

Platelet function analysis: At the edge of meaning

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Anti-platelet agents are a cornerstone in the treatment of symptomatic coronary artery disease and acute coronary syndromes. Despite the proven benefits of acetylsalicylic acid (ASA) and clopidogrel, ischaemic events still occur in patients receiving dual anti-platelet therapy. Over the course of the last 10 years, a series of published studies has examined the effects of anti-platelet therapy on ex-vivo assays of platelet function and has clearly established substantial inter-individual variability in the degree of inhibition of platelet function observed in individuals taking ASA or clopidogrel or both (1–3). Individuals who display residual platelet function in ex-vivo assays despite standard doses of anti-platelet therapy have been classified as “non-responders” or “resistant” to anti-platelet medications (4, 5). A major unknown is whether patients who are non-responsive to anti-platelet medications, as judged by testing *ex vivo*, are at higher risk of suffering an ischaemic event and, if so, whether tailoring anti-platelet medications based on ex-vivo functional assays will result in protection from ischaemic events.

A recent, small study supports the use of a tailored approach based on ex-vivo platelet function testing to dose-adjust clopidogrel and reduce ischaemic events following percutaneous coronary intervention (PCI) (6). However, the most compelling evidence of a benefit from higher platelet inhibition comes from studies of prasugrel, a novel P2Y₁₂ receptor antagonist that more completely inhibits ex-vivo platelet function than does clopidogrel when assessed by light transmittance aggregometry (LTA) (7). In the Trial to Assess Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38 trial (NCT00699998), dual anti-platelet therapy with prasugrel and ASA reduced ischaemic events as compared to clopidogrel and ASA, albeit at the expense of increased bleeding (8). One possibility is that the improved ischaemic outcomes in TRITON reflect a reduction in the number of patients that are “non-responders” to dual anti-platelet therapy.

Several point-of-care assays of platelet function have been developed in recent years to rapidly screen individuals on anti-platelet therapy. The ideal test would be capable of distinguish-

ing individuals at risk of ischaemic and bleeding events and could be used to guide dose adjustments in therapy. The clinical data indicating that a significant number of patients respond incompletely to clopidogrel, the reduction in ischaemic outcomes but increased bleeding with prasugrel in the TRITON trial, and the availability of point-of-care assays would suggest the time is right to use platelet function analysis to individualize anti-platelet therapy. Indeed, the most recent guidelines for PCI from the American College of Cardiology/American Heart Association/Society for Coronary Angiography and recommend that platelet function be monitored in patients undergoing high-risk procedures and that the dose of clopidogrel be increased in patients identified as non-responsive (9). However, before widespread use of platelet function assays is adopted, several key issues need to be resolved. First, for each platelet function assay, the reliability of the test in predicting ischaemic and bleeding risk must be determined. This may require defining non-responsiveness in a manner that includes individuals who may benefit from higher doses of therapy and excludes those at risk of bleeding. Second, the optimal timing for platelet function analysis needs to be determined (i.e. on admission, prior to balloon inflation, following PCI). Third, the strategy of dose-adjustment based on ex-vivo platelet function results must be prospectively evaluated to assure that an improvement in clinical outcomes can be realized.

In this issue of *Thrombosis and Haemostasis*, Gremmel et al. (10) provide fundamental information on the performance of four commonly used platelet function assays. Their findings are a key step in addressing the value and importance of the tests. Gremmel et al. compared the results of four platelet function assays to those of the “gold standard” LTA in a group of patients presenting for elective percutaneous stent implantation. A total of 80 patients were enrolled following stent placement in the coronary, peripheral or cerebrovascular bed. All patients were treated with ASA (100 mg/day for at least 2 weeks) and received a 300 mg loading dose of clopidogrel 24 hours before the procedure followed by 75 mg daily. Blood was sampled 24 hours after the intervention and stored in a manner consistent with the requirements of each assay. Each assay was performed by a

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Table 1: Characteristics of platelet function assays. ADP = adenosine diphosphate; PGEI = prostaglandin E1.

