb/IIIa Inhibitors
Platelet–Platelet Aggregation					
Platelet aggregometry: Turbidometric	Historical "gold standard"	Variable reproducibility; expensive; large sample volume; sample preparation; time-consuming	Yes[a]	Yes[a,b]	Yes
Platelet aggregometry: Impedance	Whole-blood assay	Expensive; large sample volume; sample preparation; time-consuming	Yes[a]	Yes[ab]	Yes
VerifyNow	Simple; rapid; POC; small sample volume; no sample preparation; whole-blood assay	No instrument adjustment	Yes[c]	Yes[d]	Yes[e]
Plateletworks	Little sample preparation; whole-blood assay	Not well studied	Yes[a]	Yes[b]	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[a]	Yes[b]	Yes
Platelet contribution to clot shear elasticity					
Thromboelastogram	POC; whole-blood assay; platelet clot formation and clot lysis data	Limited studies	Yes[a]	Yes[b]	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	Yes[a]	Yes[b]	NR
Cessation of blood flow by platelet plug (for PFA, at high shear)					
PFA-100	Simple; rapid; small sample volume; no preparation; whole-blood assay	No instrument adjustment; depends on vWF, Hct	Yes	NR	NR
Activation-dependent signaling					
VASP phosphorylation	Low sample volume; whole-blood assay; $P2Y_{12}$-dependent	Sample preparation; expensive; requires flow cytometer, experienced staff	No	Yes	No
Activation-dependent release from platelets					
Serum thromboxane B_2	COX-1–dependent	Indirect; not platelet-specific	Yes	No	No
Urinary 11-dehydro-thromboxane B_2	COX-1–dependent	Indirect; not platelet-specific; depends on renal function	Yes	No	No

Adapted with permission from Michelson AD, et al.[4] *Abbreviations:* ASA = aspirin; COX = cyclooxygenase; GP = glycoprotein; Hct = hematocrit; NR = not recommended; PFA = platelet function analyzer; POC = point of care; VASP = vasodilator-stimulated phosphoprotein; vWF = von Willebrand factor; [a]With arachidonic acid; [b]With ADP; [c]With ASA cartridge; [d]With $P2Y_{12}$ cartridge; [e]With TRAP cartridge.

rapid, point-of-care method that has several other advantages: required sample volumes are small, it uses whole blood, and no pipetting is required.[5] VerifyNow has been used to monitor the pharmacodynamic effects of the 3 main classes of antiplatelet therapies—ASA, thienopyridines, and GP IIb/IIIa inhibitors.

Other methods assess activation-dependent changes on the platelet surface. These tests include measurement of levels of platelet surface P-selectin, activated GP IIb/IIIa, and leukocyte–platelet aggregation. Their advantages include the small sample volumes required and the use of whole blood; disadvantages include complex sample preparation, the requirement for flow cytometry and experienced operators, and lack of commercial availability. They have been used to monitor the various classes of antiplatelet therapies.

The thromboelastogram (TEG) Platelet Mapping System (Figure 4) measures platelet contribution to clot strength.[5] It is a point-of-care method that uses whole blood to assess platelet clot formation and clot-lysis data. It is able to monitor all 3 classes of antiplatelet therapies. However, it requires pipetting and has undergone only limited study. The Impact cone and plate(let) analyzer is a simple, rapid, point-of-care method that assesses shear-induced platelet adhesion.[6] It uses whole blood, requires low-sample volumes, and needs no sample preparation. The drawbacks include the need for pipetting and lack of widespread availability. It is not recommended for monitoring of GP IIb/IIIa inhibitor therapy.

