High platelet reactivity and clinical outcome – Fact and fiction

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Summary
In patients suffering from acute coronary syndromes or undergoing percutaneous coronary intervention, oral antiplatelet treatment is routinely administered with the primary aim of inhibiting platelet-mediated thrombus formation and subsequent abrupt vessel occlusion. Simultaneous inhibition of blood platelet cyclooxygenase-1 by aspirin and of the P2Y12 receptor by clopidogrel or prasugrel is currently recommended in this setting. Inter-individual response variability to aspirin and especially to clopidogrel is the subject of much debate as evidence has grown over the years linking an attenuated response to treatment with the occurrence of ischaemic events. Consequently, the clinical entity of high (on-treatment) platelet reactivity (HPR) was born and subsequently characterised in numerous studies over the last decade. Until recently, alternative treatment options were limited in patients exhibiting HPR. At present the antiplatelet therapy landscape is changing with the advent of prasugrel and ticagrelor as alternative and more potent treatment options. Different tests for monitoring platelet function are available and are being increasingly employed in research projects and clinical routine. These tests may prove useful for achieving optimal platelet inhibition for the individual patient, and several centres now incorporate such testing in day-to-day practice. Widespread adoption of this practice and incorporation into clinical guidelines awaits the results of ongoing trials in which treatment is changed based on platelet function monitoring. This review aims to summarise available facts and fiction in relation to platelet function testing and reactivity with a particular focus on P2Y12 receptor inhibition in patients undergoing coronary stent placement.

Keywords
Platelets, antiplatelet agents, platelet function testing, thrombosis, P2Y12 receptor

Introduction
Platelet activation and aggregation are pivotal in the pathogenesis of acute coronary syndromes (ACS). Coronary artery obstruction is precipitated or exacerbated by reactive platelets that deposit at sites of disrupted atherosclerotic plaques with subsequent platelet aggregation, thrombus formation and total or subtotal vessel occlusion. To specifically target these processes, oral antiplatelet treatment with the primary aim of inhibiting platelet-mediated thrombus formation and subsequent abrupt vessel occlusion is routinely administered in patients suffering from acute coronary syndromes (ACS) or undergoing percutaneous coronary intervention (PCI). Simultaneous dual inhibition of blood platelets by the cyclooxygenase-1 (COX-1) inhibitor aspirin and by clopidogrel or prasugrel, which target the adenosine diphosphate (ADP) P2Y12 receptor on platelets, is currently recommended in this setting (1).

Aspirin is considered as the cornerstone of any dual antiplatelet treatment regimen whereas a variety of P2Y12 receptor inhibitors are currently available or are on the immediate horizon, including the newly developed 3rd generation thienopyridine prasugrel (2) and the non-thienopyridine derivative ticagrelor (3). These agents may prove useful as a substitute for clopidogrel in specific circumstances as major shortcomings of clopidogrel include its delayed onset of action (4), its large inter-individual response variability (5, 6) and the observation that a significant proportion of patients (~20%) exhibit a status of high (on-treatment) platelet reactivity (HPR) following in vitro stimulation with ADP (7, 8). Until recently, alternative treatment options were limited in patients exhibiting HPR. However, the recent advent of more potent and more predictably acting drugs has changed dramatically the antiplatelet treatment landscape (2, 3). Notwithstanding, it seems unlikely that all patients will benefit from more potent antiplatelet drugs as the value of antithrombotic treatment is determined by the balance between the prevention of ischaemic complications and the induction of bleeding (9, 10); the latter is – not surprisingly – a major shortcoming of the more potent agents in general (2, 3).

Along with the development of more potent drugs, different tests for platelet function monitoring have become available and are being increasingly employed in numerous research projects as well as in clinical routine. Although all of these tests claim to reliably assess the amount of platelet inhibition, they differ significantly in terms of their value to predict clinical outcome (7), the costs needed for testing and their applicability to allow near-patient or point-of-care platelet function monitoring. Some of these tests may prove useful for drug monitoring with the aim to achieve an optimal amount of platelet inhibition for the individual patient.

