

Application of platelet function testing to the bedside

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Summary

The ability to test platelet reactivity in clinical practice could help in making informed decisions on both initiation and titration of anti-platelet drug therapies. However, many barriers still remain to the effective implementation of such techniques. Many tests used in the research literature are not yet available for practical, clinical use. Platelet aggregometry, while informative and currently available for bedside use, needs addi-

tional research before routine clinical use can be recommended. This review will highlight and update contemporary issues of bedside platelet testing for the clinician and comment on future areas of clinical research.

Keywords

Clopidogrel, aspirin, platelet aggregation, platelet function tests, anti-platelet tests

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Introduction

Five years before the mechanism of action of aspirin was described, inter-individual variability in the bleeding response was noted (1–3). Since then, methods to quantify the effects of aspirin and other, newer anti-platelet agents have proliferated (4–7). In addition to the more established turbidimetric aggregometry techniques first described 20 years ago, several newer point-of-care testing modalities are now available (4). Many questions remain, however (8–12). Which tests can or should be used in the clinical setting? Are they appropriate in acute or chronic care? What is the correlation of these tests with future clinical events? Many of the current studies which attempt to address these questions are retrospective and confounded by the use of non-randomised groups for comparison (13, 14). Thus, while high post-treatment platelet reactivity and future ischaemic events are clearly related, it remains unclear if this relationship is causal (12, 15, 16). While definitive answers can only come from prospectively designed trials, there is a lot that the practicing clinician should know about currently available tests and the evidence both for and against their use.

Discussion

Aspirin, clopidogrel or both (dual therapy) have become standard therapy for acute and chronic atherothrombotic diseases (17). The occurrence or reoccurrence of cardiovascular disease (CVD) events despite indicated doses of these agents has been termed “resistance” (18). But this definition is too broad, implying that optimal dosing of antiplatelet agents could eliminate CVD events. More recently, functional tests of platelet reactivity have increased awareness of the

range of response that patients have to any class of anti-platelet medications (19–22). Subsequently, the term resistance has become somewhat arbitrary as different methods of *ex vivo* testing define their own cut-offs for what constitutes a response to therapy and what constitutes resistance (4). A brief review of platelet activation helps to explain this high rate of variability of surrogate testing.

Platelet physiology and pharmacology

Following the initial adhesion of platelets to a site of vascular injury, a variety of platelet and vascular-derived mediators are produced to accelerate adhesion and vascular repair (23, 24). Known mediators include adenosine diphosphate (ADP), thrombin, epinephrine and thromboxane A₂ (24). When stimulated, the capacity of platelets to make thromboxane A₂ rises nearly 1,000-fold but is variable (23). The variation in these pathways of activation mean that any one therapeutic modality may fail to adequately suppress platelet responsiveness (5, 11, 15, 25). While this has been labelled drug ‘resistance,’ it is more accurately described as platelet resistance and it contributes to the poor inter and intra-subject reproducibility of surrogate platelet testing (9, 12, 24).

Bedside testing

Several modalities are currently available which offer true bedside platelet testing. These include VerifyNow[®], PFA-100[®], and the Impact[®] cone and platelet analyzer.

VerifyNow[®], also identified in the literature as rapid platelet function assay (RPFA), was used early in the acute care setting in patients receiving glycoprotein (GP) IIb/IIIa receptor antagonists (20, 26). This method uses fibrinogen-coated beads which adhere to stimulated platelets. This adhesion or agglutination causes the beads to precipitate out of solution which increases the transmission of light through the sample. The change in light transmission is compared to a control and a unit of platelet activation is assigned to the result. Three different VerifyNow[®] assays are available (27–29). For clopidogrel testing, adenosine diphosphate is added to the assay and the result is reported in P2Y₁₂ reaction units (PRU). For aspirin testing, arachadonic acid is added to the assay and the result is reported in aspirin reactive units (ARU). The original RPFA for GP IIb/IIIa receptor antagonists reports a result in platelet activation units (PAU). Because the assay for GP IIb/IIIa receptor antagonists does not have arachadonic acid or ADP present, clopidogrel and aspirin do not cross react or interfere with that assay (27). However, because fibrinogen binding to GP IIb/IIIa receptors is the final step in aggregation, the presence of a GP IIb/IIIa receptor antagonist interferes with both the clopidogrel and aspirin assay and these assays should not be used in a patient who has received a GP IIb/IIIa receptor antagonist within 48 hours for eptifibatid and tirofiban and 14 days for abciximab (28, 29).

