Acetylsalicylic acid and clopidogrel hyporesponsiveness following acute coronary syndromes

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Abstract
This review discusses the response variability to acetylsalicylic acid (ASA) and particularly to clopidogrel, and their relation to adverse recurrent ischaemic events in patients with arterial diseases. The higher rate of ASA resistance reported in the literature may be mainly due to the cyclooxygenase-1 non-specific assays, non-compliance, and underdosing. Clopidogrel response variability and non-responsiveness are established concepts. Moreover, high platelet reactivity (HPR) to adenosine diphosphate during clopidogrel therapy is now a known risk factor for recurrent ischaemic events in high-risk percutaneous coronary intervention/acute coronary syndrome patients. Variable active metabolite generation is the primary explanation for clopidogrel response variability and non-responsiveness. Variable levels of active metabolite generation following clopidogrel administration could be mainly explained by functional variability in hepatic cytochrome (CYP)P450 isoenzyme activity that is influenced by drug–drug interactions and single nucleotide polymorphisms of specific genes encoding CYP450 isoenzymes. Treatment with more potent P2Y\textsubscript{12} receptor blockers, such as prasugrel and ticagrelor are credible alternative strategies to overcome HPR during clopidogrel therapy.

Key words: acetylsalicylic acid, clopidogrel, high platelet reactivity, platelets, coronary artery disease, stent thrombosis, P2Y\textsubscript{12} receptor

INTRODUCTION
Multiple lines of evidence from translational research support the pivotal role of platelet function in arterial thrombotic event occurrences, particularly in patients with acute coronary syndromes (ACSs) [1]. In ex vivo experimental models of arterial thrombogenesis using human blood samples, combined therapy of a P2Y\textsubscript{12} receptor blocker, clopidogrel, with acetylsalicylic acid (ASA) dramatically potentiated the antithrombotic effect of each drug alone [2]. Large scale landmark clinical trials have demonstrated the superior efficacy of clopidogrel and ASA therapy vs. ASA therapy alone in patients with coronary artery diseases (CADs). Based on this evidence, dual antiplatelet therapy of ASA and a P2Y\textsubscript{12} receptor antagonist is the cornerstone of secondary prevention therapy for patients presenting with ACS and in those treated with percutaneous coronary intervention (PCI) with stenting [3]. Multiple observational pharmacodynamic studies have demonstrated the response variability to ASA and particularly to clopidogrel, and their relation to adverse recurrent ischaemic event occurrence in patients with arterial diseases. The pharmacological limitations and treatment failure associated with clopidogrel therapy fostered the development and clinical implementation of the more effective P2Y\textsubscript{12} receptor blockers, prasugrel and ticagrelor [4, 5]. Landmark clinical trials involving patients with ACS unequivocally demonstrated the greater anti-ischaemic effects of these agents compared to clopidogrel, supporting the “platelet hypothesis” concept that greater platelet inhibition results in greater reduction in ischaemic outcomes [6, 7]. In this review article, we discuss the recent developments in hyporesponsiveness/resistance to ASA and clopidogrel in patients with ACS.

ACETYLSALICYLIC ACID
Acetylsalicylic acid irreversibly inhibits cyclooxygenase (COX)-1 activity, thereby inhibiting thromboxane A\textsubscript{2} (TxA\textsubscript{2})-induced platelet activation and aggregation, whereas P2Y\textsubscript{12} re-
Receptor blockers inhibit adenosine diphosphate (ADP)-induced platelet activation and aggregation [8]. In addition, ASA has been shown to exhibit numerous pleiotropic effects that might influence its antithrombotic efficacy [9]. Based on laboratory findings, earlier studies have reported that up to 50% of patients with atherosclerotic disease are “resistant” to ASA therapy (“laboratory ASA resistance”), and these patients may be at increased risk of recurrent ischaemic events. A reliable and specific laboratory method to identify ASA resistance has not yet been uniformly accepted by investigators. Because TxA₂ is an important secondary agonist that is released following platelet activation in response to shear, ADP, collagen, and epinephrine, various assays utilising the latter agonists have been wrongly used to indicate antiplatelet response of ASA. Therefore, laboratory methods including point-of-care methods using the latter agonists do not solely indicate COX-1 activity (COX-1 non-specific methods) and thus are fundamentally flawed in indicating antiplatelet response to ASA. The optimal definition of resistance or non-responsiveness to ASA is the demonstration of residual activity of the COX-1 enzyme. Measurement of serum thromboxane B₂ (TxB₂) and arachidonic acid-induced platelet aggregation are the most specific assays to indicate COX-1 ASA responsiveness (COX-1–specific methods). The ASA resistance is more meaningful only when it is associated with elevated risk for ischaemic event occurrences.