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Available assays for platelet function testing

In contrast to other laboratory measurements such as testing of electrolyte concentrations or specific enzymes, which detect a defined concentration or activity of a certain salt or biomarker, platelet function measurements rely on complex cellular functions that act in concert and finally determine the level of platelet reactivity. The latter may be influenced not only by the action of anti-platelet drugs, but also by the baseline platelet reactivity (5) of the individual as well as a number of other factors that are summarised below. Platelet function tests applied today differ in respect to the active reagents used, in respect to flow conditions, signal reactions, the anticoagulant used, and the analysis of whole blood vs. plasma. Therefore it is not surprising that the correlation of different methods is weak and a number of assays lack standardisation (11).

From a logistical point of view, platelet function assays can be differentiated according to the potential to perform the assays rapidly in a near patient setting. Laboratory-based methods require skilled personnel and are comparatively time-consuming. On the other hand near-patient or point-of-care tests are rapid and less laborious and consequently can more easily be incorporated into clinical practice. Table 1 summarises available assays classified according to these two groups. A detailed description of the tests including their specific advantages and disadvantages is discussed elsewhere (12). Only rapid, standardised and easy-to-use methods

Table 1: Overview of commonly used platelet function assays. ADP, adenosine diphosphate; MEA, multiple electrode aggregometry; PGE, prostaglandin E.

<table>
<thead>
<tr>
<th>Test</th>
<th>Platelet stimulation</th>
<th>Detection</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td><strong>Laboratory-based methods</strong></td>
<td></td>
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</tr>
<tr>
<td>Light transmission aggregometry (LTA)</td>
<td>ADP, collagen, arachidonic acid, TRAP</td>
<td>reduction of optical density after stimulation in PRP</td>
<td>instrument adjustment possible, good predictivity, long experience</td>
<td>time consuming, complex sample preparation, no standardization</td>
</tr>
<tr>
<td>VASP</td>
<td>ADP and ADP + PGE1 in parallel</td>
<td>flow cytometric detection of VASP phosphorylation</td>
<td>whole-blood assay, longer sample storage possible, P2Y12 receptor specific</td>
<td>very time consuming, complex sample preparation, need for a flow cytometer, weak sensitivity and predictivity</td>
</tr>
<tr>
<td>Impedance aggregometry (Chronolog)</td>
<td>ADP, collagen, arachidonic acid</td>
<td>coating of 2 arch-shaped electrodes by platelets</td>
<td>whole-blood assay, instrument adjustment possible</td>
<td>cleaning of reusable electrodes required, limited study results</td>
</tr>
<tr>
<td>Impact cone-and-platelet analyser</td>
<td>ADP, arachidonic acid</td>
<td>shear induced platelet adhesion on polystyrene surfaces</td>
<td>whole-blood assay, instrument adjustment possible</td>
<td>limited study results, complex procedure, requires pipetting</td>
</tr>
<tr>
<td><strong>Point-of-care methods</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiplate Analyser (MEA)</td>
<td>ADP, ADP+PGE1, arachidonic acid, TRAP-6</td>
<td>coating of 2 electrode pairs by platelets</td>
<td>whole-blood assay, simple and rapid, standardized procedure, good predictivity for bleeding and stent thrombosis</td>
<td>semi-automated (requires pipetting), rapid processing of samples necessary</td>
</tr>
<tr>
<td>VerifyNow</td>
<td>ADP+PGE1 (P2Y12 assay), arachidonic acid (Aspirin assay), TRAP-6 (lib/lla assay)</td>
<td>platelet-mediated aggregation of fibrinogen-coated polystyrene beads</td>
<td>whole-blood assay, simple and rapid, standardized procedures</td>
<td>no assay adjustment possible, expensive cartridges</td>
</tr>
<tr>
<td>PFA-100</td>
<td>ADP+collagen+shear stress (COL-ADP) / epinephrin+collagen+shear stress (COL-EPI) / ADP+PGE1+collagen+shear stress (P2Y)*</td>
<td>closure of an aperture of a collagen-coated membrane</td>
<td>whole-blood assay, simple and rapid, standardized procedures</td>
<td>no assay adjustment possible, dependent on hematocrit and vWF, limited experience with P2Y12 inhibitors, limited study results</td>
</tr>
<tr>
<td>Plateletworks (single platelet counting)</td>
<td>ADP, collagen, arachidonic acid</td>
<td>counting of single platelets following aggregation</td>
<td>whole-blood assay, simple and rapid, standardized procedures</td>
<td>not widely used, limited study results</td>
</tr>
<tr>
<td>TEG Platelet Mapping Assay</td>
<td>ADP + reptilase+ FXIIIa, arachidonic acid + reptilase + FXIIIa</td>
<td>Clot formation</td>
<td>whole-blood assay</td>
<td>complex procedure, time-consuming, requires pipetting</td>
</tr>
</tbody>
</table>
have the potential to be used in future on a large-scale basis in clinical practice. Currently the research and clinical uses of antiplatelet therapy monitoring has focused on antagonists of the P2Y12 receptor. This is due to the fact that P2Y12 antagonists are widely used, HPR to ADP has shown in many studies to be a risk factor for ischaemic events, and the fact that several alternative treatment options have become available targeting this receptor. Therefore, the focus of the present review article is on P2Y12 receptor inhibition and the phenomenon of high on-treatment platelet reactivity (HPR) in this context.

