

Platelet function testing and risk of bleeding complications

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Summary

Antiplatelet therapy has a key role in preventing atherothrombotic events in patients with coronary artery disease, particularly in those undergoing revascularisation procedures. However, this may occur at the expense of an increase risk of bleeding. Therefore, the balance between thrombotic and bleeding events is critical in order to achieve optimal outcomes. Since there is a broad variability in individual response profiles to antiplatelet therapy, these outcomes (thrombosis vs. bleeding) may depend on the level of platelet inhibition achieved in a given subject. Platelet function assays have emerged as a useful tool for its

potential to determine patients at a higher risk of ischaemic and bleeding complications. The present manuscript will review the available evidence associating platelet function testing with adverse clinical outcomes, in particular bleeding, and their potential applications in lieu of novel and more potent antithrombotic agents that will be introduced into clinical practice in the near future.

Keywords

Platelet pharmacology, antiplatelet agents, bleeding

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Introduction

Antiplatelet therapy has a key role in preventing atherothrombotic events in patients with coronary artery disease (CAD). Aspirin, a cyclooxygenase-1 (COX-1) inhibitor, and clopidogrel, an adenosine diphosphate (ADP) P2Y₁₂ receptor inhibitor, are the antiplatelet agents most commonly used in clinical practice in CAD patients. Several clinical trials have shown that more aggressive antiplatelet treatment by means of adjunctive clopidogrel therapy in addition to aspirin is associated with reduced rates of recurrent ischaemic events in high-risk patients, such as those with acute coronary syndrome (ACS) and undergoing percutaneous coronary intervention (PCI) (1–3). However, these studies have also shown that more potent platelet inhibition increases the risk of bleeding. The impact of the degree of platelet inhibition on bleeding is underscored in studies using more potent agents administered either intravenously (i.e. glycoprotein IIb/IIIa inhibitor) or orally (i.e. prasugrel). Since bleeding has shown to have important prognostic implications (4, 5), understanding the degree of platelet inhibition in a given patient treated with one or more antiplatelet agent has become an emerging clinical problem. In fact, the degree of platelet inhibition in patients treated with the same antiplatelet treatment regimen is highly variable (6, 7). Therefore, in some patients a given treatment regimen may lead to no or very little response (“hypo-responders”), while in others this may induce profound platelet inhibitory effects (“hyper-responders”).

Platelet function testing may be useful to determine the degree of platelet inhibition in patients treated with antiplatelet therapy. Currently there is a plethora of data showing how these tests may predict recurrent ischaemic events in hypo-responder patients (6, 7). Less established is their value in predicting bleeding among hyper-responders. The present editorial focus provides an overview of the available evidence associating platelet function testing with adverse clinical outcomes, in particular bleeding, and its potential application with the introduction of novel and more potent antithrombotic agents into clinical practice in the near future.

Impact of platelet inhibition on clinical outcomes

Several platelet function tests have been used to assess antiplatelet drug effects (8–10). Some important features to consider in order to determine the potential clinical utility of these assays in a clinical setting include the following: 1) consistent data showing a relationship with clinical outcomes; 2) potential use in daily clinical practice either at the bedside or in an office setting, either by being a truly point-of-care system (no pipetting required) or an easy-to-use assay, in which the presence of an expert technician is not needed and results are obtained in a relatively short time; 3) ability to assess the whole spectrum of response profiles, and 4) small

blood volume required (8–10). A brief description of the most utilised and tested assays are described in Table 1.