Depending on the patient population, type of assay, cut-off points for “normal” value dose, and ASA dose, estimates of the prevalence of ASA resistance have been reported between < 1% and 55% [8]. A true pharmacological ASA “resistance” is rare when measured by methods that solely indicate inhibition of COX-1 activity such as arachidonic acid (AA)-induced platelet aggregation [8–13]. The higher rate of ASA resistance reported in the literature may be mainly due to the COX-1 non-specific assays described above, non-compliance, and underdosing—particularly in high-risk patients.

In the recently reported ADPT-DES study of 8582 patients with CAD treated with ASA and clopidogrel after successful drug-eluting stent implantation, ASA hyporesponsiveness (> 250 ASA reaction units by VerifyNow Aspirin assay) was observed in only 5.6% of patients, and it was not associated with adverse outcomes [14].

Using urinary 11-dh-TxB₂ metabolite levels to assess ASA responsiveness among patients at high risk for cardiovascular events enrolled in the Heart Outcomes Prevention Evaluation (HOPE) trial, Eikelboom et al. [15] found that patients in the upper quartile have a higher risk of myocardial infarction (MI), stroke, or cardiovascular death compared to the lowest quartile (adjusted hazard ratio [HR] 1.66, 95% CI 1.06–2.61, p = 0.03) in patients treated with ASA. Moreover, treatment with ≥ 150 mg/dl ASA was associated with lower urinary 11-dh-TxB₂ levels, indicating a dose-dependent effect. These are the only studies that demonstrated a strong link between ASA responsiveness and clinical outcomes. However, it should be noted that 11-dh-TxB₂ represents whole-body TxB₂ production and may be influenced by non-platelet sources, especially in pathological conditions of inflammation and high-risk cardiovascular disease [16]. Currently, there is no established method to assess ASA responsiveness, nor is there a definition of “ASA resistance,” and its relevance in patients with ACS is unknown. Moreover, the clinical effectiveness of altering therapy based on a laboratory finding of ASA “resistance” has not been explored. Therefore, other than in research trials, it is not currently recommended to test for ASA resistance in patients or to change therapy based on such tests.

**CLOPIDOGREL RESPONSE VARIABILITY, CLOPIDOGREL RESISTANCE, AND HIGH ON-TREATMENT PLATELET REACTIVITY**

Clopidogrel is still the most widely used P2Y₁₂ receptor blocker to treat patients with arterial diseases. Clopidogrel is a prodrug that requires hepatic conversion into an active metabolite to exert its antiplatelet response. Only ~15% of the absorbed clopidogrel is metabolised by hepatic cytochrome (CYP) P450 isoenzymes in a two-step process involving cytochrome (CYP) 2C19, CYP3A, CYP1A2, CYP2B6, and CYP2C9. The highly unstable active metabolite covalently binds to platelet P2Y₁₂ receptor specifically and irreversibly resulting in inhibition of ADP-induced platelet activation-aggregation for the lifespan of the platelet [17, 18].

