Monitoring the effectiveness of antiplatelet therapy: opportunities and limitations

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Previous clinical studies have shown heterogeneity in individual patient responses to antiplatelet therapy and high residual platelet reactivity is associated with increased risk of adverse clinical events. Monitoring response to antiplatelet therapy and tailoring treatment accordingly is currently not recommended in routine clinical practice largely due to the lack of a standardized definition of antiplatelet therapy hyporesponse and the need for a widely accepted point-of-care platelet function test that can be reliably utilized in frontline clinical practice. Recent data have shown that titrating the dose of clopidogrel in patients undergoing percutaneous coronary intervention significantly reduces the incidence of major adverse cardiovascular events and large-scale clinical trials are currently underway to investigate whether individually tailored treatment based on results of platelet function testing leads to improved clinical outcome. Furthermore, genetic testing has demonstrated a link between CYP2C19 genetic polymorphisms, altered clopidogrel metabolite concentrations and adverse clinical events. Clinical studies are currently underway to investigate the potential clinical benefit associated with genotype-guided tailoring of antiplatelet therapy. With the advent of newer, more potent antiplatelet agents and their associated increased bleeding risks, it will become imperative in the future to select the most appropriate, safe and effective drug.

Introduction

Platelets are key mediators in the pathophysiology of atherothrombosis. Antiplatelet therapy with aspirin and clopidogrel is the cornerstone of treatment in patients with acute coronary syndromes (ACS) and following percutaneous coronary intervention (PCI), to reduce the risk of thrombotic events and thus improve long-term clinical outcomes. However, despite apparently adequate antiplatelet treatment approximately 10% of patients experience recurrent ischaemic events and this is a cause for major concern. Findings from clinical studies consistently demonstrate heterogeneity in individual patient responses to antiplatelet therapy. Relative hyporesponsiveness and high on-treatment platelet reactivity measured by *ex vivo* platelet function assays is overwhelmingly associated with increased risk of adverse events including stent thrombosis (ST) and cardiovascular death [1–4]. Nonetheless, current clinical guidelines recommend a 'one-size-fits-all' approach to antiplatelet therapy prescribing that does not take into account the well documented inter-individual variability in response to therapy with either aspirin or clopidogrel.

Clinical studies have shown that titrating the dose of clopidogrel based on results of platelet function testing improves clinical outcome in patients undergoing PCI [5, 6]. However, monitoring responses to antiplatelet therapy and tailoring treatment accordingly has not been widely implemented and is not recommended in routine clinical practice largely due to (i) lack of a standardized definition for poor response or 'resistance' to antiplatelet therapy, (ii) lack of a widely accepted platelet function test appropriate for use in frontline clinical practice and (iii) controversy surrounding the interpretation of laboratory findings of 'resistance' and whether this directly translates into an inadequate biological response which has direct clinical relevance. Large-scale clinical trials are currently underway and will provide much needed definitive answers to these clinically relevant issues.

The objectives of this paper are (i) to review the currently available platelet function tests and their specific limitations; (ii) to examine the data on antiplatelet therapy response variability and the role of tailored therapy in improving clinical outcomes; and (iii) to discuss the potential role of genetic testing in monitoring the effectiveness of clopidogrel therapy in the future. As most of the current

evidence based data focus on aspirin and clopidogrel therapy in the context of ischaemic heart disease and PCI, this will be the predominant focus of this review.

Current platelet function tests and their limitations

Platelet function testing was initially used as a screening tool to identify patients with clotting disorders and subsequently in the clinical management of bleeding to guide transfusion requirements. More recently, their role has expanded to include the assessment of the effectiveness of pro-haemostatic and antiplatelet therapy in both clinical and research settings. Specifically, in ACS patients and following PCI, high residual platelet reactivity as determined using platelet function testing is a predictor of clinical risk and poor outcome. In this particular setting, the ideal platelet function test would be rapid, simple, reproducible and appropriate for use at the point of patient contact.

Historically, turbidometric light transmittance aggregometry (LTA) was considered the 'gold standard' platelet function assay. LTA measures platelet aggregation in platelet-rich plasma following *in vitro* stimulation with various agonists and is the most widely investigated method to predict clinical outcome [7–9]. However, the assay is not standardized and is subject to many methodological variables [10]. Furthermore, several limitations preclude its use in routine clinical practice including the need for an experienced technician and lengthy processing times. Electrical aggregometry, in contrast to LTA, measures increase in electrical impedance rather than light transmittance and utilizes whole blood instead of platelet-rich plasma [11, 12].

Other platelet function assays employ flow cytometry either to assess activation-dependent changes on platelet surface membrane receptors such as P-selectin and glycoprotein (GP) IIb/IIIa or alternatively to measure intracellular signalling by vasodilator-stimulated phosphoprotein (VASP) which is a specific biomarker for P₂Y₁₂ receptor activation [13]. The advantages of flow cytometric techniques include small sample volumes and the use of whole blood but they are limited by complex sample preparation and the requirement for skilled technicians. They cannot therefore offer near-patient testing.