Platelet function testing using the array of assays currently available have shown that the degree of platelet inhibition among patients treated with the same antiplatelet treatment regimen is highly variable (6, 7). In particular, in some patients a given treatment regimen may lead to no or very little response (hypo-responders), while in others this may induce profound platelet inhibitory effects (hyper-responders). There has been emerging interest on identifying the prognostic implications of these laboratory findings. Although the best methods and cut-off values to predict the risk for ischaemic events are not standardised, there is overwhelming data showing an association between hypo-responsiveness or “resistance” to a given antiplatelet treatment regimen and recurrent ischaemic events, including stent thrombosis (6, 7). Less robust is the information associating hyper-responsiveness with bleeding. This is likely attributed to the fact that bleeding events are considerably less frequent than ischaemic events and platelet function studies typically include a limited number of patients. Therefore, studies evaluating the utility of platelet function assays to predict the risk of ischaemic events are not sufficiently powered to find a statistically significant difference in bleeding outcomes, which are often not even mentioned. Of note, the complexity of mechanisms leading to bleeding events, likely greater than those determining thrombotic complications, is a further challenge in understanding the association between platelet function testing and bleeding complications. In addition, the assessment of bleeding risk by methods other than platelet function is also difficult. Indeed, several bleeding risk scores have been developed (11, 12), the clinical value of which warrant further validation. Predictors which inherently increase the risk of bleeding include many clinical variables such as age, sex, diabetes mellitus, renal function, and anaemia (11, 12). This is in addition to the risk of bleeding associated with the revascularisation procedure (percutaneous or surgical) per se. Further, many platelet function tests does not take into account abnormalities in coagulation processes which also are key in haemostasis.

The importance of bleeding is outscored by the negative impact that haemorrhagic events have on prognosis, as they have been shown to be associated with an increased risk of adverse cardiovascular outcomes, including death (4, 5). An analysis of combined data of different studies evaluating ACS patients ($n=34,146$) showed an association between major bleeding and the risk of myocardial infarction, stroke and death. In particular, patients with major bleeding had a five-fold- and a 1.5-fold-higher incidence of death during the first 30 days and between 30 days and six months, respectively, after hospitalisation for an ACS (4). A pooled analysis combining data from randomised trials in patients undergoing PCI showed that the presence of bleeding events within 30 days after enrollment was an independent predictor of 1-year mortality after PCI (c -statistics 0.79), underscoring the need for including bleeding as an endpoint for the assessment of outcomes (5). Notably, the prognostic implications of bleeding on one-year mortality are similar or even higher than that of having had a recurrent myocardial infarction (13).

Escalating doses of aspirin have different effects on platelet function depending on which test is being used. In fact, it is important to distinguish between COX-1 specific (e.g. light transmission aggregometry [LTA] or other assays using arachidonic acid stimuli; serum thromboxane) and non-specific assays (PFA-100® or assays using stimuli other than arachidonic acid). Doses of aspirin in the 30–40 mg range are able to achieve complete COX-1 inhibition as assessed by COX-1-specific assays and higher aspirin doses do not have a further impact on assay results (14). On the contrary, a dose-dependent effect on laboratory results is observed when using COX-1 non-specific assays, which has been suggested to be attributed to a dose-dependent effect on COX-1-independent pathways. Importantly, higher aspirin doses are not associated with better clinical outcomes and are associated with increased bleeding rates, particularly upper gastrointestinal bleeding (15–18). This has been shown in patients treated with aspirin alone as well in combination with clopidogrel. The CURE (Clopidogrel in Unstable angina to prevent Recurrent Events) trial randomised patients with ACS without ST-segment elevation to receive either clopidogrel (300 mg loading dose, followed by 75 mg once daily) or placebo in addition to aspirin for a mean time of nine months. In the overall population, clopidogrel use was associated with a significant lower rate of ischaemic events, at the expense of an increased risk of major bleeding (19). A subanalysis of this trial showed that bleeding risk increased with the dose of aspirin, with or without clopidogrel, without any effect in efficacy (20). These findings strongly support the use of low-dose aspirin, particularly when used in combination with clopidogrel.