Assay	LTA	MEA	VerifyNow P2Y12	VASP	Impact-R
Measurement	aggregation	aggregation	aggregation	activation	adhesion/aggregation
Method of detection	optical transmission	impedance	optical transmission	fluorescence intensity	surface coverage
Specimen type	platelet-rich plasma	whole blood	whole blood	whole blood	whole blood
Standard conditions	variable ADP concentration	variable ADP concentration	fixed ADP and PGEI	fixed ADP and PGEI	recommended ADP 1.36 µM
Technical skills required	specialized lab and personnel	specialized lab and personnel	none	specialized lab and personnel	specialized lab and personnel
Advantages	well-studied	physiologic environment	user-friendly and fast	P2Y12 receptor-specific	user-friendly and fast
	tied to clinical outcomes	minimal sample manipulation	no sample processing	small blood volume required	small blood volume required
Disadvantages	time and extent of processing	less clinical evidence with assay	unadjustable agonist concentration	measures earlier marker (signaling)	limited experience with assay
	lack of standardisation	smaller range for response		expensive	

*All assays lack established ranges for clopidogrel responsiveness. LTA, light transmission aggregometry; MEA, multiple electrode platelet aggregometry; VASP, vasodilator-stimulated phosphoprotein.

single operator and included LTA (10 µM ADP as the agonist), the VerifyNow P2Y₁₂ assay (Accumetrics, San Diego, CA, USA), the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay (Diagnostica Stago, Biocytex, Marseille, France), multiple electrode platelet aggregometry (MEA, Dynabyte, Munich, Germany) and the Impact-R test (Diamed, Crescier, Switzerland). The properties of these assays are compared in Table 1.

The results highlight the problems encountered with platelet function analysis. Gremmel et al. (10) report a reasonable correlation between residual platelet activity as measured by each of the four assays and LTA. However, when binary comparisons were performed for responders and non-responders, the correlations were less robust. For instance, VerifyNow and LTA yielded discordant results for identification of responder/non-responders in 18 of 80 cases: nine individuals were classified as non-responders by LTA but not by VerifyNow, and nine individuals were identified as non-responders by VerifyNow but not by LTA. When LTA results were used as the standard for responsiveness, the sensitivity and specificity of the VerifyNow assay were only 55% and 85%, respectively. The other assays of platelet function fared worse than the VerifyNow assay with respect to identifying non-responders by LTA.

The findings of Gremmel et al. indicate considerable variability among five methods to assay platelet function in patients treated with ASA and clopidogrel and are in accord with the results of similar studies from other groups (11, 12). The results may not be surprising, given that these five assays measure different platelet events/endpoints (Table 1). These findings emphasize that different platelet function tests probably cannot substitute for one another. It is worth noting that LTA was chosen as the gold standard for platelet function analysis based on historical rather than clinical evidence and that universal standardisation has yet to be established. As of yet, platelet function analysis has not been put to the ultimate test. That is to say, there is no proof that a group of patients who are prospectively and re-

producibly identified by these techniques will gain more than they lose following adjustment of their anti-platelet therapy.

Several ongoing studies should provide valuable insight into the role of platelet function testing in the setting of PCI. In the Assessment of Dual Anti-Platelet Therapy with Drug Eluting Stents (ADAPT-DES, NCT00638794) registry, platelet function analysis with the VerifyNow assay will be performed in a large group of patients receiving drug-eluting stents to identify a cohort of patients at risk for early or late stent thrombosis. Additionally, the platelet function substudy of the ongoing A Comparison of Prasugrel and Clopidogrel in Acute Coronary Syndrome Subjects (TRILOGY, NCT00699998) trial aims to correlate ex-vivo testing with clinical outcomes in the under-studied population of medically managed patients.

Most importantly, the first major trial using platelet function analysis to tailor therapy is enrolling. In the Gauging Responsiveness with A VerifyNow Assay-Impact on Thrombosis and Safety (GRAVITAS, NCT00645918) trial, patients undergoing PCI are randomized into one of three arms depending on results of the VerifyNow assay. Responders will receive standard therapy with ASA and clopidogrel (75 mg daily). Non-responders will receive either standard therapy or tailored therapy with an additional 450 mg loading dose of clopidogrel followed by 150 mg daily. These ongoing trials should provide data on the VerifyNow assay as a point-of-care test to assess platelet function in the setting of anti-platelet therapy. The large number of patients tested in these trials should help determine the optimal “cut-off” value for non-responsiveness, establish a set of clinical characteristics that predict the need for platelet function testing, and provide important information regarding platelet function and the risk of adverse ischaemic and bleeding events. With GRAVITAS, the first foray into tailored anti-platelet therapy is underway. However, based on the results of Gremmel et al., it is not clear which, if any, of the current tests represents the best strategy for individualized anti-platelet therapy.

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