The desire for easy access to assessment of responses to antiplatelet therapy combined with the technical limitations associated with aggregometry and flow cytometry have led to the development of point-of-care platelet function tests that can be performed with relative ease outside of the clinical laboratory setting, require minimal sample handling and provide results within a relatively short period of time. This has facilitated an expansion in the role of platelet function testing to include the identification of patients in the acute clinical setting who are 'hyporesponsive' to antiplatelet therapy and are, as a result, at increased

risk of adverse events. The currently available point-of-care assays include VerifyNow, Thrombelastograph (TEG) Platelet Mapping, Multiple Platelet Function analyser (Multiplate), Platelet Function Assay (PFA)-100 and PlateletWorks. These tests are not standardized and utilize different methodologies and cut-off values to define antiplatelet therapy 'resistance'. Furthermore, the data on the correlation between the various platelet function assays are conflicting. For example, recent studies have shown a modest to good correlation between the various assays in measuring responses to clopidogrel therapy [14] and in predicting clinical outcome [15]. On the contrary, Lordkipanidzé *et al.* [16] observed a poor correlation between platelet function assays in measuring responses to aspirin therapy with the prevalence of aspirin resistance rates varying between 59.5% for the PFA-100 assay, 18% for whole blood aggregometry and 6.7% for VerifyNow. The currently available platelet function tests and their individual strengths and limitations are summarized in Table 1. The principles and methodology of the individual point-of-care assays are described in detail below.

VerifyNow

The VerifyNow (Accumetrics, CA, USA) system [17] is a rapid automated whole blood assay that measures agglutination of fibrinogen-coated beads in response to specific agonists. The agonists used include arachidonic acid (AA) for the aspirin assay, a combination of adenosine diphosphate (ADP) and prostaglandin E₁ for the P₂Y₁₂ receptor assay [18] and a thrombin receptor activating peptide for the GP IIb/IIIa assay. The principle of platelet function measurement is based on the increase in light transmission that occurs following platelet aggregation. Although results are delivered within 5 min it is recommended that the blood sample is incubated for a minimum of 30 min prior to aspirin testing and a minimum of 10 min prior to processing the P₂Y₁₂ assay. Data have shown a good correlation between VerifyNow and LTA in assessment of responses to clopidogrel therapy [19, 20]. Furthermore, several studies have reported that resistance to aspirin and clopidogrel as defined by the VerifyNow assay in patients undergoing PCI is associated with an increased risk of periprocedural myocardial infarction [21] and adverse clinical outcome [4, 15, 22, 23]. Large prospective clinical trials are ongoing to determine the clinical benefit associated with tailored doses of antiplatelet therapy using the VerifyNow assay [24–26].

Thrombelastography (TEG) platelet mapping

Thrombelastography (Haemonetics Corp, MA, USA) is a point-of-care test that provides an overall assessment of *ex vivo* haemostatic function. It incorporates the interaction of all the components of coagulation including platelets, fibrin, clotting factors and thrombin [27, 28]. A small volume of blood is pipetted into a cylindrical cup into which a stationary pin is suspended by a torsion wire. The

Table 1

Platelet function assays

Assay	Advantages	Limitations	Predicts outcome	Able to monitor		
				Aspirin	Clopidogrel	GP IIb/IIIa
Platelet aggregometry						
Turbidometric light transmittance	Historical 'gold standard'	Time consuming, sample preparation, poor reproducibility, experienced technician required	Yes	Yes	Yes	Yes
Electrical impedance	Whole blood assay	Time consuming, sample preparation, experienced technician required	Yes	Yes	Yes	Yes
Flow cytometry						
Assessment of platelet surface P-selectin, activated GPIIb/IIIa, leucocyte-platelet aggregates	Whole blood assay, small sample volume	Sample preparation, experienced technician required	Yes	Yes	Yes	Yes
VASP phosphorylation	Whole blood assay, sample volume, P ₂ Y ₁₂ specific	Sample preparation, experienced technician required	Yes	No	Yes	No
Point-of-care						
VerifyNow	Whole blood assay, ease of use, small sample volume, rapid	Further data required on optimal cut-off values	Yes	Yes	Yes	Yes
Thrombelastogram	Whole blood assay, measures platelet-fibrin clot formation and clot lysis	Sample preparation and manual pipetting required	Yes	Yes	Yes	Yes
Multiplate	Whole blood assay, rapid	Manual pipetting required	Yes	Yes	Yes	Yes
PFA-100	Whole blood assay, ease of use, rapid, small sample volume	Affected by VWF, haematocrit levels and platelet count, manual pipetting required	Yes	Yes	NR	NR
PlateletWorks	Whole blood assay, ease of use, rapid, small sample volume	Not extensively studied	No	Yes	Yes	Yes

NR, not recommended; VASP, vasodilator stimulated phosphoprotein; VWF, von Willebrands factor.

