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 additional 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

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, arachidonic 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 arachidonic 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 epifibatide and tirobotabin 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 shear rate and a clinical version which uses a shear 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 surface activated GP IIB/IIIa receptors) as well as markers specific for aspirin (thromboxane B2 [TXB2]) or clopidogrel (vasodilator stimulated phos-phosprotein [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 qualify 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). Tubidimetric 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