Clopidogrel per se also has an impact on bleeding events as recently confirmed in the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischaemic Stabilization, Management, and Avoidance) trial (21). In this study, patients at high risk for atherothrombotic events were randomised to receive clopidogrel in addition to low-dose aspirin (75–162 mg). Although a benefit of clopidogrel treatment was suggested in patients with symptomatic atherothrombosis, overall results did not show a benefit of clopidogrel in the rates of ischaemic events. The rate of moderate bleeding was significantly higher in the clopidogrel group and a trend was found for severe bleeding. These overall findings are in line with the synergistic effect on platelet inhibition obtained with aspirin and clopidogrel combination therapy as shown in platelet function studies.

Platelet function studies have consistently showed greater P2Y₁₂ inhibitory effects with high clopidogrel dosing (e.g. ≥ 600 mg loading dose and 150 mg maintenance dose) (6, 7). The safety and efficacy of these doses have been recently explored in the OASIS-7 (Clopidogrel optimal loading dose Usage to Reduce Recurrent Events-Organization to Assess Strategies in Ischaemic Syndromes) trial (presented at ESC Congress 2009, Barcelona, Spain), which randomised ACS patients scheduled to undergo angiography within 72 hours of hospital arrival to high dose (600 mg loading dose of clopidogrel the first day, then 150 mg once a day for seven days, followed by 75 mg daily for the rest of the month) or standard dose of clopidogrel (300 mg loading dose followed by 75 mg daily) for a month. In the subgroup of patients undergoing

Table 1: Description of the platelet function tests most commonly used for monitoring aspirin and P2Y₁₂ inhibitors response.

Test	Basis	Able to monitor	Advantages	Disadvantages
Laboratory-based methods				
Turbidometric aggregometry	Platelet aggregation	Aspirin P2Y ₁₂ inhibitors	Historical gold standard	Large sample volume Complex sample preparation Time-consuming
Impedance aggregometry	Platelet aggregation	Aspirin P2Y ₁₂ inhibitors	Whole-blood assay	Large sample volume Complex sample preparation Time-consuming
VASP phosphorylation state (flow cytometry)	P2Y ₁₂ activation-dependent signalling	P2Y ₁₂ inhibitors	Whole-blood assay Very small sample volume Most specific for assessing P2Y ₁₂ blockers effect Can be shipped to core lab	Complex sample preparation Requires flow cytometer and experienced technician
Thromboxane A ₂ metabolites	Serum thromboxane B ₂ (stable blood metabolite) Urinary 11-dehydro thromboxane B ₂ (stable urine metabolite) /creatinine	Aspirin	Evaluation of COX-1 inhibition (aspirin target) Most specific for assessing aspirin effect Can be shipped to core lab	Not entirely platelet-specific
Platelet surface P-selectin, activated GP IIb-IIIa, leukocyte-platelet aggregates (flow cytometry)	Changes in platelet surface due to activation	Aspirin P2Y ₁₂ inhibitors	Whole-blood assays Small sample volume Can be shipped to core lab	Complex sample preparation Requires flow cytometer and experienced technician
Potential bedside testing				
Bleeding time	Cessation of blood flow by platelet plug after a blade incision (e.g. in the forearm)		Physiological In vivo surrogate for potential of clinical bleeding	Crude approach Operator-dependent Low reproducibility (e.g. dependant of temperature, cuff pressure, direction of the incision)
VerifyNow®	Platelet aggregation	Aspirin P2Y ₁₂ inhibitors	Whole-blood assay Small sample volume Very simple and rapid No sample preparation True point-of-care (no pipetting required)	Limited by haematocrit and platelet count range No instrument adjustment
Multiplate® analyser	Multiple electrode aggregometry (electric impedance)	Aspirin P2Y ₁₂ inhibitors	Whole-blood assay Small sample volume Simple and rapid	Requires pipetting
TEG® Platelet Mapping™ system	Platelet contribution to clot strength	Aspirin P2Y ₁₂ inhibitors	Whole-blood assay Global evaluation of haemostasis (clot formation and lysis)	Limited studies Requires pipetting
Plateletworks™	Platelet aggregation	Aspirin P2Y ₁₂ inhibitors	Whole-blood assay Minimal sample preparation	Not well studied yet Requires pipetting
Impact® cone-and-plate(let) analyser	Shear-induced platelet adhesion	Aspirin P2Y ₁₂ inhibitors	Whole-blood assay Small sample volume Importance of shear for platelet function No sample preparation Simple and rapid	Not widely used Requires pipetting
PFA-100®	Cessation of high shear blood flow by platelet plug	Aspirin	Whole-blood assay Simple and rapid Small sample volume No sample preparation	Dependent on vWF and haematocrit Minimal pipetting Does not correlate well with clopidogrel therapy Do not assess the whole range of platelet response

