PLATELET FUNCTION TESTING-GUIDED ANTIPLATELET THERAPY

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ABSTRACT

Cardiovascular diseases are the leading cause of death in the Western world. Several factors have led to the increase in vascular disorders, including the aging population, unhealthy lifestyles, increasing rates of diabetes and raised lipids, and further risk factors resulting in inflammation and calcification of the vascular endothelium. Activated platelets in damaged blood vessels can trigger arterial thrombus formation, leading to vascular occlusion with subsequent organ hypoperfusion and clinical manifestation of myocardial infarction, stroke, or peripheral artery disease. Platelet inhibitors such as aspirin and clopidogrel (Plavix® and generics) are prescribed as primary or secondary prevention to attenuate chronic platelet activation. However, a significant proportion of patients do not respond adequately to uniform antiplatelet treatment. These 'non-responders' have an increased risk for stent thrombosis, stroke, and other ischemic complications. Platelet function (PF) tests can identify these patients thus enabling physicians to offer personalized and alternative treatment strategies. Recent alternatives to clopidogrel include prasugrel (Efient®) and ticagrelor (Brilique®) – that are both more potent than clopidogrel but also more expensive and associated with a higher risk of bleeding complications. Given these drawbacks, PF testing might help clinicians to prescribe optimal antiplatelet agent to maximize patient safety and efficacy while minimizing costs. While randomized studies using different test systems have left clinicians puzzled about the medical value of tailored antiplatelet therapy, accumulated evidence from recent studies on tailored antiplatelet therapies and the association with improved outcomes have now resulted in a consensus expert opinion for the specific adoption of PF diagnostics into clinical practice.

INTRODUCTION

Platelets are an essential component of blood coagulation and are responsible for maintaining primary hemostasis under conditions of blood flow. When an arterial blood vessel is injured, platelets adhere to and aggregate at the vessel wall to initiate primary hemostasis as well as promoting secondary hemostasis to stop bleeding. In dysfunctional blood vessels (due to plaque formation, inflammation, or lipid deposition), however, platelets become activated and platelet aggregation may cause excessive thrombus formation and vascular occlusion, resulting in myocardial infarction (MI) or stroke. Activated platelets play a pivotal role in the pathogenesis of acute coronary syndrome (ACS) and are a major concern in relation to ischemic complications

following percutaneous coronary intervention (PCI) [1, 2]. Even though stent thrombosis (ST) is a rare event, it is a severe or fatal complication and is associated with high mortality rates [3, 4]. Dual antiplatelet therapy consisting of aspirin and a P2Y12-inhibitor (clopidogrel, prasugrel, or ticagrelor) is state-of-the-art therapy in ACS patients undergoing PCI for prevention of ischemic adverse events [5]. This therapy is maintained for 1 year in the majority of patients undergoing PCI. However, inter-individual response to platelet inhibiting drugs has led to treatment failure in selected patients: up to 25% of patients respond inadequately to clopidogrel [6] and around 10% have an inadequate response to aspirin [7]. The reason for this is multifactorial: genetic factors, drug interactions, altered drug absorption, diet, age, lack of compliance, lifestyle, co-morbidities, and platelet turnover may contribute to insufficient drug efficacy. The phenomenon of clopidogrel low responsiveness or 'resistance', which is also termed high on-treatment platelet reactivity (HPR), has been associated with an increased risk of ischemic complications, including ST [8–10]. Several publications and meta-analyses have also reported the increased incidence of arterial ischemia with 'aspirin resistance' [11–13].

PHAMACOKINETICS/PHARMACODYNAMICS OF CLOPIDOGREL, PRASUGREL, AND TICAGRELOR AND COMPARATIVE EFFICACY

One factor in the pathogenesis of clopidogrel low responsiveness is the complex biotransformation of this drug in the body. Clopidogrel is a pro-drug and is converted to the active metabolite via several enzymatic steps. The cytochrome P450 (CYP)-isoenzymes involved in the conversion of clopidogrel to its active metabolite have several polymorphisms. Variants of theses enzymes with reduced activity (CYP2C19*2, 'loss of function' polymorphism) and also variants with increased activity (CYP2C19*17, 'gain of function' polymorphism) have been described and linked to both clopidogrel responsiveness and to clinical events [14–16]. More potent and less variable novel antiplatelet drugs have been developed and launched to market. Prasugrel is also a thienopyridine pro-drug, which irreversibly blocks the P2Y12 receptor (adenosine diphosphate [ADP] receptor) but with much higher potency compared with clopidogrel. The main difference between clopidogrel and prasugrel is that prasugrel is more readily transformed to its active metabolite, fewer enzymes are involved, and the reaction time is much faster and less variable. However, low responsiveness to prasugrel has also been reported [17].

