Point-of-care platelet function testing in patients undergoing PCI: between a rock and a hard place

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Since recent studies have linked an impaired response to antiplatelet therapy with a higher incidence of atherothrombotic events, the monitoring of the efficacy of antiplatelet therapy in the individual patient has attracted much attention. In the present report, we demonstrate that platelet function testing with several point-of-care assays results in ambiguous and conflicting results: some assays indicated that the patient's platelets were insufficiently inhibited by clopidogrel whereas other assays reported an adequate response. Therefore, platelet function assays should not be used solely to guide treatment decisions, and tailor-made antithrombotic treatment has to wait for the most predictive platelet function test to emerge for measuring the risk for thrombotic complications after stenting. Until then, daily clinical practice should not be guided by point-of-care platelet function testing. (*Neth Heart J* 2007;15:299-305).

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A 44-year-old man was admitted to our hospital in June 2006 with an acute inferoposterior myocardial infarction (AMI). The patient had no relevant medical history and cardiovascular risk factors included positive family history and smoking.

Aspirin (900 mg Aspegic) and unfractionated heparin (5,000 IU) were given in the ambulance and a 600 mg loading dose of clopidogrel was administered immediately before the primary percutaneous coronary intervention (PCI) procedure. At presentation, his blood pressure was 100/75 mmHg and his heart rate was 65 beats/min. His lungs were clear on auscultation and cardiac examination revealed no abnormalities. Coronary angiography revealed a calcified and occluded left anterior descending branch (LAD), a subtotal stenosis in the ramus circumflexus (RCX) and a fresh thrombus occluding the proximal right coronary artery (RCA). Recanalisation of the occluded artery was achieved by implantation of two (24 x 3.5 mm and 30 x 3.5 mm) Driver stents ((Medtronic Vascular, Santa Rosa, California) without evidence of residual stenosis or dissection. An intra-aortic balloon pump (IABP) was inserted immediately after the procedure because of severe hypotension. One day after the primary PCI, the IABP was removed and the patient was doing well. The maximal creatine kinase-myocardial band (CK/MB) was 6743/4230 U/l (normal 0-175). On day five of his hospital admission, the patient was included in a large single-centre observational pilot study. This study included measurements with several 'point-of-care' platelet function tests to assess the patient's response to clopidogrel therapy. The results of various platelet function assays are depicted in table 1. Eight days after hospital admission, the patient again experienced sudden onset chest pain.

Electrocardiography revealed an acute inferoposterior myocardial infarction. Repeated coronary angiography revealed a totally occluded proximal RCA with a massive thrombus load in the proximal stent which was unsuccessfully treated with a balloon angioplasty. Despite the insertion of an intra-aortic balloon pump, the patient remained haemodynamically unstable and emergency coronary artery bypass grafting with the

Table 1. Results of various platelet function assays.

Platelet function assay	Results in our patient	Adequate clopidogrel response (normal reference)	Conclusion
VerifyNow P2Y12 assay	P2Y12 reaction units: 263 Base: 284 Inhibition=7%	Unknown	?
ADP-induced optical aggregometry (20 µM)	Peak aggregation: 82% for 20 µM=82%	0-70%*	Resistant
Cone and plate(let) analyser (after pre-incubation with 1.38 µM ADP)	Surface coverage: 4.4%	Surface coverage: >2.8%	Responder
Impedance aggregometry (10 µM ADP-induced)	Impedance: 8 Ω	<5 Ω	Resistant
Platelet-function analyser (PFA-100) COL/ADP cartridge	COL/ADP closure time=125 s	Unknown (Normal values for controls <120 sec)	?
Plateletworks	IPA=6%	Unknown	?

*Given the multiple definition of 'clopidogrel resistance' with optical aggregometry, we defined clopidogrel resistance according to White et al.²⁰ ADP=adenosine diphosphate, COL=collagen, IPA=inhibition of (micro)aggregation.

use of two single venous grafts to the RCA and the LAD was performed to restore coronary blood flow. The maximal CK-MB rise was 350/41 U/l. Echocardiography showed akinesia of the posterior and lateral wall and a calculated ejection fraction of 20%. The patient was discharged on day 16. He was prescribed lifelong aspirin, clopidogrel for at least one year, an ACE inhibitor, a β-blocker and a statin.