vWF, von Willebrand factor. Adapted in part from Michelson AD. Platelet function testing in cardiovascular diseases. *Circulation* 2004;110:e489–93. with permission.

PCI, a benefit of the high clopidogrel dose regimen was observed in terms of a reduced risk of stent thrombosis and myocardial infarction. The latter was at the expense of increased rates of major and severe bleeding according to the study definition (CURRENT bleeding), but not of Thrombolysis In Myocardial Infarction (TIMI) major bleeding, intracerebral, fatal bleeds or coronary artery bypass grafting (CABG)-related bleeds. The study also included an open-label randomisation to high (300–325 mg daily) vs. low (75–100 mg daily) dose of aspirin. No significant differences in efficacy or bleeding between high and low-dose aspirin were found, and only a trend towards a higher rate of gastrointestinal bleeds in the high-dose group was observed. Of note, the high-clopidogrel-dose regimen was associated with major CURRENT-bleeding in those patients receiving a low dose of aspirin.

Recently, the publication of phase III trials evaluating more potent antiplatelet agents has provided important information regarding the efficacy and safety of novel and more potent agents. Prasugrel is a third generation thienopyridine which achieves more potent ADP P2Y₁₂ receptor blockade than clopidogrel even when used at high doses (22). The TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel – Thrombolysis In Myocardial Infarction 38) trial showed that in high risk ACS patients undergoing PCI, prasugrel compared with clopidogrel was associated with significantly reduced rates of ischaemic events at 15 months, including stent thrombosis, but with an increased risk of TIMI major non-CABG related bleeding, including life-threatening bleeding (23).

Ticagrelor is a cyclopentyltriazolopyrimidine which directly and reversibly inhibits the platelet P2Y₁₂ receptor, achieving a higher inhibition of platelet aggregation than clopidogrel (24). The PLATO (Platelet Inhibition and Patient Outcomes) trial compared

ticagrelor (loading dose of 180 mg followed by 90 mg twice daily) with clopidogrel (loading dose of 300 to 600 mg followed by 75 mg daily) for preventing cardiovascular events in ACS patients with or without ST-segment elevation (25). In this trial, ticagrelor was associated with a significant reduction in the rates of ischaemic events at 12 months, including cardiovascular death, death from any cause, and stent thrombosis. Although no increase in major bleeding was found using the protocol definition, ticagrelor was associated with a higher rate of TIMI major non-CABG related bleeding (25).

Platelet function testing and bleeding outcomes

The association between platelet function measurement and bleeding risk was evaluated in a recent study in which different doses of clopidogrel and prasugrel were administered in an animal model (rabbits) and inhibition of ADP-induced platelet aggregation assessed by LTA as well as bleeding times were measured (26). Clopidogrel was shown to be 4–5 times less potent in terms of platelet inhibition than prasugrel. A moderate inhibition of platelet aggregation (30–40%) was associated with a two-fold increase of bleeding time, while higher inhibition (50–60%) was associated with a five- to six-fold increase of bleeding time. Therefore, this investigation observed that higher levels of platelet inhibition were associated with higher bleeding times, a surrogate for clinical bleeding.