Ticagrelor, in contrast to clopidogrel and prasugrel, is a direct-acting ADP antagonist and not a pro-drug. As a reversible ADP receptor antagonist, it was expected that ticagrelor's action would cease much faster than clopidogrel's. However, the ONSET/OFFSET trial showed that the platelet function (PF) inhibition of both agents was very similar, although ticagrelor showed much higher potency compared with clopidogrel [18]. The drug approval trials TRITON-TIMI 38 [19] and PLATO [20] each compared head-to-head the efficacy of prasugrel or ticagrelor versus standard care with clopidogrel. Both novel drugs showed benefit with regard to ischemic events but at the same time were associated with an increased risk of bleeding. In fact, for prasugrel an excess of life-threatening bleeding was observed [19]. Of particular note, a landmark analysis of the TRITON-TIMI 38 trial data showed that the bleeding risk for prasugrel was similar to clopidogrel during the first 3 days of treatment (in-hospital bleeding events) but was significantly greater during Day 3 after the PCI procedure until the end of the study (i.e. post-hospital discharge bleeding events). Thus, the excess of bleeding events observed with the use of prasugrel occurred predominantly during the maintenance phase of treatment [21]. Moreover, most of the benefit seen with prasugrel versus clopidogrel treatment in ACS patients with regard to thrombotic risk reduction was seen in the early and acute periods of treatment [19, 21]. As a consequence, strategies to optimize P2Y12 receptor-directed antiplatelet treatment beyond the acute phase and during the maintenance phase of treatment may significantly improve the outcome for ACS patients undergoing PCI. It is also important to mention that both prasugrel and ticagrelor are significantly more costly than treatment with generic clopidogrel [22, 23]. An individualized approach to treat clopidogrel low-responders with more potent drugs and to prescribe generic clopidogrel (which is well tolerated and cheap) for patients without HPR might be a valid strategy to maintain the benefit for patients and reduce healthcare costs. Therefore, it is of increasing interest to identify patients who are non-responders to antiplatelet drugs and to tailor therapy to the most effective individual option.

PLATELET FUNCTION TESTING

There are several methods for platelet function testing (PFT), which differ with regard to the underlying detection principle, agonists, and sample material used for testing. Light transmission aggregometry (LTA) is the historical gold standard for PF analysis and monitoring the pharmacodynamic response to antiplatelet agents. This optical method is based on the change in turbidity of the platelet-rich plasma after the addition of an agonist [24]. Even though this method is still widely used and has shown significant clinical value, LTA is complex, time-consuming, and poorly standardized [25]. Flow cytometric analysis of the phosphorylation state of the vasodilator stimulated phosphoprotein (VASP) is a very specific method for the evaluation of ADP-receptor inhibition. However, this technique requires a specialized laboratory environment and experienced staff and is thus not suitable for routine clinical use. The VerifyNow® (Accumetrics, USA) device measures PF based on turbidimetric optical detection of the aggregation of platelets with fibrinogen-coated plastic beads in whole blood. VerifyNow is a fully automated

and standardized point-of-care (POC) device. In the PFA-100 system, citrated whole blood is aspirated at high shear rates through a capillary containing an aperture within a collagen-coated membrane [26]. Besides sensitivity to the antiplatelet drug's effect, the PFA system demonstrates high sensitivity for von Willebrand syndrome due to the measurement principle [27]. A further method for PFT that is based on impedance aggregometry, the Multiplate® analyzer (Roche Diagnostics, Switzerland), is described below. The characteristics of the most commonly used assays are also summarized in Table 1, which illustrates the wide variability in the approach taken. Thus, it is not surprising that the results differ considerably from method to method [28]. It is, therefore, important that every assay should be validated with regard to the relationship between the result provided and the association with patient outcome. If this validation of assay-specific predictability is not available, the results should be interpreted with caution, and be based on the physician's experience and in line with the current guideline recommendations.