cup oscillates and, as blood clots, changes in its viscoelasticity are transmitted to the pin which acts as a torque transducer converting the oscillations into an electrical signal to produce a TEG trace. Analysis of the TEG trace provides information on the speed and strength of clot formation as well as clot stability. Recent modifications to the original TEG methodology including the use of specific platelet activators have allowed TEG to be used more specifically to assess the effects of antiplatelet therapy [29, 30] and, in this context, it has been shown to correlate closely with the historical 'gold standard' method LTA [7, 31–33]. Conventionally the maximum amplitude (MA) of the TEG trace, which is representative of clot strength, has been used to determine response to antiplatelet therapy. Previous studies have shown that TEG MA is a predictive tool for ischaemic events following PCI in patients on antiplatelet therapy [7, 34]. However, it can take up to 1 h for the MA value to be obtained and, thus, a novel TEG parameter known as area under the curve (AUC15) which provides an assessment of the effects of antiplatelet therapy in 15 min has been developed and validated [27, 28, 35, 36]. AUC15 incorporates both the speed of clot formation as well as clot strength and has been shown to correlate strongly with the traditionally used MA [27].

Multiple platelet function analyser (Multiplate)

The Multiplate analyser (Dynabyte, Munich, Germany) is a whole blood assay that utilizes impedance aggregometry

to measure responses to aspirin, clopidogrel and GPIIb/IIIa antagonists [37]. It employs five test channels containing various agonists that stimulate platelet aggregation. The attachment of platelets to the Multiplate sensors generates an increase in impedance which is transformed into an aggregation tracing that is plotted against time and from which various parameters are measured. It requires only a small amount of blood (0.3 ml per test), no sample preparation and has a rapid 10-min test time. Multiplate has been shown to correlate well with LTA in measuring responses to antiplatelet therapy [38–40] and large prospective studies have shown that clopidogrel [41, 42] and aspirin [43] hyporesponsiveness, as determined using Multiplate in patients undergoing PCI, is an independent predictor for the occurrence of ischaemic events including periprocedural myocardial infarction and stent thrombosis. Furthermore, a recent study in 2533 patients undergoing PCI has shown that an enhanced response to clopidogrel measured using the multiplate analyser is associated with a higher risk of major bleeding [44].

Platelet function assay (PFA)-100

The PFA-100 system (Dade Behring, Marburg, Germany) is a whole blood assay that utilizes cartridges containing collagen and epinephrine agonists to measure the antiplatelet effects of aspirin [45]. Platelet aggregation is determined by the time taken for the occlusion of an aperture in a membrane under high shear conditions. The assay

does not require any sample preparation and the aperture closure time is up to 5 min. However, blood samples require manual pipetting and need to stand for at least 15 min prior to assaying. The disadvantages of this system are that the assay is affected by Von Willebrand factor levels (which are increased following PCI [46]), platelet count and haematocrit levels. Furthermore, although the PFA-100 assay has a collagen/ADP cartridge, it has been shown to be insensitive to the effects of clopidogrel [47–49] and there are limited data to support its use in monitoring thienopyridines. Several meta-analyses have shown that high residual platelet reactivity and aspirin resistance determined by the PFA-100 assay is associated with increased risk of cardiovascular events and may be useful in predicting adverse outcome. Specifically, Reny *et al.* [50] and Crescente *et al.* [51] observed a significant association between aspirin resistance and risk of vascular and recurrent ischaemic events in patients with cardiovascular disease (odds ratio (OR) 2.1, 95% CI 1.4, 3.4 and relative risk 1.63, 95% CI 1.16, 2.28, respectively). However, the cut-off aperture closure times used to define aspirin hyporesponse vary significantly between individual studies (from 130 to 300 s), signifying the need for better standardization of this assay.

PlateletWorks

The PlateletWorks system (Helena Laboratories, TX, USA) utilizes impedance aggregometry to measure the degree of platelet aggregation by way of single platelet counting [52]. Platelet aggregation is stimulated by ADP, AA or collagen agonists. Only a small amount of whole blood is required and the results are available within 2–10 min depending on the agonist. However, blood samples must strictly be analysed within 10 min of collection which can be difficult in a busy clinical setting leading to unreliable test results. The Plateletworks assay has been shown to correlate well with LTA [53] but there are very limited data on its use in the prediction of cardiovascular outcomes. A recent study has shown that high residual platelet reactivity determined by Plateletworks in PCI patients on clopidogrel therapy was associated with adverse clinical outcome [15].

How do we define antiplatelet therapy 'resistance'?

There is lack of consensus regarding the definition of antiplatelet therapy 'resistance'. The use of arbitrary cut-off values to define high residual platelet reactivity and thus differentiate 'responders' from 'non-responders' in the clinical setting remains challenging and controversial. Unsurprisingly therefore the reported prevalence of hyporesponsiveness to aspirin and clopidogrel varies significantly depending upon the assay and methodology used, the definition applied and the population group studied.