Correlation between platelet function tests results and bleeding has been assessed in humans mainly in patients undergoing CABG surgery (27, 28) (Table 2). Among platelet function tests, thrombo-

Table 2: Clinical studies relating inhibition of platelet aggregation assessed by various platelet function assays with a higher risk of bleeding outcomes.

	N	Scenario	Test	Outcomes
Poston et al (27)	82	Patients on aspirin (>90%) undergoing off-pump CABG	TEG*	Haemoglobin loss at 24 hours
Chen et al (29)	45	Patients on clopidogrel within 6 days of CABG	LTA [‡]	In-hospital platelet and packed red blood cell transfusions
Rahe-Meyer et al (30)	60	Patients undergoing elective cardiac surgery	MEA	In-hospital transfusion of platelet concentrates
Cuisset et al (31)	597	NSTEACS patients undergoing coronary angiography (DAPT 1 month after PCI)	VASP	Non-CABG related bleedings at 30 days
Sibbing et al (32)	2533	Patients undergoing PCI (600-mg clopidogrel loading dose)	MEA	In-hospital TIMI major bleedings
Serebruany et al (33)	363	Patients with documented CAD or ischaemic stroke	LTA	BleedScore minor bleedings until clopidogrel discontinuation
Michelson et al (34)	125	ACS patients scheduled for PCI receiving prasugrel or clopidogrel (substudy of TRITON-TIMI 38 trial)	VASP	Serious and non-serious haemorrhagic events ≥3 days post-PCI

ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; CAD, coronary artery disease; DAPT, dual antiplatelet therapy; LTA, light transmittance aggregometry; MEA, multiple electrode platelet aggregometry; NSTEACS, non-ST elevation acute coronary syndrome; PCI, percutaneous coronary intervention; TEG, thromboelastography; TIMI, thrombolysis in myocardial infarction; VASP, vasodilator-stimulated phosphoprotein phosphorylation assay. *No significant association using whole blood aggregometry. [‡]No significant association using Platelet Function Analyzer (PFA)-100 or PlateletWorks systems.

elastography (TEG) has been extensively studied and has been reported as a useful tool to reduce bleeding risk and the need for blood transfusion (28). Poston et al. evaluated patients (n=82) with a high preoperative use of aspirin (>90%) undergoing CABG. In a receiver operating characteristic curves (ROC) analysis, aggregation assessed by TEG, but not with impedance aggregometry, was found to be a good predictor for haemoglobin loss at 24 hours (area under the curve 0.77) (27). Other functional assays have also shown their value for predicting bleeding complications and need for blood transfusion in patients taking clopidogrel and undergoing CABG. Chen and colleagues found that in patients on clopidogrel therapy undergoing CABG (n=45) lower platelet aggregation assessed by LTA was significantly associated with a higher risk of receiving platelets and packed red blood cells transfusions, while other tests, such as Platelet Function Analyzer (PFA)-100 (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and PlateletWorks (Helena Laboratories, Beaumont, TX, USA), did not show any association (29). Recently, the multiple electrode aggregometry (MEA) (Multiplate[®] analyser, Dynabyte Medical, Munich, Germany) was also assessed in cardiac surgery, showing a significant higher number of platelet concentrates transfusions in patients in the lower tertile of platelet aggregation compared to patients in the higher tertile. In a ROC analysis, the postoperative ADP test in relation to platelet concentrates transfusion had a value of 0.76 for the area under the curve (30). Several ongoing studies are evaluating the degree of platelet inhibition which will allow clopidogrel-treated patients (e.g. ACS patients pre-treated with clopidogrel before coronary anatomy is known) to safely undergo CABG using the VerifyNow[®] P2Y₁₂ assay (Accumetrics, San Diego, CA, USA) and the TEG[®] Platelet Mapping[™] system (Haemoscope, Niles, IL, USA).