In order to translate laboratory findings into clinical benefits, it is paramount that standardized PFT methods which are comparable across centers and have high clinical predictivity to determine patient risk for thrombosis or bleeding are utilized. Evidence is required for the PFT result being a modifiable risk factor, i.e. the drug intervention based on the test result has to translate into superior clinical outcomes. According to the consensus document on the definition of HPR to ADP the Multiplate analyzer demonstrated the best predictability of ischemic events with an odds ratio of 12 (12 times increased risk for clopidogrel low-responders for early ST), while other studies using, for example, the VerifyNow system or LTA showed an odds ratio of only 2–4 and 2–6, respectively [10, 28, 30]. A large meta-analysis in 4213 patients on the efficacy and safety of intensified antiplatelet therapy on the basis of platelet reactivity testing in patients after PCI concluded that for high-risk patients "intensifying antiplatelet therapy on the basis of platelet reactivity testing reduces cardiovascular mortality and ST after PCI" [31]. Several registry studies confirmed this finding using the Multiplate ADPtest [17, 32, 33]. In contrast, randomized studies (ARCTIC [34], GRAVITAS [35] and TRIGGER-PCI [36]) using the VerifyNow device failed to confirm the concept of improving patient benefit based on tailored anti-platelet therapy. There are different reasons which led to the negative results of these studies, in particular the studies mostly recruited patients with stable coronary artery disease patients who exhibited a low-to-intermediate risk for recurrent thromboembolic complications. Furthermore, the studies made little use of switching patients to more potent drugs in the case of clopidogrel low-response, a relatively high proportion of patients were defined as low-responders (>40% in GRAVITAS), and non-PFT-related study endpoints were chosen in ARCTIC (periprocedual MI), which allowed complicated drug treatment protocols according to the physicians discretion [37]. In fact, in the open-label ARCTIC trial involving 2440 patients, antiplatelet therapy was intensified in the monitoring arm with either an additional bolus plus intensified maintenance dose of clopidogrel (80.2%), by switching to prasugrel (only 3.3%), by increasing the dose of aspirin, or by additional treatment with glycoprotein IIb/IIIa inhibitors during PCI [34]. It is important to remember that neither prasugrel nor ticagrelor were available at the time of the GRAVITAS trial and a strategy of single re-loading and doubling the maintenance dose of clopidogrel versus standard dose clopidogrel has been shown to be non-efficacious in 2200 patients with HPR [35]. The fact that HPR resolved over time in 40% of patients in the guided group of the GRAVITAS trial might be a further possible explanation for the negative

Table 1
Comparison between the most commonly used platelet function assays (modified from [27]).

Method	Test	Medium	Detection	Advantages	Disadvantages
Laboratory based	Light transmission aggregometry (LTA)	Platelet-rich plasma (PRP)	Reduction of optical density after stimulation in PRP	• Instrument adjustment possible • Good predictivity • Long experience	• Time-consuming • Complex sample preparation • No standardization
	Vasodilator-stimulated phosphoprotein (VASP) assay	Whole blood	Flow cytometric detection of VASP phosphorylation	• Whole blood assay • Longer sample storage • P2Y12 receptor specific	• Very time-consuming • Complex sample preparation • Need of flow cytometer
Near patient	Multiplate analyzer (multiple electrode aggregometry [MEA]		Coating of two electrode pairs by platelets	• Whole blood assay • Simple and rapid • Standardized procedures	• Semi-automated (requires pipetting) • Rapid processing of samples necessary
	VerifyNow	Whole blood	Platelet-mediated aggregation of fibrinogen-coated polystyrene beads		• No assay adjustment possible • Expensive cartridges
	PFA-100		Closure of an aperture of a collagen-coated membrane		• No assay adjustment possible • Dependent on hematocrit and von Willebrand factor • Limited experience and study results with P2Y12-inhibitors

outcome of the study [38]. Furthermore, the pre-specified cut-off for the VerifyNow test of 230 P2Y12 reaction units (PRU) that was used in GRAVITAS to identify clopidogrel low-responders defined a high proportion of patients with HPR who did not exhibit any greater thrombotic risk compared to the control cohort of clopidogrel normal responders in the observational substudy. According to a post hoc analysis of the GRAVITAS results, a lower cut-off for the definition of HPR of 208 PRU was associated with a lower risk for cardiovascular events [38]. This cut-off was used in the Testing Platelet Reactivity in Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel (TRIGGER-PCI) trial which randomized subjects with HPR on clopidogrel after successful PCI [36]. However, this study was halted prematurely by the steering committee after randomization of 423 patients (of the planned 2150) because of a lower than expected clinical event rate. Therefore, it is not surprising that these studies on personalized antiplatelet therapy approaches failed to confirm a significant benefit. In contrast, several studies involving more than 2500 patients did show the proven benefit of individualized treatment guided by the Multiplate analyzer [32, 33, 39]. In fact, a switch from clopidogrel to prasugrel significantly improved clinical outcome in patients with HPR in the MADONNA study [32]. According to a cut-off value of 47 U for the Multiplate ADPtest, the percentage of clopidogrel low-responders is about 20–25% [8, 33]. The results of the ISAR -HPR registry from Munich University (Germany) which included 999 high-risk ACS patients with HPR could demonstrate a significant reduction in the risk of death or ST (HRadj=0.18, 95% confidence interval (CI)=0.07–0.50; P=0.0008, Cox model) in the Multiplate tailored cohort receiving prasugrel or repeated loading doses of clopidogrel versus the control cohort treated with conventional clopidogrel therapy [39].