The Platelet Function Analyzer (PFA)-100 measures in vitro the cessation of high-shear blood flow by the platelet plug. It is a simple, rapid, point-of-care, whole blood method that requires low sample volumes and no sample preparation. Its disadvantages are that it is dependent on von Willebrand factor (vWF) and hematocrit levels and that it requires pipetting. It is not recommended for monitoring of thienopyridines.[6]

Vasodilator-stimulated phosphoprotein (VASP) phosphorylation measures activation-dependent platelet signaling. Its advantages include small required sample volumes, the use of whole blood, stability (allowing samples to be shipped to a remote laboratory), and dependency on the $P2Y_{12}$ receptor, the site of action for thienopyridines. Its disadvantages are that it requires complex sample preparation, flow cytometry, and experienced technicians, and cannot be used to monitor ASA or GP IIb/IIIa inhibitor treatment.[6]

The serum thromboxane B_2 level reflects its activation-dependent release from platelets. Its chief advantage is that it is dependent on cyclooxygenase (COX)-1, the specific enzyme inhibited by ASA. However, thromboxane B_2 levels may be influenced by prostaglandins produced by leukocyte-derived COX-2. Therefore, thromboxane B_2 is not entirely

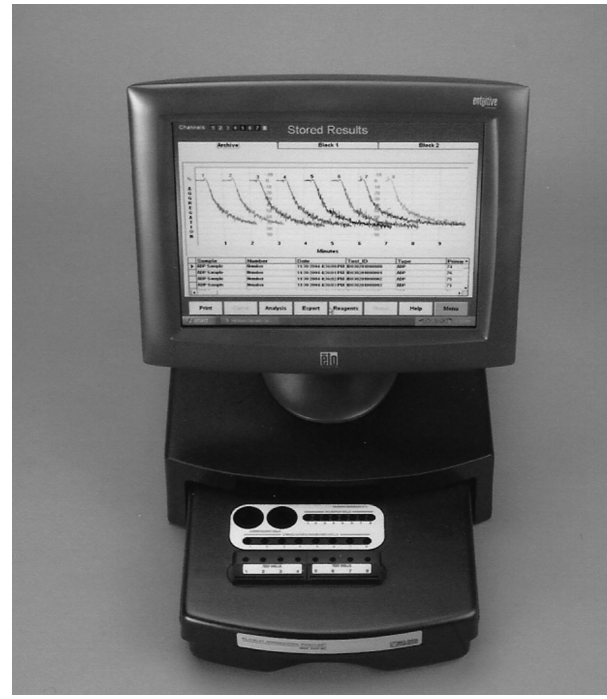

Figure 1: Turbidometric platelet aggregometer. It measures the decrease in light transmittance that results from platelet coaggregation in platelet-rich plasma, when samples are exposed to an agonist such as ADP. Reproduced with permission from Harrison P et al.[5]

COX-1 or platelet specific. This indirect measure is not completely platelet-specific, however. These characteristics also apply to measurements of the ratio of the stable urinary metabolite of thromboxane B_2, 11-dehydro-thromboxane B_2 (UDTB) to creatinine. These tests cannot be used to monitor thienopyridines or GP IIb/IIIa inhibitor therapy.

The Relationship between Results of In Vitro Platelet Function Tests and Clinical Response to Antiplatelet Therapy

When reviewing studies that have reported a link between laboratory measures of platelet function and outcomes of antiplatelet therapy in patients with CVD, it is important to keep several cautions in mind. First, these studies have generally included small numbers of patients (resulting in relatively small numbers of events) and have been conducted at single centers, precluding definitive conclusions. There is an absence of serial measurements. The known bias toward publication of "positive" studies must also be considered. However, the concordance in reports should also be recognized. It may also be difficult to assess the relationship between in vitro platelet reactivity and clinical outcomes because of inter- and intrapatient variability in platelet responses to agonists such as ADP.[7] Patient noncompliance with study medication is also an important practical issue because a noncompliant patient's platelets will appear not to respond well to the putative ingestion of the antiplatelet agent.

Aspirin: Eikelboom and colleagues studied whether hyporesponse to ASA treatment was related to the risk of cardiovascular events, in a substudy of the Heart Outcomes Prevention Evaluation (HOPE) secondary prevention trial.[8] Baseline UDTB levels were compared among 488 patients

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Figure 2: Impedance platelet aggregometer. It measures the increase in electrical impedance across two precious metal wires that results from platelet coaggregation in whole blood, when samples are exposed to an agonist such as ADP. Reproduced with permission from Harrison P et al.[5]

receiving ASA who had myocardial infarction (MI), stroke, or cardiovascular death over 5 years of follow-up and 488 sex- and age-matched control patients taking ASA who had no such event. The adjusted odds ratio (OR) for the composite endpoint was 1.8 times higher for patients in the upper quartile of UDTB level compared with patients in the lowest UDTB quartile ($p = 0.009$) (Figure 5). Similar patterns were shown for the component events of MI (adjusted OR, 2.0; $p = 0.006$) and cardiovascular death (adjusted OR, 3.5; $p<0.001$), but not for stroke. Thus, elevated UDTB levels appeared to identify patients whose platelets responded suboptimally to ASA treatment in this study.