There is some emerging data for the utility of platelet function assays in predicting bleeding in patients undergoing PCI (Table 2). Cuisset et al. observed in a population of patients (n=597) admitted with non-ST-elevation ACS that a higher response to clopidogrel therapy assessed by LTA and flow cytometry analysis of the status of phosphorylation of vasodilator-stimulated phosphoprotein (VASP) was associated with a higher risk of non-CABG related bleedings at 30 days (31). Recently, the impact of clopidogrel responsiveness on the risk of bleeding was evaluated in clopidogrel-treated patients undergoing PCI (n=2,533), using the MEA technique (32). As established cut-off points or definitions for “enhanced clopidogrel responsiveness” or “hyper-responders” are still lacking, ROC analysis was used in this study to derive the optimal level of platelet aggregation value defining enhanced clopidogrel responders for the association of platelet aggregation measurements with the primary endpoint of in-hospital TIMI major bleeding. The risk for major bleeding was significantly higher in patients (n=975) with an enhanced response to clopidogrel as compared to the remaining patients (n=1,558) (unadjusted odds ratio [OR] 2.6, 95% confidence interval [CI]: 1.3–5.2; adjusted OR 3.5, 95% CI: 1.6–7.3).

A recently published retrospective analysis of a cohort of stable patients with CAD or ischaemic stroke (n=363) treated with chronic low-dose aspirin and clopidogrel observed a strong corre-

lation between higher value of inhibition of platelet aggregation and minor, but not severe, bleeding events, using a novel bleeding classification, named “BleedScore”, which is more sensitive to superficial bleeding episodes than other bleeding classifications (TIMI and GUSTO) (33).

Bleeding concerns have particularly emerged with the clinical results of studies using more potent antiplatelet agents, such as prasugrel. A platelet function substudy (n=125) of the TRITON-TIMI 38 trial using VASP and LTA confirmed greater platelet inhibition with prasugrel (34). Although the study included a limited number of patients, reduced platelet reactivity assessed by VASP was significantly associated with haemorrhagic events during follow up (a combination of serious and non-serious haemorrhagic events occurring >3 days post-PCI; OR 0.971, CI 0.944 – 0.998, p=0.033 after adjusting for age, sex and beta-blocker use). However, this association lost statistical significance when adjusted for study site and baseline result. Platelet function testing with other novel and potent P2Y₁₂ receptor antagonists (ticagrelor, cangrelor, elinogrel) currently under clinical investigation will provide further insights to this topic (35).

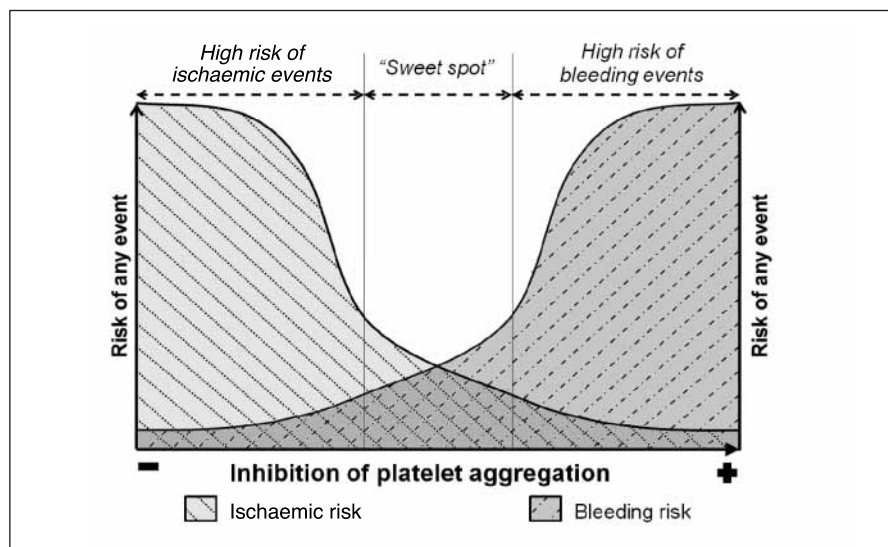
The POPular (Do Point-of-Care Platelet Function Assays Predict Clinical Outcomes in Clopidogrel Pretreated Patients Undergoing Elective PCI) study prospectively compared the value of assessing clopidogrel-induced platelet inhibition with five different tests (LTA using ADP as agonist, VerifyNow[®] P2Y₁₂ assay, Plateletworks[®] assay, IMPACT-R, and PFA-100[®] System) for predicting ischaemic and bleeding outcomes in patients (n=1,069) undergoing elective PCI and receiving both aspirin and clopidogrel therapy. While high on-treatment platelet reactivity assessed by LTA, the VerifyNow[®] P2Y₁₂ assay and the Plateletworks[®] was associated with ischaemic events at one-year follow-up, none of the tests predicted the risk of bleeding complications (36). These findings support the need for further understanding on the association between platelet function testing and bleeding complications.