**Figure 1: High on-clopidogrel treatment platelet reactivity.** The distribution of platelet function measurements in a study cohort (87) of PCI-treated patients following (A) loading with 600 mg clopidogrel and in study cohort (56) of patients under steady-state treatment (B) with 75 mg/day clopidogrel is shown. Values obtained with the Multiplate assay are shown as aggregation units (AU) x min. The red arrows illustrate the proportion of patients with HPR. ADP, adenosine diphosphate; HPR, high platelet reactivity.

**High platelet reactivity (HPR)**

The clinical entity of high (on-treatment) platelet reactivity (HPR) has been extensively characterised in numerous studies over the last decade (7). For the vast majority of these studies the focus has been set on P2Y12 receptor inhibition and specifically the antiplatelet action of clopidogrel. The pharmacokinetics and pharmacodynamics of clopidogrel are beyond the scope of the current review and are summarised elsewhere (13). Keeping in mind the number of steps that are required for generation of the active metabolite of clopidogrel – which is ultimately responsible for the irreversible inhibition of the P2Y12 receptor on platelets via a disulfide bridge binding – it is not surprising that the drug is susceptible to numerous factors that may negatively impact its antiplatelet action and may ultimately lead to HPR at an individual patient level. Interferences with clopidogrel bioactivation may occur at various
points in the metabolism of the drug: (a) intestinal absorption, (b) bioactivation or (c) during inhibition of the active thiol metabolite at the P2Y12 receptor.

Somewhat surprisingly, it was about six years after clopidogrel approval by the Food and Drug Administration (FDA) in 1997 that the phenomenon of inter-individual response variability or high on-treatment platelet reactivity – nowadays regarded as a defining characteristic of this drug – was fully recognised and first described by Gurbel et al. (5). As demonstrated in Figure 1, both following loading with a single high-loading dose of 600 mg clopidogrel as well as under steady-state treatment with 75 mg/day clopidogrel a wide dispersion of platelet reactivity measurements is observed after stimulation with adenosine diphosphate (ADP). Importantly, a relevant proportion of patients (approximately 20% in both settings) exhibit a status of HPR and are therefore at risk for suffering ischaemic complications (7). As outlined in Table 2, a number of genetic and non-genetic variables have been identified as causative for HPR. These factors may be summarised as follows:

### Non-genetic factors for HPR

Before investigation of HPR in an individual patient, non-compliance must always be considered and should be excluded (14). Apart from drug non-compliance, multiple clinical variables and medication interactions have been found to contribute to HPR on clopidogrel treatment. Whereas some of these factors have shown a high reproducibility across different studies, for some other factors conflicting results have been reported. A high body mass index (15, 16), diabetes mellitus (17, 18) and the presence of acute coronary syndromes (19) have been consistently shown to be associated with HPR. Patients of older age (18) or with renal insufficiency (18), as well as female patients (20) may also suffer from higher platelet aggregation values on clopidogrel therapy as compared to patients without these characteristics. Specifically in patients suffering from myocardial infarction complicated by cardiogenic shock clopidogrel shows little or no antiplatelet action presumably due to both reduced intestinal absorption of the pro-drug and markedly reduced bioactivation in the acute setting (21). In addition, the phenotype of HPR on clopidogrel treatment has also been associated with systemic inflammation (22), a reduced left ventricular ejection fraction (18) and smoking (23). In the case of cigarette smoking however, existing data is somewhat conflicting (24, 25) regarding a causative role in HPR and further investigations are certainly warranted.