Gum and colleagues assessed the relationship between hyporesponse to ASA treatment and the composite incidence of death, MI, or stroke in 326 US patients with stable CVD.[9] Hyporesponse was defined as mean platelet aggregation of $\geqslant 70\%$ in response to 10μmol/L ADP agonism and aggregation $\geqslant 20\%$ with 0.5 mg/mL arachidonic acid

Figure 3: The VerifyNow platelet aggregometer. Reprinted with permission from Harrison P et al.[5]

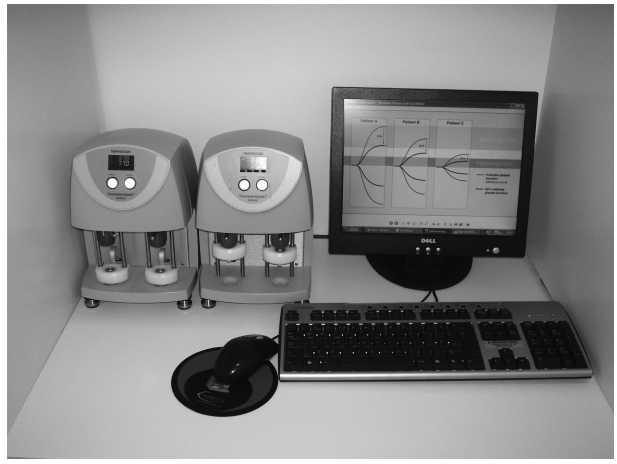

Figure 4: The Thromboelastogram (TEG) Platelet Mapping system. Reproduced with permission from Harrison P et al.[5]

stimulation. In all, 5.2% of patients showed hyporesponse to ASA. Of these patients, 24% reached the composite endpoint over a mean 1.8 years of follow-up versus only 10% of patients with a "normal" response ($p = 0.03$). However, the relationships between hyporesponse (whether a categorical or continuous variable) and component event rates were not statistically significant. Of note, these same investigators had previously shown that hyporesponse as defined above did not correlate well with hyporesponse as assessed by the PFA-100 test (defined as having a normal collagen and/or epinephrine closure time) in this population.[10]

Ohmori and colleagues assessed the relationship between platelet hyporesponse to ASA therapy and clinical outcomes in 136 patients with stable CVD.[11] They assessed platelet responses to collagen stimulation by both standard light-transmittance aggregometry and by light-scattering intensities with a PA-20 aggregation analyzer (reflecting small, medium, or large aggregates). Patients in the upper quartile of aggregation by light transmittance, or who had large aggregates, had a significantly and independently increased risk of MI, cerebrovascular infarction, or cardiovascular death at 12 months (hazard ratio [HR], 7.76; $p = 0.008$ for light transmittance aggregometry; HR, 7.98; $p = 0.007$ for large aggregates).

Chen and colleagues examined the relationship between serum markers of myonecrosis after nonurgent percutaneous coronary intervention (PCI) and hyporesponse to ASA therapy among 151 patients pretreated with clopidogrel.[12] Response to ASA was assessed by a commercially available, point-of-care assay, the VerifyNow Ultegra Rapid Platelet Function Assay-ASA (RPFA-ASA). Hyporesponse to ASA was defined as an ASA reaction unit of $\geqslant 550$ at baseline (before clopidogrel treatment and PCI) blood sampling. Myonecrosis was measured by creatine kinase-myocardial band (CK-MB) and troponin I (TnI) elevations after PCI. In all, 19.2% of the patients were hyporesponsive to ASA, and significantly more of these patients were women. After clopidogrel treatment and PCI, 51.7% of the ASA-hyporesponsive patients showed elevated CK-MB levels compared with 24.6% of the ASA-responsive patients ($p = 0.006$); 65.5% and 38.5% of patients, respectively, showed elevations in TnI

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levels ($p = 0.012$). Hyporesponse to ASA was one of two independent predictors of CK-MB elevation after PCI in multivariable analysis (OR, 2.9; $p = 0.015$). Thus, despite adequate pretreatment with clopidogrel, patients with a lesser response to ASA treatment in this study remained at increased risk of myonecrosis after PCI.