Recent findings support that blockade of P2Y₁₂ signalling pathway has an impact on thrombin generation processes (37). The latter plays also a key role in haemostasis and therefore on bleeding. This is in line with the greater risk of bleeding when adding clopidogrel to aspirin and with the greater risk of bleeding associated with prasugrel compared to clopidogrel. High doses of clopidogrel have shown to reduce thrombin generation processes as assessed by TEG (37). How other more potent P2Y₁₂ receptor antagonists compare with clopidogrel regarding thrombin generation processes is currently under investigation.

Future considerations

The main challenge in the future for platelet function testing is identifying the “optimal” test and cut-off values of platelet inhibition which will reduce ischaemic event rates without increasing the risk of bleeding (38) (Fig. 1). Large-scale studies are needed to identify this therapeutic window of platelet inhibition and define its adjunctive value to clinical predictors of adverse outcomes.

Figure 1: Platelet inhibition is related to the risk of both ischaemic and bleeding events. In particular, low levels of platelet inhibition increase the risk of recurrent ischaemic events, while high inhibition increases the risk of bleeding. Therefore, the objective of antiplatelet therapies should be to inhibit platelet function to an extent that the risk of ischaemic as well as bleeding outcomes is minimised. This optimal range of platelet inhibition or "sweet spot" may be tailored to specific populations or clinical scenarios with different ischaemic and bleeding risk.



Only through these studies will we be able to perform dedicated trials evaluating the efficacy and safety of titrating antithrombotic treatment according to the identified cut-off values. In addition, the use of more potent antiplatelet treatments, such as high clopidogrel doses or newer P2Y₁₂ inhibitors (e.g. prasugrel, ticagrelor), may be of special benefit in those patients that are hypo-responders to standard clopidogrel dosing. In particular, a randomised platelet function study comparing the functional impact of high (150 mg daily) vs. standard (75 mg daily) clopidogrel maintenance dosing in patients (n=40) undergoing elective PCI showed that the high-dose regimen increased platelet inhibition in all patients, but to a greater extent in those with higher platelet reactivity, assessed by LTA, while on standard clopidogrel dosing (39). These findings support the usefulness of platelet function testing to identify hypo-responders to standard antiplatelet therapy, who will potentially benefit of a tailored treatment, which, however, needs to be confirmed in dedicated randomised studies.

To date, some studies have evaluated tailored treatment based on platelet function testing. Cuisset et al. evaluated the effect of adding abciximab to dual antiplatelet therapy in clopidogrel non-responders (n=149), defined by LTA, referred for elective PCI. The rate of cardiovascular events at one month was significantly lower when abciximab was added compared to conventional dual antiplatelet therapy (40). Bonello et al. randomised patients undergoing PCI with low response to clopidogrel (n=429), assessed by VASP, to receive or not up to three additional 600 mg loading doses of clopidogrel showing reduced stent thrombosis rates in the VASP-guided group (41). The 3T/2R trial randomised poor responders (n=263) to aspirin or clopidogrel, assessed by the VerifyNow, who underwent elective PCI to receive tirofiban vs. placebo and showed that patients in the tirofiban group had a significant reduction of major adverse cardiovascular events within 30 days (42). In none of the above platelet function guided trials was the use of more aggressive antiplatelet therapy in hypo-responders associated with increased bleeding. Ongoing trials are evaluating the