In 2003, clopidogrel response variability and resistance was first demonstrated using conventional platelet aggregometry and flow cytometry studies in patients undergoing PCI, who had received a 300-mg loading dose followed by 75-mg daily maintenance dose of clopidogrel [19]. Since then, numerous studies using various assays to measure ADP-induced platelet reactivity and involving thousands of patients conducted around the world reported pharmacological “resistance” to clopidogrel and indicated clopidogrel non-responsiveness where a substantial percentage of patients (up to 35%) exhibited either negligible or no antiplatelet response to clopidogrel [4, 5]. Multiple lines of evidence strongly suggest that variable active metabolite generation is the primary explanation for clopidogrel response variability and non-responsiveness. Variable levels of active metabolite generation following clopidogrel administration could be mainly explained by functional variability in hepatic P450 isoenzyme activity that is influenced by drug-drug interactions and single nucleotide polymorphisms (SNPs) of specific genes encoding CYP450 isoenzymes [4].
Single nucleotide polymorphisms of CYP2C19 genes (loss-of-function allele carriers) have been associated with variable pharmacokinetic and pharmacodynamic response as well as elevated ischaemic risk in clopidogrel-treated patients who have undergone PCI [20]. It should be noted that the CYP2C19 isoenzyme is not the only factor determining the antiplatelet response to clopidogrel, because even in poor metabolisers some degree of platelet inhibition has been observed where no enzyme activity is expected [21]. In addition, numerous demographic and epigenetic factors influence antiplatelet response independently of genetic polymorphisms of CYP2C19 gene (Figs. 1, 2) [5, 22]. Therefore, SNPs of CYP2C19 may not be a surrogate indicator of clopidogrel resistance or platelet reactivity to ADP, and the assessment of platelet reactivity to ADP is more suitable to identify high-risk patients and to treat with personalised antiplatelet therapy strategy.

**HIGH PLATELET REACTIVITY TO ADP**

The level of on-treatment platelet reactivity may be a superior risk predictor compared to the assessment of clopidogrel resistance (difference between baseline and post-treatment platelet reactivity) because platelet reactivity to ADP has been shown to be variable before clopidogrel treatment in patients on ASA therapy [23]. It has been demonstrated that high platelet reactivity (HPR) to ADP during clopidogrel treatment is a strong and independent risk factor for post-PCI thrombotic events. Numerous observational studies confirmed the relation of HPR to post-PCI ischaemic events including periprocedural events, long-term events, and stent thrombosis (ST). A consensus opinion on the definition of HPR based on various methods of platelet function testing (PFT) has been reported [4, 5]. Based on accumulating data from the observational studies, American and European practice guidelines have issued a class IIb recommendation for PFT to facilitate the choice of P2Y<sub>12</sub> receptor inhibitor in selected high-risk patients treated with PCI, although routine testing is not recommended (class III) [24–26].

In the Assessment of Dual Anti-Platelet Therapy with Drug-Eluting Stents (ADAPT-DES) study involving more than 8500 patients, nearly half of these patients had ACS (unstable angina 28%, non-ST-segment elevation MI 15%, and ST-segment elevation MI 9%). In this study, 43% of patients met the criteria of HPR (> 208 P2Y<sub>12</sub> reaction units [PRU]), and PRU > 208 was independently associated with an approximately 4-, 1.5-, and 1.8-fold increase in the risk of definite/probable ST at 0 to 30 days (HR 3.90; 95% CI 1.90–8.00; p < 0.001), 30 days to one year (HR 1.55; 95% CI 0.76–3.18; p = 0.23), and two years (HR 1.84; p = 0.009) in landmark analyses, respectively [27, 28]. The relation of HPR and ischaemic event occurrences was more pronounced in patients with ACS compared to patients with stable CAD (adjusted HR 2.60, p < 0.005 and 1.44, p = 0.47, respectively). The ADAPT-DES study also demonstrated a strong
inversed relationship between > 208 PRU and major bleeding (HR 0.65, p = 0.04) [28].