Clopidogrel: Matetzky and colleagues examined the relationship between hyporesponse to clopidogrel treatment and clinical outcomes in 60 consecutive patients with MI undergoing primary PCI with stenting at one center.[13] All received clopidogrel, ASA, and eptifibatide. Controls were 10 consecutive patients undergoing primary PCI alone who received no clopidogrel. Platelet aggregation was assessed by cone and plate(let) analysis and aggregation responses to 5μmol/L ADP and 10μmol/L epinephrine. Patients were first grouped into quartiles by percent reduction in ADP-induced aggregation from baseline to Day 6; values ranged from 103% of the baseline value in the first quartile (clopidogrel hyporesponse; $n = 15$) to 33% of the baseline value in the fourth quartile ($p<0.01$ across groups). Similar patterns were shown for epinephrine-induced aggregation and the cone and plate(let) analysis. Patients in the clopidogrel-hyporesponse group experienced 7 of the 8 major cardiac events that occurred during the 6-month follow-up. In all, 40% of these patients had another ischemic event, whereas 13% of patients in the fourth quartile had major bleeding. Angiographic findings and infarct size were not different across groups. Of note, patients with recurrent events were older and had a higher Killip class at presentation, and the analysis did not adjust for these or other risk factors. The investigators did not report data regarding a correlation between cone and plate(let) analysis and clinical outcomes.

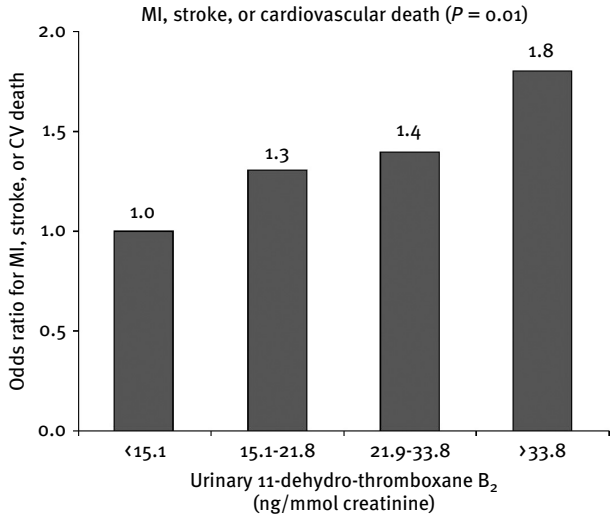

Figure 5: Risk of myocardial infarction, stroke, or cardiovascular death by quartile of urinary 11-dehydro-thromboxane B_2 level among patients in a substudy of the HOPE secondary prevention trial of ASA. Adapted with permission from Eikelboom JW et al.[8]

Gurbel and colleagues used VASP phosphorylation to examine the relationship between platelet reactivity to clopidogrel treatment and stent thrombosis.[14] They compared platelet reactivity to ADP and $P2Y_{12}$ activity in 20 patients who developed subacute stent thrombosis after clopidogrel therapy versus that of 100 age-matched controls. Both measures were significantly increased in patients with thrombosis, but there was substantial overlap in values between groups. Of note, patients with <40% aggregation had no stent thrombosis, representing a possible threshold effect. Other investigators have reported a relationship between incomplete $P2Y_{12}$ receptor blockade by clopidogrel as assessed by VASP phosphorylation and stent thrombosis and greater shear-induced platelet aggregation in patients with stent thrombosis compared with thrombosis-free patients or volunteers.[15,16]