The PFA-100[®] is an *in vitro* surrogate test for bleeding time. The test measures the slowing and cessation of blood flow under conditions of shear stress and reports the time for closure of an aperture with a platelet plug. Similar to VerifyNow[®], the test uses whole blood which does not require any additional preparation. The application of shear stress to the testing modality is attractive since it begins to mimic the *in vivo* conditions that platelets experience in patients with occlusive arterial diseases. Two different testing cassettes are available, one with collagen and epinephrine and one with collagen and ADP. While the PFA-100[®] has been widely used for aspirin resistance testing and can be used to qualify GP IIb/IIIa receptor antagonist response, neither cassette is sensitive for testing clopidogrel response. At the time of this writing, an application for a new cassette and testing method for clopidogrel was pending before the U.S. Food and Drug Administration.

The Impact[®] cone and platelet analyzer also uses a method that incorporates shear stress. There is both a research version of the device which has an adjustable sheer rate and a clinical version which uses a sheer rate that approximates atherosclerotic disease. While it does require pipetting of blood, no additional sample preparation is necessary.

Other platelet function testing

It is worth commenting on other tests not yet available at the bedside but frequently used in the drug literature to define resistance. These tests can generally be qualified as either biochemical or functional. Biochemical tests measure products which are affected by platelet activation or by inhibition of activation by antiplatelet drugs. These include general markers (P-selectin and platelet sur-

face activated GP IIb/IIIa receptors) as well as markers specific for aspirin (thromboxane B₂ [TXB₂]) or clopidogrel (vasodilator stimulated phos-phosphoprotein [VASP]).

Biochemical tests

Biochemical tests take advantage of signalling changes which occur as quiescent platelets become activated. Recent advancements in flow cytometry techniques have allowed wider use of these tests (4). In this way, P-selectin and activated GP IIb/IIIa receptors can be measured and standard cut-offs developed to quantify platelets as either being active or inactive. Other biochemical measures of note take advantage of the many autocrine and paracrine mediators that are involved with platelet-mediated vascular repair (12).

TXB₂ is the stable metabolite of thromboxane A₂ and can be measured in either the serum or urine. This test is frequently used in the aspirin resistance literature (4, 13, 19).

VASP is important for conversion of GP IIb/IIIa to the active state (30). The phosphorylated form of VASP (VASP-P) is inactive. By binding to the P2Y₁₂ receptor, ADP-initiated intracellular signalling inhibits formation of VASP-P. Inhibition of the ADP receptor by clopidogrel, ticlopidine or prasugrel therefore promotes the conversion of VASP to VASP-P and a mathematical expression of this ratio can be used as measure of the degree of P2Y₁₂ inhibition (30).

Functional tests

Functional platelet testing which is not available at the bedside includes primarily other laboratory-based methods of measuring aggregation. The historical gold standard is turbidimetric aggregometry but impedance aggregometry and electrical conductance aggregometry are also available (31, 32). Turbidimetric aggregometry utilises the previously described method of measuring the change in light transmission through platelet-rich plasma in response to a platelet agonist. Impedance aggregometry is performed on whole blood and is essentially an automated platelet count (similar to the Coulter method).

Aggregometry measured by electrical impedance is termed multiple electrode aggregometry (MEA) (32). In this technique, electrodes are placed in saline and whole blood and electrical conduction is measured. As activated platelets adhere to the electrode, the resulting impedance change is measured and compared to the control using aggregation curves (16, 32).

Since all three of these tests rely on direct platelet to platelet aggregation by GP IIb/IIIa receptors, both methods can be used for testing response to any class of antiplatelet agent. Current disadvantages of both techniques include the need for immediate processing of a fairly large sample volume, variable reproducibility, sample preparation time and technician experience (4, 9).

A simpler laboratory-based functional test closely related to impedance aggregometry is Plateletworks[®] which also uses whole blood and simply compares platelet counts before and after the use of an agonist (33). This test has been used in studies of drug interactions with antiplatelet agents (34) but has had very limited use in clinical settings.

Limitations of platelet function testing in clinical practice

A major limitation to the implementation of platelet testing in clinical practice is the lack of clear indications or guidelines for how such tests should be used. This is due directly to both a lack of adequate prospective trials with clinical endpoints as well as intrinsic variability in the testing modalities themselves (35).