The optimal definition of antiplatelet therapy 'resistance' should encompass failure of the antiplatelet agent to inhibit the activity of its specific target. It therefore follows that the ideal laboratory test to measure responses to aspirin and clopidogrel should employ techniques that can reliably and specifically detect changes in activity of the target receptor before and after administration of therapy. However, in 'real-world' acute clinical practice pre-treatment baseline platelet reactivity levels would be difficult to ascertain and the clinical requirement is for a snapshot assessment. Thus, an absolute measure of platelet reactivity during treatment (i.e. on-treatment platelet reactivity) is generally used to define poor response instead [54].

Thromboxane A₂ (TXA₂) is a potent platelet agonist that is produced via the AA pathway. It is well established that the antiplatelet effect of aspirin is mediated through irreversible inactivation of the enzyme cyclo-oxygenase-1 (COX1) which is required for the conversion of AA into the precursors of TXA₂. Thus, the pharmacological definition of 'aspirin resistance' should strictly refer to its inability to inhibit platelet COX-1 dependent TXA₂ generation despite evidence of aspirin intake. This can be determined by directly measuring AA-induced platelet activation or serum TXB₂ concentrations. However, a number of clinical studies investigating aspirin resistance utilize platelet function assays that are not specific to the COX-1 pathway and variably reflect the TX-dependent component of platelet aggregation [55].

Low-dose aspirin (40 to 60 mg) has been shown to inhibit successfully over 95% of platelet COX-1 activity [56, 57], although the only data so far that have suggested any direct clinical benefit from low-dose therapy is confined to the stroke population [58]. It has been suggested that the prevalence of aspirin resistance is rare when assessed by methods directly dependent on platelet COX-1 activity [31]. However, other platelet-independent sources of TXA₂ exist such as monocytes, macrophages and endothelial cells which produce large amounts of TX synthase particularly in conditions associated with infection and inflammation and this may need to be taken into consideration when solely using TX concentrations to determine aspirin resistance.

In addition to COX-1, platelets contain a variable amount of COX-2. Aspirin is 170-fold more potent in inhibiting COX-1 than COX-2 [59] and it has been speculated that one possible explanation for aspirin 'resistance' is residual TX generation via platelet COX-2. Specifically, newly formed immature platelets express COX-2 [60] and, thus, in conditions associated with increased platelet turnover, the large sub-population of immature platelets could generate elevated platelet COX-2 concentrations that may be sufficient to produce detectable concentrations of TX despite aspirin therapy. This could be misinterpreted as aspirin 'resistance' when, in fact, aspirin is adequately suppressing COX-1 as it should be. Clinical studies have shown

that individuals on aspirin [61, 62] or a combination of aspirin and clopidogrel [63] who have high residual platelet reactivity also have a greater fraction of immature platelets. These findings demand further investigation.

With regards to the mechanism of action of clopidogrel, its active metabolite irreversibly binds to platelet P_2Y_{12} ADP receptors, thereby inhibiting ADP-induced platelet aggregation. Thus, the pharmacological definition of 'clopidogrel resistance' should strictly refer to evidence of increased P_2Y_{12} reactivity despite clopidogrel treatment, and this can be determined using assays that specifically measure ADP-induced platelet activation [64].

However, there is accumulating evidence to suggest that clopidogrel also influences AA-TXA₂-COX pathways, thereby potentiating the effect of aspirin [65, 66]. A recent small study has shown that standard doses of clopidogrel suppress the production of *in vivo* TXA₂ urinary metabolites (11-dehydro-TXB₂) to the same extent as aspirin in healthy volunteers. However, *ex vivo* production of TXA₂ in response to platelet stimulation was virtually abolished following aspirin treatment, as would be expected, and although clopidogrel also influenced the production of TXA₂, this was not to the same extent as the dramatic effect observed with aspirin [67]. Further data have also shown that patients who were initially labelled as 'non-responders' to aspirin as determined by LTA and TEG were converted to 'normal responders' as a result of increased inhibition of AA-induced platelet aggregation following the addition of clopidogrel therapy [68, 69]. In addition, Lev *et al.* [4] investigated the response to clopidogrel among aspirin-resistant vs. aspirin-sensitive patients undergoing PCI and found that almost 50% of aspirin-resistant patients were also resistant to the effects of clopidogrel as determined using LTA and VerifyNow. In this study, aspirin resistance was more common in women and diabetics, and dual drug resistance was more likely in patients with a higher body mass index. It is possible that the observed incidence of dual drug resistance may be due to a global increase in platelet reactivity or increased platelet turnover precipitated by other potentially reversible factors. Patient compliance is also an important consideration although, in this particular study, antiplatelet therapy was administered under direct supervision. These data raise important questions with regards to the clinical implications of abruptly discontinuing clopidogrel therapy in patients who are on long-term aspirin, which is currently recommended at 1 and 12 months following PCI in patients with bare metal and drug-eluting stents, respectively. There may be an argument for routinely measuring antiplatelet therapy responses in all such individuals prior to discontinuing clopidogrel.