In addition to underlying clinical factors, the co-administration of several drugs influences the efficacy of clopidogrel (see also Table 2). A number of different proton-pump inhibitors (PPIs) interact to a different extent with the hepatic cytochrome P450 system and this determines their ability to influence clopidogrel bioactivation. Although this interaction is clearly proven at the level of platelet aggregation testing (26, 27), the clinical relevance of this drug-drug interactions remains to be determined in light of the conflicting results that have been reported across numerous observational and randomised studies (27–33). Indeed the detailed interaction of PPIs with clopidogrel treatment is a subject of its own and has been summarised in numerous editorial comments and review articles (31, 34, 35). Other potentially significant interactions, arising due to interference with CYP-dependent bioactivation of clopidogrel, may be caused by calcium channel blockers, statins, and coumarine derivatives (36–39). For statins and calcium channel blockers, however, available data is conflicting and studies with larger cohorts of patients have shown no relevant impact of these drugs on the platelet response and/or clinical outcome of patients (40–43).

### Genetic factors for HPR

In the past years, multiple genetic variants in different candidate genes, which are involved in clopidogrel absorption and bioactivation, have been associated with both high and low on-treatment platelet reactivity to clopidogrel as well as both ischaemic and bleeding events after PCI (44–49). The polymorphically expressed isoenzyme CYP2C19 seems to be involved both metabolic steps of clopidogrel’s active metabolite generation (50, 51) and several studies and meta analyses have proven that the presence of the CYP2C19*2 loss-of-function allelic variant is associated with an attenuated response to clopidogrel and with a higher risk for ischaemic events including stent thrombosis in PCI-treated patients (44–46, 49, 52–54). As a result, the FDA issued a “boxed warning” about the diminished effectiveness of clopidogrel in patients who are homozygous carriers for CYP2C19*2 (55). Results of a recently published meta analysis (53) with data from >9,000 PCI-treated patients clearly confirmed a significant impact of both homozygous and indeed heterozygous

### Table 2: Variables causing high on-clopidogrel treatment platelet reactivity.

<table>
<thead>
<tr>
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<th>Genetic factors</th>
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<tbody>
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<td>CYP2C19 gene variants:</td>
</tr>
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<td>CYP2C19*2</td>
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<tr>
<td>Renal insufficiency</td>
<td>CYP2C19*17</td>
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<tr>
<td>Body mass index</td>
<td>CYP3A4 gene variants</td>
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<tr>
<td>Diabetes mellitus</td>
<td>CYP3A5 gene variants</td>
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<td>Systemic inflammation</td>
<td>MDR1 gene variants</td>
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<td>Acute coronary syndromes</td>
<td>P2Y12 gene variants</td>
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<td>Cardiogenic shock</td>
<td>ITGB3 gene variants</td>
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<tr>
<td>Ejection fraction</td>
<td>PON-1 gene variants</td>
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<td>Smoking</td>
<td>Co-medication:</td>
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<td></td>
<td>Proton pump inhibitors</td>
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<td>Calcium-channel blockers</td>
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<td>Coumarin derivatives</td>
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<td>Statins</td>
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<td></td>
<td>Non-compliance</td>
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</table>

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*2 allele carriage on clinical outcome, which is in line with the results of platelet function studies (56). The latter finding is of high clinical relevance since it expands the population at risk from less than 2% of patients to more than 20%. In contrast, additional information on risk associated with the *2 allele variant from post-hoc analyses of the CURE and ACTIVE trials has provided conflicting results, showing no impact of *2 on the clinical outcome of patients (57). However, the rate of PCI-treated patients in CURE was low, and it may well be that the negative impact of CYP19*2 is confined to patients treated with coronary stenting. In support of this, a number of studies have shown that the greatest impact of *2 on adverse events was observed for the occurrence of stent thrombosis (48, 53).