Causes of stent thrombosis

Multiple studies have identified several possible causes and risk factors for the occurrence of stent thrombosis (either acute, subacute or late) in both bare metal stents (BMS) stent and drug-eluting stents (DES).¹⁻⁵ These include the use of longer stents and/or multiple stents, stent overlap, stent underexpansion, stent implantation for myocardial infarction, bifurcation lesions, complexity of PCI, small final lumen diameter, residual dissection, renal insufficiency, diabetes mellitus and premature withdrawal of antiplatelet therapy.¹⁻⁵ Furthermore, stent thrombosis can be the result of an impaired or inadequate responsiveness to aspirin and/or clopidogrel therapy.⁶⁻⁹ As a result, the monitoring of the individual patient response with platelet function tests has gained widespread attention.

Monitoring the individual response

Laboratory evaluation of the platelet response to the fixed 'one size fits all' dosing regimen of aspirin and/or clopidogrel has revealed a wide individual response variability.¹⁰⁻¹² Moreover, several recent studies have demonstrated an inverse relationship between the level of platelet inhibition and the occurrence of atherothrombotic events.^{8,13-15} However, most of these studies

were performed with 'classical' light transmittance aggregometry (LTA), a method that is labour intensive, and therefore not suitable for use on a patient-to-patient basis. To overcome this problem, several commercial point-of-care platelet function assays (e.g. the VerifyNow system, the cone and plate(let) analyser, the platelet function analyser (PFA-100), the Plateletworks assay and impedance aggregometry) have been introduced for the rapid evaluation of the individual response to antiplatelet therapy.¹⁶⁻²¹ However, the problem with the widespread introduction of these new, bedside platelet function assays is the fact that they have been insufficiently validated and standardised. Moreover, the most important three key questions 1) are all these assays capable of predicting the occurrence of thrombotic events?, 2) what is the cut-off level to segregate responders from non-responders? and 3) does a change in therapy (based on the results of these assays) improve clinical outcome? have not yet been answered.

Currently available platelet function assays

The following assays are currently available for the monitoring of the individual response to antiplatelet therapy.

The VerifyNow assay

Principle of the test

The VerifyNow assay (Accumetrics, San Diego, USA) is based upon the principle that agonist-induced activated platelets bind to fibrinogen-coated polystyrene beads that agglutinate in whole-blood (figure 1).¹⁶

Figure 1. A schematic presentation showing how the rate and extent of platelet aggregation is quantified with the VerifyNow system. The mixing chamber contains fibrinogen-coated beads as well as specific platelet agonist (either TRAP, AA or ADP+PGE1). Activated glycoprotein (GP) IIb/IIIa receptors on the platelets bind via fibrinogen on the beads and cause agglutination of the platelets and the beads. Infrared-light transmittance through the chamber is measured and increases as the agglutinated platelets and beads fall out of the solution (reprinted with permission from Dr A.D. Michelson, Platelets).

Description of the test

After whole blood is added in the mixing chamber, the platelets become activated by a specific agonist. As a result, they bind via GP IIb/IIIa receptors to the fibrinogen-coated beads and cause agglutination. Infrared-light transmittance through the chamber increases as the platelet-bead complexes fall out of the solution. Currently, three different cartridges are available: VerifyNow aspirin assay (using arachidonic acid as the agonist), VerifyNow P2Y12 assay (using ADP as the agonist, antagonised by prostaglandin E₁) and VerifyNow IIb/IIIa assay (using thrombin receptor-activating peptide (TRAP) as the agonist).

Strengths and drawbacks

It is an automated, cartridge-based bedside device that allows a rapid evaluation of platelet function in patients treated with either acetylsalicylic acid, clopidogrel or GP IIb/IIIa-receptor antagonists. Several relatively small studies have indicated that the results obtained from the VerifyNow aspirin assay and the VerifyNow IIb/IIIa assay correlate with the (re)occurrence of cardiovascular events.^{22,23} This in contrast to the recently introduced P2Y12 assay, which has not been evaluated in large groups of patients.

Plateletworks single platelet counting

Principle of the test

The Plateletworks system (Helena Laboratories, Beaumont, USA) is based upon the single platelet disappearance (SPD), expressed as the platelet count ratio before and after exposure to ADP, to calculate the percentage of platelet inhibition.

Description of the test

A total reference platelet count is performed in whole blood in a Plateletworks tube containing K₃-EDTA as the anticoagulant on a cell counter. This process is repeated with a second sample of fresh whole blood in a Plateletworks tube containing both D-Phe-Pro-Arg-chloromethyl ketone (PPACK) 50 µmol/l as an anticoagulant and 20 µmol/l adenosine diphosphate (ADP). In the presence of ADP, platelets associate and aggregate. As the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio of the platelet count in the ADP tube and the reference tube containing EDTA is expressed as a percentage of inhibition of platelet aggregation (IPA).