Large-scale studies are clearly warranted but, for now, defining true 'resistance' to aspirin and clopidogrel and determining the most appropriate assay that should be used to measure individual responses to specific antiplatelet agents will remain a challenging and contentious issue.

Antiplatelet therapy response variability and risk of adverse events

Clinical studies have shown significant heterogeneity in individual patient responses to both aspirin and clopidogrel. Specifically, large systematic reviews and meta-analyses of data [2, 70] have shown that the incidence of aspirin hyporesponse in patients with cardiovascular disease varies between 0.4 to 65% depending on the assay and definition used, with seemingly higher rates of 'resistance' observed with the PFA-100 assay [16, 51, 71]. Similarly, the reported incidence of clopidogrel hyporesponse varies significantly between 4 to 30% [3, 72].

A number of factors have been identified that contribute to the observed variability in physiological response which include the methodology, assay and definition used to determine hyporesponse, patient compliance, genetic variability in drug absorption and metabolism [73], the presence of specific cardiovascular risk factors such as diabetes, smoking, obesity and dyslipidaemia and drug-related factors such as dosing regimens and drug-drug interactions.

Clinical studies have demonstrated a clear link between antiplatelet therapy hyporesponse, high on-treatment platelet reactivity, and adverse clinical outcome in patients with peripheral vascular disease, ischaemic stroke, stable coronary artery disease, ACS and following PCI. A meta-analysis by Krasopoulos *et al.* [2] concluded that 'patients who are resistant to aspirin are at greater risk of clinically important adverse cardiovascular events, regardless of the assay used to measure aspirin resistance'. This observation was echoed by Snoep *et al.* [70] who reported on a meta-analysis of 15 studies conducted in patients with cardiovascular disease.

Peripheral vascular disease (PVD)

Mueller *et al.* [74] investigated 100 patients with PVD on long-term aspirin therapy undergoing peripheral limb balloon angioplasty. They observed a poor response to aspirin in 60% of patients as determined by whole blood aggregometry and this was a predictor of vessel re-occlusion at 18 months. The risk of re-occlusion was at least 87% higher ($P = 0.0093$) in aspirin hyporesponders. Similarly, Ziegler *et al.* [75] examined the incidence of vessel restenosis or re-occlusion at 12 months following angioplasty for PVD in 98 patients. They observed an increased risk of re-occlusion in clopidogrel hyporesponders compared with clopidogrel responders (55% vs. 13%) measured using the PFA-100 assay.

Ischaemic stroke

Clinical studies have reported an association between aspirin resistance in ischaemic stroke and the frequency of stroke recurrence, severity of neurological deficit and

cardiovascular death. Furthermore, Englyst *et al.* [76] reported a high prevalence of aspirin resistance of 67% in 45 stroke patients measured using unmodified TEG. In this study, aspirin resistance was independently associated with stroke severity and was more common in lacunar than embolic strokes. The aspirin resistant group had a higher Rankin score compared with the aspirin responsive group (4.0 vs. 2.0, $P = 0.013$). Jeon *et al.* [77] reported a positive association between aspirin resistance in 117 stroke patients determined using the VerifyNow assay and early recurrent ischaemic lesions seen on brain imaging at 1 week. The reported prevalence of aspirin resistance in this study was 13.7% and this was independently related to early recurrent ischaemic lesions occurring outside the vascular territories of index stroke within the first week of stroke (OR 6.01, 95% CI 1.29, 28.09, $P = 0.023$). Grotemeyer *et al.* [78] measured platelet reactivity prior to discharge in 180 stroke patients on aspirin and reported a significantly higher prevalence of recurrent stroke, myocardial infarction and vascular death at 2 years in aspirin non-responders vs. aspirin responders (40% vs. 4.4%, $P < 0.0001$).

Stable coronary artery disease (CAD)

Gum *et al.* [79] investigated aspirin resistance using LTA in a prospective study of 326 patients with stable CAD. They observed a 5.2% prevalence of aspirin resistance and a greater than threefold increase in the composite endpoint of death, myocardial infarction and stroke in this group at a mean follow-up of 679 ± 185 days. Chen *et al.* [80] reported a higher incidence of aspirin resistance of 27.4% in 422 stable CAD patients using VerifyNow and a significant increase in the primary composite outcome of cardiovascular death, ACS and stroke and was reported in the aspirin resistant vs. aspirin sensitive group (15.6% vs. 5.3%, $P < 0.001$). By contrast, the recent ASCET study [81] investigated 1001 patients with stable, symptomatic CAD on aspirin 160 mg daily who were hyporesponsive to treatment measured using PFA-100 and platelet aggregometry AA assays. Patients were randomized to either continued treatment with aspirin or to maintenance clopidogrel therapy. At 2-year follow-up, aspirin hyporesponders randomized to clopidogrel had a 40% non-statistically significant reduction in the combined primary endpoint of all-cause death, non-fatal MI, ischaemic stroke and unstable angina compared with the aspirin group (7.8% vs. 13.1%, $P = 0.16$). Of note, at randomization, 26% of patients were hyporesponsive to aspirin and although aspirin hyporesponsiveness was associated with a higher clinical event rate, the occurrence of the primary endpoint in the hyporesponder vs. responder group was not statistically significant (13.1 vs. 10.5%, $P = 0.41$). The ASCET study has been criticized for being underpowered and, thus, the latter finding should be interpreted with caution.