Another variant within the CYP2C19 gene, namely the CYP2C19*17 gain-of-function allelic variant results in an increased enzyme function (58). We were among the first to show in a large cohort of 1524 PCI-treated patients that the presence of *17 is associated with lower ADP-induced platelet aggregation values and a substantially higher bleeding risk especially for homozygous (*17/*17) allele carriers (47). These results were confirmed by a genetic sub-study of the PLATO trial (59) which reported more bleeding events in *17 carriers treated with clopidogrel during the trial period. Other rare variants within the hepatic CYP system that may have an influence on platelet aggregation in clopidogrel treated patients are summarised in Table 2. Polymorphisms within the ABCB1 gene, particularly 3435C→T, may also affect drug transport and efficacy of clopidogrel treatment (60). The ABCB1 gene encodes for the enteric Multidrug Resistance Protein 1 (MDR 1) and this protein is responsible for the absorption of orally administered clopidogrel in the intestine. In a number of different study cohorts, patients with the T allelic variant demonstrated a higher risk of ischaemic events in comparison to those with the wild-type genotype (49, 61). In contrast, a genetic sub-study of the PLATO trial found no significant influence of common single nucleotide polymorphisms (SNPs) within the ABCB1 gene on the clinical outcome of ticagrelor or clopidogrel treated ACS patients (59). Thus, the impact of variants within ABCB1 on clopidogrel treatment efficacy warrants further investigation.

More recently, another genetic variant within the gene encoding for the paraoxonase-1 (PON1) enzyme was linked to clopidogrel bioactivation, response to clopidogrel treatment and the clinical outcome of clopidogrel treated patients (62). The authors identified PON1 as a key factor for the second step of clopidogrel bioactivation. While showing a significant impact of the PON1 Q192R genotype on the clinical outcome of clopidogrel treated patients, the authors were not able to confirm the established association of CYP2C19*2 and stent thrombosis in clopidogrel treated patients. In summary, the available data on both (a) clopidogrel bioactivation steps and (b) the impact of linked genetic variants on clinical outcomes is conflicting and further studies are clearly needed to confirm these results and to better characterise the genetic influence on clopidogrel bioactivation.

Facts on HPR and clinical outcome

In 1997 following on from the release of results from the Clopidogrel vs. Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial (63), the 2nd generation thienopyridine clopidogrel was approved by the FDA. Since then a number of trials that aimed to further characterise its pharmacological properties and to identify possible shortcomings of the drug. In relation to platelet function testing, it was in 2001 when Steinhubl et al. first drew attention to the association between the amount of platelet reactivity and clinical outcomes in PCI-treated patients in the GOLD (AU-Assessing Ullegra) trial (64). Although not investigating clopidogrel responsiveness but the level of platelet inhibition achieved by administration of glycoprotein (GP) IIb/IIIa inhibitors, the principle message to be taken from this trial is that better clinical outcome is observed in patients with higher levels of platelet inhibition. In the following years, a number of different studies (see Table 3) sought to re-address this platelet activation/clinical outcome relationship in the setting of clopidogrel treatment. In 2003, Barragan et al. provided first evidence for an association between HPR and the occurrence of stent thrombosis in a case control study (16 cases vs. 30 controls) using the VASP technique for platelet function testing-treated patients (65). Using light transmission aggregometry (LTA), Mateszky et al. were among the first to report on higher rates of ischaemic events in ST-segment elevation myocardial infarction (STEMI) patients (n=60) undergoing PCI that showed a low response to clopidogrel treatment (66). In a larger cohort, the Platelet reactivity in patients and recurrent events post-stenting (PREPARE POST-STEMTING) study, the authors found a similar relationship in 192 patients undergoing PCI (67). The first dedicated study that prospectively enrolled clopidogrel-treated patients to test for an association of single platelet function measurements and 30-day outcome after PCI was the EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) trial, reported by Hochholzer et al. in 2006 (68). The authors reported on significantly higher rates of major adverse cardiac events in patients with platelet aggregation values greater than the median. Of note, the incidence of stent thrombosis in relation to platelet reactivity was not reported specifically. The RECLOSE (Low Responsiveness to Clopidogrel and Sirolimus- or Paclitaxel-Eluting Stent Thrombosis) trial (n=804) aimed to close this scientific gap by reporting on the cumulative incidence of a combined endpoint consisting of definite or probable stent thrombosis according to ARC criteria. The authors found a higher incidence of the combined endpoint in non-responders to clopidogrel as compared to responders (69). Interestingly, this difference was mainly driven by a higher rate of probable stent thrombosis in the non-responders group and no significant differences for definite stent thrombosis risk were observed between the two groups, the reason for which remains unclear. The above mentioned studies are notable for all employing laboratory-based methods (VASP or LTA, see Table 1) for platelet function testing. Although these methods are excellent research tools for addressing certain aspects of platelets from a researcher point of view, from a clinical point of view they are too...
It is costly in terms of labor to be implemented in clinical routine. Against this, assays that allow near-patient or point-of-care testing would be desirable in a clinical setting.