In the Pécs registry from the Heart Center Balatonfüred (Pécs, Hungary), 741 consecutive high-risk ACS patients undergoing PCI were enrolled, comprising almost 50% ST segment elevation myocardial infarction and 5% cardiogenic shock patients with an overall mortality of 8.1% and an ST rate of 5.3% during 1 year follow-up. The authors concluded that tailoring antiplatelet treatment based on the Multiplate result and "switching ACS patients with HPR to prasugrel reduces thrombotic and bleeding events to a level similar to those without HPR…, while high-dose clopidogrel resulted in higher risk for both thrombotic and bleeding complications" [33]. These findings clearly demonstrate the clinical utility of PFT with Multiplate for guided antiplatelet treatment with intensification of P2Y12-receptor inhibition via switch to prasugrel in clopidogrel low-responders.

In general, randomized studies testing the value of PF-guided antiplatelet treatment regimens have been conducted focusing on the acute phase to guide therapy. Thus, it is not known at present if ACS patients may benefit from a personalized antiplatelet treatment regimen with potent third-generation ADP receptor antagonists beyond the acute phase. In addition to the possible benefits of individualized antiplatelet treatment on the clinical outcome of ACS patients, the economic impact and the cost-saving potential of such a treatment regimen remains to be determined. Cost-effectiveness analyses in such scenarios must focus on both the cost savings driven by the use of generic clopidogrel and the costs associated with adverse events (bleeding and thrombotic complications).

Recently, Straub et al. described a model-based analysis of cost-effectiveness of PFT with Multiplate [40]. The aim of this model-based health economic analysis was to use the available evidence to assess the clinical and economic impact of a personalized antiplatelet treatment regimen based on PFT with the Multiplate analyzer compared with the unrestricted use of prasugrel and ticagrelor in ACS patients undergoing PCI. According to this model which utilizes peer-reviewed, published data for the model analysis, PFT-guided therapy for selection of P2Y12-inhibitors in ACS patients undergoing PCI appeared to have fewer adverse events than general treatment with clopidogrel and may be more cost-effective than prasugrel and ticagrelor therapy. However, these results require further investigation and confirmation in large randomized controlled studies. At present, a randomized trial has not been conducted to directly compare the PFT-guided selection of clopidogrel or novel P2Y12-inhibitors in ACS patients with either generic clopidogrel or with prasugrel/ticagrelor. This gap may be overcome by a recently announced study entitled Testing Responsiveness to Platelet Inhibition on Chronic Antiplatelet Treatment for Acute Coronary Syndromes (TROPICAL-ACS) [41]. TROPICAL-ACS is an investigator-initiated, multicenter study designed to evaluate the benefit of personalized antiplatelet therapy in ACS patients treated with PCI. The objective of TROPICAL-ACS is to determine whether a PFT-guided approach that employs the Multiplate ADPtest with a controlled switch from prasugrel to clopidogrel antiplatelet maintenance treatment is non-inferior to standard 12-month treatment with prasugrel with regards to a combined ischemic and bleeding event. It will address both the clinical and economic impact of an individualized P2Y12 receptor-directed antiplatelet treatment in ACS patients undergoing PCI. If the TROPICAL-ACS study meets its primary endpoint, the tailored antiplatelet therapy approach will carry a significant cost-saving potential alongside the benefit to patients, with generic clopidogrel being up to 20 times less costly than prasugrel or ticagrelor. This would translate into cost savings of up to 1500 US$ per patient for annual drug therapy, based on US drug pricing [22, 23].

The expert opinion of the European Society of Cardiology Working Group on Thrombosis regarding the role of PFT in patients undergoing coronary stenting was published in the European Heart Journal in October 2013 and emphasized the utility of PF diagnostics in clinical practice. The paper states "based on the currently available evidence, the recommended assays for

monitoring platelet inhibition during P2Y12-inhibitors are the VerifyNow P2Y12 assay, the Multiplate device with the ADP kit and the VASP assay". LTA "is only recommended when no standardized assays are available" [25]. In addition, the US Working Group published a Consensus and Update on the Definition of On-Treatment Platelet Reactivity stating that, based on available evidence, "a risk algorithm that includes PFT along with biomarker testing and clinical factors may improve risk prediction and facilitate personalization of antiplatelet therapy" [42].