While association between platelet resistance and major acute coronary events (MACE) has been shown in numerous trials (13, 14, 16, 36, 37), the studies are often retrospective (14), underpowered (36), or non-randomised (16, 37). The largest retrospective analysis illustrates the typical problems of this study design. In an analysis of 5,529 patients taking aspirin in the Heart Outcomes Prevention Evaluation (HOPE) study, case patients who experienced MACE had higher TXB₂ levels compared to age- and sex-matched controls (14). However, these patients had significantly higher rates of prior myocardial infarction (74.6% vs. 63.4%, $p < 0.001$), hypertension (44.9% vs. 31.6%, $p < 0.001$), diabetes (32.6% vs. 21.5% $p < 0.001$) and current smoking (16.6% vs. 11.7%, $p = 0.03$). While statistical adjustments can be employed to try and minimise the impact of these differences, this sort of data is hypothesis generating but inadequate to justify changes in clinical practice.

Poor correlation among different testing modalities is another limitation. Factors such as haematocrit and platelet count affect different testing modalities differently (38). This makes it especially challenging to establish testing cut-offs or treatment goals and contributes to the high rate of intra-patient variation that is seen with these tests. In a study using aspirin 100 mg for one to eight weeks in 48 subjects, serum TXB₂ was adequately suppressed by 99% from baseline but both VerifyNow[®] and turbidimetric aggregometry routinely showed that patients classified as responders one week could be non-responders a week later (19) and *vice versa*.

In practice, factors such as poor compliance (25), handling and processing of *in vitro* samples (39, 40) and genetic variability in drug absorption, metabolism and clearance (41) will also contribute to intra-patient as well as inter-patient variability in test results.

Despite these flaws in existing study data and techniques, the strong association between residual platelet activity and vascular events continues to be consistently found in newer prospective trials, especially in patients undergoing PCI (16, 37, 42, 43). While these data are not testing the benefit of randomised interventions, the results of such trials are eagerly awaited.

Dose titration and dual therapy

A last major limitation to implementation of surrogate platelet testing is the question of what the clinical response should be if a test shows a patient to be a 'non-responder' to a given therapy. While higher doses of aspirin (44), clopidogrel (45, 46) or dual therapy (46) can increase the average number of patients defined

as 'responders' based on platelet aggregometry, data from the Anti-platelet Trialists Collaboration does not support improved outcomes from higher doses of an individual antiplatelet agent (47). While it is tempting to assume that non-responders to one agent would be good candidates for dual therapy, resistance to either aspirin or clopidogrel increases the likelihood of resistance to the second agent (48). This suggests some common underlying mechanisms of platelet resistance (49) which might involve pathways of activation which are not blocked by current drug therapy (50). Therefore, the patient who seems most likely to benefit from dual therapy based on surrogate testing may in fact be the least likely (51).

Future directions

Both newer agents and larger trials with clinical endpoints will help illuminate more effective therapeutic strategies.

One early study using up to four loading doses of 600 mg of clopidogrel based on daily VASP measurements did find a reduction in vascular endpoints compared to the group who received a single, 600 mg dose without VASP testing prior to percutaneous coronary intervention (PCI) (30). A larger study, GRAVITAS (Gauging Responsiveness with A VerifyNow[®] assay-Impact on Thrombosis And Safety, NCT00645918) is ongoing with a target enrolment of nearly 3,000 and an estimated completion date of September, 2009. The study is using the VerifyNow[®] assay to give higher 'tailored' clopidogrel doses to subjects at the time of PCI and to continue a higher than average dose (150 mg) for six months post-PCI in patients who are labelled non-responders to the standard 75 mg dose. The ASCET trial (ASpirin non-responsiveness and Clopidogrel Endpoint Trial, NCT00222261) is using PFA-100 to identify aspirin non-responders and randomise them to either continue aspirin or receive standard dose clopidogrel for at least two years. Both GRAVITAS and ASCET are using MACE as outcomes.

The newer thienopyridine, prasugrel, has less variability in its bioactivation compared to clopidogrel and produces greater platelet inhibition when measured by both VASP and aggregometry (52, 53). In a landmark trial comparing prasugrel to clopidogrel after an acute coronary syndrome, prasugrel was more effective but resulted in significantly more bleeding, including both life-threatening and fatal bleeds (54). The role of prasugrel in the "real-life" setting of clinical practice currently remains controversial (55–57).