Strengths and drawbacks

Plateletworks needs minimal sample preparation and the results are available within a couple of minutes. Multiple studies have demonstrated the utility of the Plateletworks assay in monitoring the effects of GP IIb/IIIa antagonists. However, whether the Plateletworks assay is capable of detecting the efficacy of thienopyridine (i.e. clopidogrel) therapy needs further evaluation in future studies.²⁴ Most importantly, there are no prospective studies evaluating the predictive value of the results obtained with the Plateletworks.

The platelet function analyser (PFA-100)

Principle of the test

The PFA-100 analyser (Dade Behring, Marburg, Germany) measures platelet function, in particular adhesion and aggregation, in whole blood under high shear conditions. Specifically, the time needed to form a platelet plug occluding the aperture cut into a collagen/epinephrine (COL/EPI) or collagen/ADP (COL/ADP)-coated membrane is determined and reported as closure time (CT) in seconds (figure 2).¹⁹

Description of the test

Whole blood is drawn in citrated tubes and 800 µl is pipetted into the disposable cartridge. The inserted whole blood is then aspirated through a capillary and is forced to flow through the aperture cut into a collagen-coated membrane infused with either ADP or

epinephrine at high shear rate. Platelets come into contact with the membrane with its agonists (COL/ADP or COL/EPI) and consequently adhere and aggregate. As a result, the platelet plug formed will occlude the aperture and stop the blood flow. However, if patients are sufficiently inhibited by antiplatelet therapy, the CT can not be measured and the results are presented as >300 seconds. The COL/EPI cartridge detects intrinsic platelet defects, von Willebrand disease as well as exposure to platelet inhibiting agents such as aspirin. The COL/ADP test cartridge is less influenced by aspirin use.

Strengths and drawbacks

The PFA-100 is a simple and rapid point-of-care assay that can be used for a variety of platelet disorders. However, several studies have demonstrated that the COL/ADP cartridge is relatively insensitive to the thienopyridine effect and international consensus based on an extensive overview of available evidence has advised restricting use to research studies and clinical trials at present.²⁵

The cone and plate(let) analyser

Principle of the test

The cone and plate(let) analyser (Diamed, Cressier s/ Morat, Switzerland) measures interaction with the subendothelium under flow conditions with subsequent shear forces (figure 3).

Figure 2. The time needed to form a platelet plug occluding the aperture cut into a collagen/epinephrine (COL/EPI) or collagen/ADP (COL/ADP)-coated membrane is determined and reported as closure time (CT) in seconds by the PFA machine (reprinted with permission from Dade Behring).

Figure 3. A schematic presentation of the clopidogrel aggregation test by the cone and plate(let) analyser. Testing a normal blood sample results in aggregate formation on the well surface (normal SC: upper part). However, pre-incubating the blood sample under gentle mixing (10 rpm) for one minute with an appropriate platelet agonist (ADP) induces microaggregate formation when platelets are insufficiently inhibited by clopidogrel. These activated platelets lose their adhesion properties temporarily until spontaneous disaggregation occurs.

Description of the test

Citrated whole blood is pre-incubated with 1.38 $\mu\text{mol/l}$ ADP under gentle mixing (10 rpm) for one minute. ADP induces microaggregate formation when platelets are insufficiently inhibited by clopidogrel. These activated platelets lose their adhesion properties temporarily. Aliquots of 130 μl of ADP pre-incubated whole blood are placed into polystyrene plates and subjected to a shear rate of 1800s^{-1} for two minutes using a specially developed Teflon cone. Under these testing conditions, von Willebrand factor and fibrinogen are instantly immobilised on the polystyrene surface, serving as a substrate for platelet adhesion and subsequent aggregation. The wells are stained with May-Grunwald stain and analysed with an inverted light microscope which is connected to an image analysis system. Platelet adhesion and aggregation on the surface are evaluated by examining the percentage of total area covered with platelets designated as surface coverage (SC) and the average size of surface-bound objects (i.e. platelet aggregates). A low surface coverage ($<2.8\%$) implies that the platelets do not sufficiently respond to clopidogrel therapy (personal communication from Professor Varon).

Strengths and drawbacks

It is generally presumed that platelet function testing under high shear 'physiological' conditions reflects the physiological milieu more precisely as compared with ex vivo aggregation-based platelet function tests. However, to date, there are no published studies that have demonstrated that a low-surface coverage is associated with a poor clopidogrel response and vice versa.