In an analysis of studies including 20,839 patients (97% were treated with clopidogrel and 3% with prasugrel), patients with HPR in contrast to optimal platelet reactivity had significantly higher risk of ST (risk ratio [RR] 2.73, p < 0.001) and a slight reduction in bleeding (RR 0.84, p = 0.04) compared to those with optimal platelet reactivity, whereas patients with low platelet reactivity (LPR) had a higher risk of bleeding (RR 1.74, p < 0.001), without any further benefit in ST (RR 1.06, p = 0.78) [29]. A systematic review and meta-analysis of individual patient data on major adverse cardiac event (MACE) outcomes (ACS, ischaemic strokes, and vascular deaths) in relation to platelet reactivity and their interaction with cardiovascular risk levels revealed that the magnitude of the association between platelet reactivity and MACE risk is strongly dependent on the level of cardiovascular risk faced by patients on clopidogrel [30].

In addition to the upper threshold for ischaemic risk (i.e. HPR), small translational research studies have demonstrated the relation of LPR with bleeding. The concept of a “therapeutic window” of P2Y12 receptor reactivity associated with both ischaemic event occurrence (upper threshold) and bleeding risk (lower threshold) has been proposed. A consensus document highlighting the above observations with a therapeutic window concept with updated cut-off for HPR and LPR for P2Y12 inhibitor therapy has been published (Fig. 2) [28, 31–34]. This approach is more meaningful while titrating the dose of more potent P2Y12 receptor blockers that are known to be associated with increased incidences of bleeding. These observations provided a strong rationale to measure platelet response to ADP during clopidogrel therapy and to personalise antiplatelet therapy based on the therapeutic window concept to reduce both ischaemic and bleeding events in high-risk CAD patients undergoing PCI. Moreover, newer P2Y12 receptor blockers such as prasugrel and ticagrelor have been shown to have superior pharmacodynamic effects and improved clinical outcomes in ACS patients. Prasugrel and ticagrelor are associated with elevated risk for bleeding, and a uniform and long-term therapy with these agents may be associated with elevated bleeding risk. Therefore, they are good alternative agents to overcome the limitations of clopidogrel in selected patients who exhibit HPR phenotype and low risk of bleeding. The utility of platelet function measurement in personalising antiplatelet therapy has been addressed in recent large-scale randomised trials.