Conclusion

A wide variety of platelet tests are available including several which offer true point-of-care testing. As combination anti-platelet regimens become more widely used and newer agents offer more potent anti-platelet effects, it will become increasingly important

to be able to optimise the risk-to-benefit ratio in individual patients. However, due to methodologic flaws in the currently available literature, prospective trials will be required before any particular modality can be recommended for routine clinical use.

References

- Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 1971; 231: 232–235.
- Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. *Nat New Biol* 1971; 231: 235–237.
- Quick AJ. Salicylates and bleeding: the aspirin tolerance test. *Am J Med Sci* 1966; 252: 265–269.
- Michelson AD. Methods for the measurement of platelet function. *Am J Cardiol* 2009; 103 (3 Suppl): 20A–26A.
- Gremmel T, Steiner S, Seidinger D, et al. Comparison of methods to evaluate clopidogrel-mediated platelet inhibition after percutaneous intervention with stent implantation. *Thromb Haemost* 2009; 101: 333–339.
- Gurbel PA, Becker RC, Mann KG, et al. Platelet function monitoring in patients with coronary artery disease. *J Am Coll Cardiol* 2007; 50: 1822–1834.
- Penz SM, Reininger AJ, Toth O, et al. Glycoprotein Ibalph α inhibition and ADP receptor antagonists, but not aspirin, reduce platelet thrombus formation in flowing blood exposed to atherosclerotic plaques. *Thromb Haemost* 2007; 97: 435–443.
- Alfonso F, Angiolillo DJ. Platelet function assessment to predict outcomes after coronary interventions: hype or hope? *J Am Coll Cardiol* 2006; 48: 1751–1754.
- Oestreich JH, Smyth SS, Campbell CL. Platelet function analysis: at the edge of meaning. *Thromb Haemost* 2009; 101: 217–219.
- Lev EI. Aspirin resistance transient laboratory finding or important clinical entity? *J Am Coll Cardiol*. 2009; 53: 678–680.
- De Miguel A, Ibanez B, Badimon JJ. Clinical implications of clopidogrel resistance. *Thromb Haemost* 2008; 100: 196–203.
- Zimmermann N, Hohlfeld T. Clinical implications of aspirin resistance. *Thromb Haemost* 2008; 100: 379–390.
- Snoep JD, Hovens MM, Eikenboom JC, et al. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. *Arch Intern Med* 2007; 167: 1593–1599.
- Eikelboom JW, Hirsh J, Weitz JJ, et al. 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: 1650–1655.
- Crescente M, Di Castelnuovo A, Iacoviello L, et al. PFA-100 closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a meta-analysis of 19 studies comprising 3,003 patients. *Thromb Haemost* 2008; 99: 1129–1131.
- Sibbing D, Braun S, Morath T, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J Am Coll Cardiol* 2009; 53: 849–856.
- Smith SC, Jr., Allen J, Blair SN, et al. AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update: endorsed by the National Heart, Lung, and Blood Institute. *Circulation* 2006; 113: 2363–2372.
- Patrono C. Aspirin as an antiplatelet drug. *N Engl J Med* 1994; 330: 1287–1294.
- Santilli F, Rocca B, De Cristofaro R, et al. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin „resistance“. *J Am Coll Cardiol* 2009; 53: 667–677.
- Steinhuyl 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: 2572–2578.
- Serebruany VL, Steinhuyl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005; 45: 246–251.
- Serebruany V, Pokov I, Kuliczowski W, et al. Baseline platelet activity and response after clopidogrel in 257 diabetics among 822 patients with coronary artery disease. *Thromb Haemost*. 2008; 100: 76–82.
- Zhang JN, Bergeron AL, Yu Q, et al. Platelet aggregation and activation under complex patterns of shear stress. *Thromb Haemost* 2002; 88: 817–821.
- Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007; 357: 2482–2494.
- Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. *Thromb Haemost* 2009; 102: 7–14.
- Hobson A, Curzen N. Improving outcomes with antiplatelet therapies in percutaneous coronary intervention and stenting. *Thromb Haemost* 2009; 101: 23–30.
- Product labelling, VerifyNow IIB/IIIA [database on the Internet]. 2009; Available at: http://www.accumetrics.com/products/docs/IIB_IIIA/14320_IIBIIIA_Eng.pdf.