ACS and PCI

There is accumulating data demonstrating a link between aspirin and/or clopidogrel hyporesponse and increased risk of ST, cardiovascular death, myocardial infarction and stroke in patients presenting with ACS or following PCI. ST is a potentially catastrophic complication of PCI that is encountered in the acute clinical setting. Thus, a rapid, reliable, near-patient test of response to antiplatelet therapy is particularly relevant in this patient group in order to determine the optimal acute and long-term treatment strategy in a timely manner. The data specifically relating to the utility of POC testing in predicting clinical risk in patients with ACS and/or coronary stents are summarized in Table 2.

In summary, the data suggest that patients who are hyporesponsive to antiplatelet therapy are at increased risk of clinically important cardiovascular events across the entire spectrum of cardiovascular and cerebrovascular disease. However, some heterogeneity has been observed depending on the laboratory assay used to measure response to therapy. What is clearly needed is a widely available, standardized and reproducible test with well-defined, validated cut-off values representative of clinical risk that can be used in everyday clinical practice to measure reliably responses to antiplatelet therapy.

Tailored antiplatelet therapy and clinical outcome

The growing body of evidence demonstrating a link between high on-treatment platelet reactivity and risk of adverse events with clopidogrel, has led to unanswered questions as to whether tailoring antiplatelet therapy according to results of platelet function tests leads to improved clinical outcome. At present, the only guideline recommendation supporting tailored therapy based on platelet function testing is in patients undergoing PCI in whom ST may be a 'catastrophic or lethal' event (such as unprotected left main stem or last patent coronary vessel). In these patients, the American College of Cardiology/American Heart Association recommends that '*platelet aggregation studies may be considered and the dose of clopidogrel increased to 150 mg per day if less than 50% inhibition of platelet aggregation is demonstrated. . . .*' (Class IIb, level of evidence C recommendation) [82]. However, the method to assess platelet inhibition is not described and there are no specific recommendations for tailoring aspirin therapy.

Bonello *et al.* [5, 6] investigated the clinical benefit of VASP-guided incremental loading doses of clopidogrel in patients with clopidogrel resistance undergoing PCI in two small prospective, randomized multicentre studies. In both studies, clopidogrel resistance was defined as a VASP index of more than 50% after a 600-mg loading dose of clopidogrel. Patients were randomly assigned to a control group, in which PCI was undertaken without an additional bolus of clopidogrel, or to a VASP-guided group, in which

Table 2

Relationship between clinical outcome and antiplatelet therapy hyporesponsiveness

Study	n	Patients	Point-of-care test	Cut-off value	Agent studied	Clinical outcome in hyporesponders
Sibbing <i>et al.</i> [41]	1608	Elective PCI	MEA	>416 AU min ⁻¹	Clopidogrel	↑ST at 6 months (4.1 vs. 0.4%; OR 5.8, $P < 0.0001$)
Breet <i>et al.</i> [15]*	1069	Elective PCI	VerifyNow Plateletworks	PRU > 236 >80.5% 20 µmol l ⁻¹ ADP	Clopidogrel	↑Death, MI, ST and ischaemic stroke at 12 months (VN – 13.3 vs. 5.7%, $P < 0.001$. PW – 12.6 vs. 6.1%, $P = 0.005$)
Eshtehardi <i>et al.</i> [43]	219	PCI	MEA	ASA test AUC > 168 ADP test AUC > 309	Aspirin and clopidogrel	↑Peri-procedural MI and death, MI and ST at 30 days (ASA: 36.8 vs. 8.8%, $P < 0.001$)
Marcucci <i>et al.</i> [23]	683	PCI; ACS	VerifyNow	PRU ≥ 240	Clopidogrel	↑CV death (HR 2.55; $P = 0.034$) and MI (HR 3.36, $P = 0.004$) at 12 months
Price <i>et al.</i> [22]	380	PCI	VerifyNow	PRU ≥ 235	Clopidogrel	↑CV death, non-fatal MI and ST at 6 months (6.5 vs. 1.0%, $P = 0.008$)
Patti <i>et al.</i> [104]	160	PCI	VerifyNow	PRU ≥ 240	Clopidogrel	↑MACE at 30 days (20% vs. 3%, $P = 0.034$)
Cuisset <i>et al.</i> [105]	120	PCI; SA	VerifyNow	Percent inhibition P2Y12 < 15%	Clopidogrel	↑peri-procedural myonecrosis (44% vs. 15%, OR 4.6, $P = 0.001$)
Bliden <i>et al.</i> [33]*	100	PCI	TEG	ADP > 70%; AA > 50%	Aspirin and Clopidogrel	↑Death, MI, ST, stroke, ischaemia requiring hospitalization at 12 months (87% of patients with ischaemic events had HPR)
Foussas <i>et al.</i> [106]	612	PCI	PFA-100	<193 s closure time	aspirin	↑Cardiac death and rehospitalization for non fatal MI at 12 months (18.7 vs. 7.6%, $P < 0.001$)
Lev <i>et al.</i> [4]*	150	Elective PCI	VerifyNow	>550 ARU	Aspirin and clopidogrel	↑Post PCI myonecrosis (ASA: 38.9 vs. 18.3%, $P = 0.04$. CLO: 32.4 vs. 17.3%, $P = 0.06$)
Pamucku <i>et al.</i> [107]	105	ACS	PFA-100	<186 s closure time	aspirin	↑MI, unstable angina, cardiac death (45 vs. 11.7%, $P = 0.001$)
Marcucci <i>et al.</i> [108]	146	PCI	PFA-100	<203 s closure time	aspirin	↑MACE at 12 months (43.9% vs. 24.8%, $P < 0.05$)
Chen <i>et al.</i> [21]	151	Elective PCI	VerifyNow	>550 ARU	Aspirin	↑post PCI myonecrosis (51.7 vs. 24.6%, $P = 0.006$)