Impedance aggregometry

Principle of the test

Impedance aggregometry (Chrono-Log, Havertown, USA) is based upon the principle of electrical impedance between two electrodes immersed in whole blood. Agonist-induced clot formation between the electrodes increases the impedance when patients are not fully protected by clopidogrel therapy (figure 4).²¹

Detailed description of the test

In a cuvette, 500 μl of whole blood is diluted with an equivalent volume of isotonic saline. The cuvette is then incubated for five minutes at 37°C . Thereafter, the impedance of each sample is monitored in

Figure 4. A schematic presentation of impedance aggregometry. Electrical impedance between two electrodes immersed in whole blood is measured after the addition of ADP. Clot formation between the electrodes increases the impedance when patients are not fully protected by clopidogrel therapy.

sequential one-minute intervals until a stable baseline is established (<5 mV drift per minute). The agonist is then added to the sample and the electrical impedance aggregation response to ADP is monitored for six minutes and the final increase in ohms over this period is displayed as a numeric light-emitting diode (LED) readout. In addition, a graphical printout (i.e. chart tracing) of each electrical impedance aggregometry tracing is provided by the aggrolink software.

Strengths and drawbacks

Impedance aggregometry has demonstrated a good correlation with optical aggregometry when testing the GP IIb/IIIa inhibitors and thienopyridines. Sample preparation takes about two minutes and results are available within ten minutes after initiating the test. However, there are no prospective studies evaluating the predictive value of the results obtained with impedance aggregometry.

Discussion

Since recent studies have linked an impaired response to antiplatelet therapy with a higher incidence of the occurrence of atherothrombotic events, the monitoring of the efficacy of antiplatelet therapy in the individual patient has attracted much attention.^{8,13,14} Consequently, a variety of techniques and terms have

been introduced to quantify the variability in platelet response to clopidogrel therapy; however, there is no uniform or consensual definition of this clopidogrel poor-responsiveness (also referred to as 'heightened post-treatment reactivity' or 'clopidogrel resistance') and many questions need to be addressed.

In the present report, we demonstrate that platelet function testing with several point-of-care assays results in ambiguous and conflicting results: some assays indicate that the patient platelets were insufficiently inhibited by clopidogrel whereas other assays report an adequate response. Furthermore, the relatively newest devices do not have any cut-off levels at all because they have not been sufficiently validated.

Given the numerous definitions of clopidogrel poor-responsiveness, the multifactorial nature of its existence, the absence of a uniform method of measurement, the lack of evidence for these new point-of-care assays and the variable prevalence of poor-responsiveness throughout different clinical scenarios, it is impossible to propose treatment recommendations for patients exhibiting poor-responsiveness to antiplatelet therapy at the time of this writing. Importantly, there are no prospective clinical trials demonstrating that a change of therapy (e.g. increasing the dose or a switch to an alternative antithrombotic strategy) in poor-responsive patients will improve clinical outcome.

Therefore, we need to await the results of currently ongoing clinical trials before we can safely titrate the dose of antiplatelet therapy to an efficient level. The ongoing Aspirin Non-Responsiveness and Clopidogrel Endpoint Trial (ASCET) is seeking to address whether aspirin resistance - defined using the PFA-100 - can be linked to adverse cardiovascular outcomes among 1000 coronary artery disease patients. The Research Evaluation to Study Individuals who Show Thromboxane Or P2Y12 Receptor Resistance (RESISTOR) trial will hopefully answer the important question whether additional GP IIb/IIIa antagonist treatment will benefit patients with an inadequate response to either clopidogrel and/or aspirin. The Do Point-of-Care Platelet Function Assays Predict Clinical Outcomes in clopidogrel pre-treated patients undergoing elective PCI (POPular study) is aiming to identify the most suitable point-of-care platelet function assay to predict the occurrence of atherothrombotic events. Finally, the large Stent Thrombosis Study, which will include about 12,000 patients, will hopefully demonstrate the predictive value of the VerifyNow assays.

Conclusion

There is an increasing interest in the use of point-of-care platelet function tests for the monitoring of the efficacy of antiplatelet therapy. The ultimate goal is to tailor the antiplatelet therapy individually by titrating the drugs to the optimal dose. However, none of the currently available platelet function assays is sufficiently validated and standardised to guide the antiplatelet therapy to its optimal effect without inducing haemorrhagic side effects. Furthermore, well-defined cut-off levels for the segregation of non-responders from responders are lacking for the different assays. Therefore, platelet function assays should not be used solely to guide treatment decisions and tailor-made antithrombotic treatment has to wait for the most predictive platelet function test to emerge for measuring the risk for thrombotic complications after stenting. Until then, daily clinical practice should not be guided by point-of-care platelet function testing. ■

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