Finally, Wenaweser and colleagues have studied the relative associations between ASA and clopidogrel hyporesponse and subacute stent thrombosis.[17] They compared 23 patients with previous stent thrombosis (median, 7 days after implantation) at 1 center with 50 patients who had undergone stenting without developing thrombosis and with 9 healthy volunteers. Platelet aggregation in response to 5 and 20μmol/L ADP was assessed via optical aggregometry during 1 month of ASA monotherapy and during a second month of combined ASA and clopidogrel treatment (after a 300 mg loading dose of clopidogrel). Maximal platelet aggregation was significantly higher in patients with stent thrombosis than in controls or volunteers. Hyporesponse to ASA, defined as >20% aggregation in response to 0.5 mg/mL arachidonic acid, was more common in patients with stent thrombosis (48%) than in control patients (32%, $p = NS$) or volunteers (0%, $p = 0.01$). Hyporesponse to clopidogrel, defined as a less than 10% relative change in aggregation, did not differ significantly among groups (4% of patients with stent thrombosis, 6% of control patients, and 11% of the volunteers, respectively, all $p = NS$). Hyporesponse to both ASA and clopidogrel, however, was more common in patients with stent thrombosis (52%) than in controls (38%, $p = NS$) or volunteers (11%, $p<0.05$). The investigators concluded that hyporesponse to ASA, but not to clopidogrel may be associated with a risk of thrombosis after stenting.

Current Role of Platelet Reactivity Testing

According to the most recent consensus statement on the use of antiplatelet agents and position paper on ASA "resistance" from the International Society on Thrombosis

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and Haemostasis, it is currently not recommended to test patients for ASA or clopidogrel resistance outside of clinical trials or to change therapy based on such testing.[18,19] There are two main reasons for these recommendations. First, a clinically meaningful, standardized definition of resistance based on data linking therapy-dependent laboratory tests to clinical outcomes has yet to be developed.[19] Second, the correct treatment of patients whose platelets are hyporesponsive to antiplatelet agents is unknown, given that no study has assessed clinical effectiveness after altering therapy based on laboratory findings of hyporesponse.[19]

The American College of Cardiology/American Heart Association/Society for Cardiovascular Angiography and Interventions guidelines recommend daily ASA therapy after PCI for patients without ASA "resistance," but no definition of resistance is offered.[20] For clopidogrel, the guidelines state that " . . .in patients in whom stent thrombosis may be catastrophic or lethal. . . platelet aggregation studies may be considered and the dose of clopidogrel increased to 150 mg per day if less than 50% inhibition of platelet aggregation is demonstrated." This is a Class IIb, level C recommendation, indicating that there is disagreement over whether the intervention is considered beneficial, and that the recommendation reflects only consensus opinion, not data from randomized clinical trials. Finally, the method to assess platelet inhibition is not described.

Adequately powered clinical trials are urgently needed to address 3 pivotal questions. First, which simple, inexpensive, and rapid test of platelet function (or combination of tests) best predicts clinical outcomes of antiplatelet therapy for specific patient subgroups? Second, are individual outcomes affected when treatment is changed in response to the test(s) results? The goal here would be development of thresholds for test results, similar to the practice of adjusting warfarin therapy by international normalized ratio (INR) results.[21,22] Third, what is the benefit of dose titration or supplementary treatment with an agent having a different mechanism of action in patients with known hyporesponse to antiplatelet agents? For example, if a patient with CVD suffers an event while already taking ASA, is it more effective to increase the dose of ASA, add a thienopyridine for additional secondary prevention, or both, rather than to simply substitute the thienopyridine for ASA? Recent reports of early and late thrombosis of drug-eluting stents after interruption of antiplatelet therapy have raised concern about stopping antiplatelet therapy altogether after an acute cardiovascular event, but there are few data to guide clinicians in this regard.[23–25] Clinical trials can provide the robust evidence needed to answer these questions, which are so important to clinicians.

Conclusions

Tests of platelet function in vitro are widely used in research and provide a mechanistic rationale for antiplatelet therapy. Correlating the results of such tests with clinical outcomes and using the results to guide therapy, however, remain challenging goals. The ideal test of platelet function for use in clinical practice would be rapid, easy-to-use, inexpensive, and would be a reliable indicator of the clinical response to the specific antiplatelet therapy or combination of therapies. Prospective clinical trials are now needed.

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