*Light transmittance aggregometry was also used in these studies. AA, arachidonic acid; ACS, acute coronary syndrome; ADP, adenosine diphosphate; ARU, aspirin reaction unit; ASA, aspirin; CAD, coronary artery disease; CLO, clopidogrel; CV, cardiovascular; HPR, high on-treatment platelet reactivity; MACE, major adverse cardiovascular events; MEA, multiple electrode platelet aggregometry; MI, myocardial infarction; PCI, percutaneous coronary intervention; PW, Plateletworks; RPFA, rapid platelet function assay; SA, stable angina; ST, stent thrombosis; TEG, thrombelastography; VN, VerifyNow.

patients received up to three additional boluses of 600-mg clopidogrel to obtain a VASP index below 50% prior to PCI. Both studies observed a significant reduction in 30 days MACE in the VASP-guided group compared with the control group (0% vs. 10%, $P = 0.007$ and 8.9% vs. 0.5%, $P < 0.001$) with no difference in bleeding events. The rate of ST was also significantly lower in the VASP-guided group (0.5% vs. 4.2%, $P < 0.01$) [6]. Of note, 8% [6] and 14% [5] of patients remained resistant to clopidogrel despite the use of up to 2400-mg doses. The use of alternative more potent antiplatelet agents such as prasugrel in patients who are hyporesponsive to high doses of clopidogrel needs to be examined in further studies. Although these findings support routine testing for antiplatelet therapy resistance in patients undergoing PCI, the VASP assay is expensive, technically demanding and not widely available hence limiting its use in routine clinical practice.

Similarly, data have shown that intensifying platelet inhibition by selective administration of GPIIb/IIIa inhibitors in patients undergoing elective PCI who are poor responders to aspirin and/or clopidogrel significantly reduces periprocedural myocardial infarction and major adverse cardiovascular events at 30 days without any increase in bleeding rates [83]. This study measured plate-

let reactivity using VerifyNow and similar findings have been reported using LTA [84].

By contrast, the recent large randomized prospective GRAVITAS study [85] investigated the outcome of doubling the dose of clopidogrel in 2214 patients undergoing PCI who exhibited high on-treatment platelet reactivity measured using the VerifyNow assay. Patients were randomized to either continuing with the usual 75-mg dose of clopidogrel or to receiving an additional loading dose of 600 mg followed by a higher maintenance dose of 150 mg daily. At 6-month follow-up, the combined primary endpoint of CV death, MI and ST was identical in both groups (2.3%) with no significant difference in bleeding events. However, one of the criticisms of the design of GRAVITAS is that it was underpowered, because the aim of achieving a 50% relative risk reduction by just doubling the maintenance dose of clopidogrel was overoptimistic. Furthermore, the investigators predictions of an event rate of around 5% turned out to be unrealistic, given that the actual event rate was half that.