**RANDOMISED STUDIES OF PERSONALISED ANTIPLATELET THERAPY**

Despite strong evidence linking HPR to ischaemic events from numerous observational studies, prospective, randomised trials have failed to demonstrate that personalised antiplatelet therapy based on platelet function is effective in reducing ischaemic event occurrences (Table 1) [35–39]. These studies have fundamental problems. The majority of them enrolled low-risk patients, and therefore the associated ischaemic event occurrence was low (i.e. underpowered), they mainly used high-dose clopidogrel, which is not optimal to reduce HPR, and therefore the risk reduction was not significant (undertreated), and all these studies used VerifyNow P2Y12 assay to identify HPR phenotype. Furthermore,
<table>
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<tr>
<th>Trial</th>
<th>Patients</th>
<th>PFT (HPR cut-off)</th>
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<td>GRAVITAS [35]</td>
<td>N = 2214</td>
<td>VerifyNow P2Y_{12} assay (≥ 230 PRU)</td>
<td>High-dose vs. standard dose clopidogrel for six months</td>
<td>— Cardiovascular death, non-fatal MI or ST: 2.3% vs. 2.3%, HR = 1.01; 95% CI 0.58–1.76; p = 0.97 &lt;br&gt; — Severe or moderate GUSTO bleeding: 1.4% vs. 2.3%, HR = 0.59; 95% CI 0.31–1.11; p = 0.10</td>
<td>Reasons for neutral outcome: &lt;br&gt; — Low-risk patients &lt;br&gt; — Suboptimal clopidogrel dose to overcome HPR &lt;br&gt; — Too high HPR cut-off ≥ 230 PRU</td>
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<td>ARCTIC [36]</td>
<td>N = 2440</td>
<td>VerifyNow P2Y_{12} assay (≥ 235 PRU)</td>
<td>Drug adjustment in patients who had ≥ 235 PRU (80% received additional clopidogrel and ~10% received prasugrel, or a conventional strategy without monitoring and drug adjustment)</td>
<td>— Death from any cause, MI, ST, stroke, or transient ischaemic attack, or urgent revascularisation, HR = 1.13; 95% CI 0.98–1.29; p = 0.10 &lt;br&gt; — Major STEEPLE bleeding: 3.3% vs. 2.3, HR = 0.70; 95% CI 0.43–1.14; p = 0.15</td>
<td>— Low-risk patients &lt;br&gt; — ~10 received prasugrel &lt;br&gt; — Non-uniform method of evaluation of MI &lt;br&gt; — Stroke and urgent revascularisation may not be related to platelet function</td>
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<td>TRIGGER-PCI [37]</td>
<td>N = 423</td>
<td>VerifyNow P2Y_{12} assay (≥ 208 PRU)</td>
<td>10 mg/day prasugrel vs. 75 mg/day clopidogrel for six months</td>
<td>— Cardiovascular death or MI only one event &lt;br&gt; — TIMI major non-CABG bleeding only 5 events</td>
<td>— Low-risk patients &lt;br&gt; — Low event rate &lt;br&gt; — ~30% of patients declined randomisation</td>
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<tr>
<td>ANTARCTIC [38]</td>
<td>N = 877</td>
<td>VerifyNow P2Y_{12} assay (PRU ≥ 208)</td>
<td>Prasugrel 5 mg (without monitoring and drug adjustment) vs. strategy of platelet-function monitoring, with drug adjustment in patients who had a poor response to antiplatelet therapy</td>
<td>— One-year incidence of cardiovascular death, MI, stroke, ST, urgent revascularisation, or BARC ≥ 2 bleeding &lt;br&gt; — No differences in the primary endpoint (28%, monitoring group vs. 28%, conventional group, HR = 1.00, 95% CI 0.78–1.29; p = 0.98) &lt;br&gt; — No differences in bleeding</td>
<td>— Therapeutic approach in the PFT group was primarily to reduce bleeding</td>
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<td>TROPICAL-ACS [39]</td>
<td>N = 2610</td>
<td>Multiplate ADP test (≥ 46 U)</td>
<td>Conventional treatment with prasugrel (without drug or dose adjustment) vs. strategy of PFT-guided de-escalation with one-week prasugrel followed by one-week clopidogrel, then clopidogrel or prasugrel from day 14</td>
<td>— One-year incidence of cardiovascular death, MI, stroke, ST, or BARC ≥ 2 bleeding &lt;br&gt; — Non-inferiority for the primary endpoint (7.3%, de-escalation group vs. 9.0%, control group, p_{non-inferiority} = 0.0004; HR = 0.81; 95% CI 0.62–1.06; p_{superiority} = 0.12) &lt;br&gt; — No differences in bleeding</td>
<td>— Provided a strong rationale for the de-escalation of prasugrel therapy in ACS patients managed with PCI</td>
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ACS — acute coronary syndrome; ADP — adenosine diphosphate; BARC — Bleeding Academic Research Consortium; CABG — coronary artery bypass grafting; CAD — coronary artery disease; CI — confidence interval; HPR — high platelet reactivity; HR — hazard ratio; MI — myocardial infarction; NSTEMI — non-ST-segment elevation myocardial infarction; PCI — percutaneous coronary intervention; PFT — platelet function testing; PRU — P12Y_{12} reaction units; ST — stent thrombosis; STEMI — ST-segment elevation myocardial infarction; TIMI — thrombolysis in myocardial infarction
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in the ANTARCTIC study, the therapeutic approach in the PFT group was primarily to reduce bleeding. Therefore, the results of these studies should not be considered to disapprove the utility of PFT in improving clinical outcomes. At the same time, smaller studies have suggested that the PFT-directed approach may be effective with proper implementation [5]. Finally, in the absence of evidence from a randomised study, selective and optional use of PFT to guide P2Y<sub>12</sub> inhibitor therapy is reasonable to consider in selected high-risk patients, such as those in whom ACS risk of excessive bleeding is low.