MONITORING OF PLATELET FUNCTION IN MAJOR SURGERY

The use of antiplatelet therapy is continuing to expand due to the increasing prevalence of cardiovascular disease. Patients taking antiplatelet drugs who are scheduled for major surgery present clinicians with the challenge of balancing the risk of thrombotic complications (if antiplatelet drugs are stopped before surgery), with the problems of excessive bleeding when surgery is performed in the absence of adequate PF, which impairs patient prognosis and increases the cost of hemostatic management.

Current guidelines recommend stopping antiplatelet drugs 3 to 7 days before surgery [43–45]. This can mean an additional cost of in-hospital patient care while waiting for surgery. However, it is important to remember that approximately 20% of patients do not respond adequately to clopidogrel initially [8, 9, 46, 47]. The Society of Thoracic Surgeons Clinical Practice Guidelines (updated in 2012) states "for patients on dual antiplatelet therapy, it is reasonable to make decisions about surgical delay based on tests of platelet inhibition rather than arbitrary use of a specified period of surgical delay" [43]. Preoperative PFT facilitates the identification of those patients who have recovered sufficient PF to allow surgery to be scheduled without unnecessary delay.

Several clinical studies have shown that the aggregation values assessed with Multiplate correlate with the risk of bleeding and transfusions in patients who are pre-treated with dual antiplatelet therapy [48–52]. Ranucci et al. suggested a cut-off of 31 U for the Multiplate ADPtest to support a clinical decision to either postpone elective surgery if the patient's PF is below this value, or if there is an urgent requirement to perform surgery, to ensure adequate precautions and appropriate therapeutic consequences (e.g. ensure availability of platelet concentrates at the blood bank, selection of a less traumatic surgical approach with good visibility, and less aggressive hemodilution), because aggregation <31 U was associated with significantly increased bleeding risk [48]. According to prospectively validated algorithms based on POC coagulation testing in surgical patients, Multiplate analysis can be used for treatment stratification in bleeding patients. In this setting, when normal aggregation is found by underlying bleeding, platelet transfusion is considered less efficient to improve the bleeding situation. In contrast, in patients who bleed and show inhibited PF, this is a sign that administration of platelets or desmopressin could improve the clinical situation [53, 54]. Weber et al. demonstrated that the hemostatic therapy algorithm guided by POC testing via Multiplate and thromboelastometry not only reduced patient exposure to allogenic blood products and decreased number of transfused units of packed erythrocytes but also lowered fresh frozen plasma and platelet concentrate usage. Furthermore, a POC-guided algorithm significantly improved clinical outcome and provided significant cost benefits compared with the standard treatment [53].

THE MULTIPLATE® ANALYZER

The Multiplate analyzer was introduced in 2005 [55] and is an advance on impedance aggregometry, which was presented by Cardinal and Flower in 1979 [56]. It assesses PF in whole blood by the attachment of thrombocytes onto metal electrodes, leading to a change in the electrical conductivity (or impedance), which is continuously recorded. The Multiplate analyzer provides five channels for parallel determinations as well as automatic analysis, calibration, and documentation using an integrated computer system. The disposable measurement cell incorporates a magnetic stirrer as well as two independent electrode pairs. Therefore, a double determination is performed during each test. Multiplate analysis allows the assessment of platelet aggregation using collagen, arachidonic acid, ADP, TRAP-6, and ristocetin stimulation. Typically, 300 µL of whole blood are added to 300 µL of isotonic saline and then analyzed.

In impedance aggregometry (as applied by the Multiplate system) the platelets have to firmly adhere to sensors, comprised of metal electrodes, in order to trigger a signal reaction, a mechanism similar to the in vivo adhesion and aggregation of platelets on a metallic stent surface or at the vessel lesion. By contrast, a pre-existing loose aggregation in solution can lead to the reduction of turbidity in classical LTA.

CONCLUSIONS

Monitoring a patient's individual response to different antiplatelet drugs can contribute to providing optimal treatment for patients at risk of arterial thrombosis. PFT represents a tangible example of personalized healthcare and ongoing randomized clinical studies will further elucidate the medical value of this biomarker. PFT has the potential to assist clinicians in determining an individual patient's risk of arterial thrombosis and bleeding and support informed decisions relating to the needs of each patient.

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