Large-scale clinical trials that will further investigate the potential clinical benefit of tailored doses of antiplatelet therapy based on the VerifyNow assay are currently ongoing (Table 3). The results from these studies will

Table 3

Ongoing clinical trials evaluating the merit of tailored antiplatelet therapy

Study	Patients (n)	Design	Endpoints
ARCTIC	Elective PCI/DES (2500)	RCT Tailored therapy based on VN assay Adjustment of antiplatelet therapy in suboptimal responders	12 months composite endpoint of death, MI, stroke, urgent revascularisation, ST
TRIGGER-PCI	PCI (2150)	RCT Tailored therapy based on VN assay Prasugrel 60 mg LD + 10 mg MD vs. Clopidogrel 600 mg LD + 75 mg MD	CV death, non fatal MI
DANTE	ACS/PCI (442)	RCT Tailored therapy based on VN assay 75 mg vs. 150 mg clopidogrel MD	6 and 12 months CV death, nonfatal MI and TVR

ARCTIC: Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy Study. TRIGGER-PCI: Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel Study. DANTE: Dual Antiplatelet Therapy Tailored on the Extent of Platelet Inhibition Study. ACS, acute coronary syndrome; CV, cardiovascular; DES, drug eluting stent; LD, loading dose; MD, maintenance dose; MI, myocardial infarction; PCI, percutaneous coronary intervention; RCT, randomized controlled trial; ST, stent thrombosis; TVR, target vessel revascularization; VN, VerifyNow.

provide the much needed evidenced-based data upon which specific recommendations on the role of point-of-care testing in antiplatelet therapy prescribing can be developed.

Genetic testing

Clopidogrel is an inactive pro-drug that is oxidized to its active metabolite via the hepatic cytochrome (CY) P450 system in a two-step oxidation process. Hepatic bioactivation of clopidogrel is achieved via a number of different CYP isoenzymes and it has been shown that the CYP2C19 isoenzyme plays a major role in this process [86]. Recent studies have linked CYP2C19 genetic polymorphisms to an alteration in the metabolite concentrations of clopidogrel [73, 87–89]. Specifically, e.g. the CYP2C19*2 allele variant is associated with higher levels of ADP-induced platelet aggregation (so-called 'loss of function' allele) and thus increased risk of major adverse cardiovascular events including ST [88–90]. By contrast, recent data have shown that the CYP2C19*17 polymorphism is significantly associated with enhanced response to clopidogrel and increased risk of bleeding [91].

The US Food and Drug Administration recently added a boxed warning to the clopidogrel label emphasizing the increased risk of adverse cardiovascular events in patients with genetic polymorphisms [92, 93]. Thus there is increasing interest surrounding genetic testing in all patients who require clopidogrel therapy in order to identify high risk groups with the CYP2C19*2 carrier status [94]. These patients may be identified as 'high risk' and therefore could be considered for alternative antiplatelet agents such as prasugrel, as it has been shown that common functional CYP genetic variants do not affect active drug metabolite levels or clinical outcomes in relation to prasugrel [89].

However, a recent study genotyped 5059 patients from two large randomized trials that showed that clopidogrel reduced the rate of cardiovascular events in patients with ACS and atrial fibrillation compared with placebo. In this study, the beneficial effect of clopidogrel in improving clinical outcome was consistent, irrespective of the 'loss of function' CYP2C19 carrier status [95]. There are limited data on the actual predictive value of genetic testing within an individual and whether tailored therapy based on CYP2C19 carrier status results in improved clinical outcomes. Furthermore genetic testing is technically demanding, time-consuming and expensive thus limiting its use in the acute clinical care setting. Several studies are currently underway to evaluate the potential clinical benefit associated with genotype-guided tailoring of antiplatelet therapy [96, 97]. Currently there seems little justification for routine genotyping because it is actually the phenotype (platelet function testing) that determines outcome. The predictive value of genotyping is poor because the distribution of responses to clopidogrel between wild type and heterozygotes for CYP2C19*2 overlaps enormously [98].

Future directions

The clinically relevant questions that need to be addressed in the future by way of large-scale clinical trials are: (i) what are the optimal cut-off values for platelet reactivity that can be used to define increased risk of thrombotic as well as bleeding events? (ii) does the optimal target for platelet reactivity vary with time, in different patient populations and in different clinical settings? and (iii) will the use of genotyping in addition to platelet function testing be a more effective and reliable combined tool for identifying high risk patients who are hyporesponsive to antiplatelet therapy?

Conclusion

A standardized and widely accepted platelet function assay that fulfils all the criteria for an ideal near-patient test and can be used with relative ease in the acute clinical care setting is still lacking. Clinical studies have shown mixed results with regards to the utility of point-of-care tests to measure response to antiplatelet therapy and thus predict clinical risk. Adequately powered clinical trials to determine whether individually tailored therapy based on laboratory findings of resistance leads to improved long-term clinical outcomes are currently underway and will form the basis for antiplatelet therapy prescribing guidelines in the future. However, these studies are largely undertaken in controlled research environments rather than point-of-care settings that precisely mimic 'real world' clinical practice.

Recent observational data has shown that enhanced responsiveness to clopidogrel is associated with increased risk of major bleeding events [99]. With the advent of newer more potent antiplatelet agents such as prasugrel [100, 101] and ticagrelor [102, 103] which have a more rapid onset of action, more predictable inhibition of platelet aggregation and less response variability it may become mandatory in the future to select the most appropriate and safe antiplatelet agent on a patient-by-patient basis based on an individualized assessment of thrombotic as well as bleeding risk using standardized and well validated platelet function tests that are suitable for routine clinical use.

Competing Interests

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