- Product labelling, VerifyNow aspirin. 2009.
- Product labelling, VerifyNow P2Y12. 2009.
- Kleiman NS. Will measuring vasodilator-stimulated phosphoprotein phosphorylation help us optimize the loading dose of clopidogrel? *J Am Coll Cardiol* 2008; 51: 1412–1414.
- Geiger J, Teichmann L, Grossmann R, et al. Monitoring of clopidogrel action: comparison of methods. *Clin Chem* 2005; 51: 957–965.
- Toth O, Calatzis A, Penz S, et al. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. *Thromb Haemost* 2006; 96: 781–788.
- Plateletworks ADP and collagen [database on the Internet]. 2009; Available at: <http://www.helena.com/Procedures/Pro190Rev6.pdf>.
- Lau WC, Waskell LA, Watkins PB, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. *Circulation* 2003; 107: 32–37.
- Gasparyan AY, Watson T, Lip GY. The role of aspirin in cardiovascular prevention: implications of aspirin resistance. *J Am Coll Cardiol* 2008; 51: 1829–1843.
- Gum PA, Kottke-Marchant K, Welsh PA, et al. 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.
- Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. *Circulation* 2009; 119: 237–242.
- Lasne D, Fiemeyer A, Chatellier G, et al. A study of platelet functions with a new analyzer using high shear stress (PFA 100) in patients undergoing coronary artery bypass graft. *Thromb Haemost* 2000; 84: 794–799.
- Morgenstern E, Boeckel N, Luscher EF. Do ADP-activated platelets in vitro aggregate as discocytes? *Thromb Haemost* 2007; 98: 73–76.
- Harding SA, Din JN, Sarma J, et al. Flow cytometric analysis of circulating platelet-monocyte aggregates in whole blood: methodological considerations. *Thromb Haemost* 2007; 98: 451–456.
- Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; 360: 363–375.
- Patti G, Nusca A, Mangiacapra F, et al. Point-of-care measurement of clopidogrel responsiveness predicts clinical outcome in patients undergoing percutaneous coronary intervention results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study. *J Am Coll Cardiol* 2008; 52: 1128–1133.
- Price MJ, Endemann S, Gollapudi RR, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* 2008; 29: 992–1000.
- Mirkhel A, Peyster E, Sundeen J, et al. Frequency of aspirin resistance in a community hospital. *Am J Cardiol* 2006; 98: 577–579.
- Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. *Circulation* 2007; 115: 708–716.
- Neubauer H, Lask S, Engelhardt A, Mugge A. How to optimise clopidogrel therapy? Reducing the low-response incidence by aggregometry-guided therapy modification. *Thromb Haemost* 2008; 99: 357–362.
- Antiplatelet Trial Collaborators. 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.
- Lev EI, Patel RT, Maresh KJ. 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.
- Guthikonda S, Mangalpally K, Vaduganathan M, et al. Increased platelet sensitivity among individuals with aspirin resistance – platelet aggregation to submaximal concentration of arachidonic acid predicts response to antiplatelet therapy. *Thromb Haemost* 2008; 100: 83–89.
- Gori AM, Marcucci R, Paniccia R, et al. Thrombotic events in high risk patients are predicted by evaluating different pathways of platelet function. *Thromb Haemost* 2008; 100: 1136–1145.

51. Shantsila E, Lip GY. Predicting thrombotic events: creating a complex approach for a complex condition. *Thromb Haemost* 2008; 100: 962–964.
52. Sugidachi A, Ogawa T, Kurihara A, et al. The greater in vivo antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite. *J Thromb Haemost* 2007; 5: 1545–1551.
53. Jakubowski JA, Payne CD, Li YG, et al. A comparison of the antiplatelet effects of prasugrel and high-dose clopidogrel as assessed by VASP-phosphorylation and light transmission aggregometry. *Thromb Haemost* 2008; 99: 215–222.
54. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; 357: 2001–2015.
55. Calatzis A. Another view on prasugrel. *Thromb Haemost* 2009; 101: 12–13.
56. Serebruany V, Shalito I, Kopyleva O. Prasugrel development – claims and achievements. *Thromb Haemost* 2009; 101: 14–22.
57. Serebruany VL, Makarov LM. Future of oral antiplatelet therapy: four challenged hypotheses. *Thromb Haemost* 2009; 101: 1041–1043.