In addition, de-escalation of P2Y<sub>12</sub> inhibitor therapy to reduce bleeding risk has been suggested. In the recently published Testing Responsiveness to Platelet Inhibition on Chronic Antiplatelet Treatment For Acute Coronary Syndromes (TROPICAL-ACS) trial, ACS patients with successful PCI were randomised to a standard treatment with prasugrel for 12 months (control group) or one week prasugrel therapy followed by one week clopidogrel and PFT-guided maintenance therapy with clopidogrel or prasugrel from day 14 after hospital discharge (guided de-escalation group). The combined primary endpoint of cardiovascular death, MI, stroke, or bleeding grade 2 or higher according to Bleeding Academic Research Consortium (BARC) criteria was similar (9% vs. 7%) (p<sub>non-inferiority</sub> = 0.0004; HR 0.81, 95% CI 0.62–1.06, p<sub>equivalence</sub> = 0.12). There was no increase in the combined risk of cardiovascular death, MI, or stroke and a non-significant increase in the BARC 2 or higher bleeding in the de-escalation group vs. in the control group [39]. The study provided a strong rationale for the de-escalation of prasugrel therapy in ACS patients managed with PCI.

CONCLUSIONS

Clopidogrel response variability and nonresponsiveness are established concepts. Moreover, HPR during clopidogrel therapy is now a known risk factor for recurrent ischaemic events in high-risk PCI/ACS patients. However, the TRILOGY-ACS platelet function substudy and other studies suggest that the clinical relevance of HPR in patients with medically managed ACS or stable CAD is less clear [40, 41]. Treatment with high-dose clopidogrel (600 mg loading dose plus 150 mg maintenance dose) is not an optimal strategy to overcome HPR and to reduce frequency of recurrent ischaemic events. Whereas, treatment with more potent P2Y<sub>12</sub> receptor blockers, such as prasugrel and ticagrelor, is associated with faster and greater platelet inhibition than clopidogrel therapy and is a credible alternative strategy to overcome HPR during clopidogrel therapy. However, recent data indicate that prasugrel and ticagrelor therapies are associated with a delayed pharmacodynamic effect after stenting in ACS patients. The two major randomised trials of personalised antiplatelet therapy failed to confirm the benefit of PFT to improve outcomes. However, these studies were underpowered given the low risk of the enrolled patients, and they used high-dose clopidogrel as the remedy for HPR. Currently we do not have an adequate evidence base to support or refute the clinical utility of platelet function testing in the high-risk patient. What we do have are an unshakable biologic rationale and a robust body of data linking HPR to thrombotic events in the high-risk patient treated with PCI.

In ACS patients enrolled in the TRITON-TIMI trial, the greatest anti-ischaemic benefit of prasugrel was observed early after ACS and PCI, whereas long-term therapy was associated with elevated bleeding risk [6]. Similarly, in the PLATO trial, in patients treated with PCI, reduction in ST associated with ticagrelor therapy was most prevalent in the first 30 days after the procedure, and again increased bleeding risk was apparent during long-term therapy [7]. These observations indicate that de-escalation of more potent P2Y<sub>12</sub> receptor blocker therapy may be considered in patients without HPR as in the TROPICAL-ACS study [39]. This strategy may be suitable for achieving the balance between ischaemic and bleeding risk, and for cost-related reasons.

Thus, currently we must rely on the guidelines and the existing observational data while keeping in mind the role that platelet physiology plays in catastrophic event occurrence in the stented patient. It is important not to ignore the body of evidence demonstrating that: (a) HPR during clopidogrel therapy is associated with post-PCI ischaemic event occurrences, (b) user-friendly and reliable assays are available to assess platelet function, (c) more potent P2Y<sub>12</sub> receptor blockers are available to overcome HPR, which is present in ~35% of patients treated with clopidogrel, (d) nearly two-thirds of patients without HPR can be optimally treated with less expensive generic clopidogrel, and (e) excessive bleeding may be reduced and net clinical outcome improved by utilising serial PFT in patients selectively treated with the more potent P2Y<sub>12</sub> receptor blockers as shown in the TROPICAL-ACS study.

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