association between platelet function measures and both ischaemic and bleeding outcomes. Among them, the GRAVITAS (Gauging Responsiveness with a VerifyNow Assay: Impact on Thrombosis And Safety) trial is evaluating the safety and efficacy of a high clopidogrel maintenance dose in patients with inadequate response to clopidogrel assessed by VerifyNow (NCT00645918) (43), and the TRILOGY-ACS (TaRgeted platelet Inhibition to cLarify the Optimal strateGy to medically manage Acute Coronary Syndromes) study (NCT00699998) will have a large scale platelet function substudy using the VerifyNow[®] assay, regarding the use of the more potent antiplatelet agent prasugrel.

The impact of gene sequence variations on outcomes, in particular bleeding, has been extensively evaluated with oral anti-coagulants (44). Pharmacogenetic modulation of antiplatelet agents and its prognostic implications has been recently investigated (45). Several recent large-scale studies have showed that carriers of the loss-of-function variant allele of the CYP2C19 enzyme, a cytochrome P450 isoform involved in clopidogrel metabolism into its active metabolite, have an increased risk of ischaemic events (46–48). Of note, a recently reported genomic substudy of the CHARISMA trial observed an association between homozygotic CYP2C19*2 genotype and a lower risk of GUSTO bleeding events when compared with the wild type (presented at TCT meeting 2009, San Francisco, CA, USA) in patients treated with clopidogrel. Therefore, patients with higher clopidogrel-induced antiplatelet effects due to an extremely efficient hepatic biotransformation, such as the increased enzymatic function of CYP2C19 due to increased transcription of the CYP2C19 gene in carriers of the CYP2C19*17 allelic variant (48), may have a higher risk of bleeding complications. The latter is in line with the results of a study in which the impact of the CYP2C19*17 genotype on platelet aggregation and the risk of bleeding was assessed in clopidogrel treated patients (n=1,524) undergoing coronary stent placement. Indeed, for both heterozygote and homozygote *17 carriers, significantly lower ADP-induced platelet aggregation values were ob-

served as compared to wild-type homozygous patients, and carriage of the CYP2C19*17 allele was associated with an increased risk of bleeding defined according to TIMI criteria (49). Interestingly, a gene-dose effect was observed and the highest risk of bleeding occurred in CYP2C19*17 homozygous patients, which also showed the lowest ADP-induced platelet aggregation values.

Ongoing studies will provide further evidence to what extent genetic variants affecting the metabolism of antithrombotic drugs may also have an impact on the occurrence of bleeding. Likely, in the future the best predictive models of ischaemic and bleeding outcomes will imply integrating both genetic and platelet function testing. The ongoing GIFT (Genotype Information and Functional Testing) study (NCT00992420) will be evaluating synergy between genetic and functional testing within the GRAVITAS trial.

In summary, a broad variety of platelet function tests are currently available which enable assessment of antiplatelet treatment effects. Although platelet function testing represents a promising tool to assess the risk of undesired outcomes, including bleeding, in patients treated with antiplatelet therapy, there is currently no gold standard test or cut-off value which justifies its routine use in clinical practice. Ongoing studies in large patient populations using point-of-care assays will provide important insights on the prognostic value of platelet function testing and hopefully define the therapeutic window or "sweet spot" of platelet inhibition which is associated with reduction in recurrent ischaemic events with minimised bleeding complications. This optimal range of platelet inhibition or "sweet spot" may be variable according to specific populations or clinical scenarios with different ischaemic and bleeding risk. Platelet function testing, potentially integrated with genetic testing, is likely to play a key role with the development and introduction into clinical practice of new antithrombotic drugs.

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