In 2008, Price et al. first described the association of HPR and clinical outcome of patients (n=307) by using a point-of-care device (VerifyNow P2Y12) for platelet function testing (70). In 2009, we reported the results of a large-scale trial that prospectively enrolled 1,608 patients and used the Multiplate analyzer for near-patient platelet function monitoring (8). In that study we were able to show a highly significant association of HPR (based on single platelet function testing directly before PCI) and the occurrence of early (<30 days) definite stent thrombosis (according to ARC criteria) (8). By investigating the occurrence of stent thrombosis across quintiles, results of the trial also lend support to the hypothesis of a threshold effect for platelet reactivity, as stent thrombosis events did not gradually increase across quintiles of measurements but in fact accumulated in the highest quintile.

However, above mentioned studies only investigated the predictive value of one single assay at any one time and until recently, there has been no study that aimed to undertake a head-to-head comparison of different assays in the same study cohort. This gap has been closed recently by the POPULAR (Do Platelet Function Assays Predict Clinical Outcomes in clopidogrel pretreated patients undergoing elective PCI) trial (71). In the POPULAR trial (71), a total of 1,069 clopidogrel-treated patients undergoing PCI were enrolled and platelet function was tested simultaneously with six different assays. Only three of the assays included – namely, LTA, VerifyNow, Plateletworks – showed predictive value for the occurrence of the primary endpoint of the study (one-year composite of death, myocardial infarction, stent thrombosis, and stroke). In particular, whereas the aggregation-based methods were found to be predictive for ischaemic event occurrence, the shear-based methods (IMPACT-R, PFA-100 with both the COL/ADP and INNOVANCE® PFA P2Y* cartridge) were un-

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<th>Device</th>
<th>Setting</th>
<th>Outcomes</th>
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<td>Barragan et al. (65)</td>
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<td>VASP</td>
<td>PCI (all comers)</td>
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<tr>
<td>Blindt et al. (77)</td>
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<td>VASP</td>
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<td>ST (6 months)</td>
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<td>LTA VerifyNow Plateletworks IMPACT-R PFA-100</td>
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<td>LTA</td>
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<td>LTA</td>
<td>PCI with DES implantation</td>
<td>ST (6 months)</td>
</tr>
<tr>
<td>Gurbel et al. (75)</td>
<td>297</td>
<td>LTA</td>
<td>Elective PCI</td>
<td>MACE (2 years)</td>
</tr>
<tr>
<td>Gurbel et al. (67)</td>
<td>192</td>
<td>LTA</td>
<td>Elective PCI</td>
<td>MACE (6 months)</td>
</tr>
<tr>
<td>Gurbel et al. (97)</td>
<td>120</td>
<td>LTA</td>
<td>Elective PCI</td>
<td>ST (over 1.5 years)</td>
</tr>
<tr>
<td>Hochholzer et al. (68)</td>
<td>802</td>
<td>LTA</td>
<td>Elective PCI</td>
<td>MACE (30 days)</td>
</tr>
<tr>
<td>Marcucci et al. (76)</td>
<td>683</td>
<td>VerifyNow</td>
<td>PCI in ACS patients</td>
<td>MACE (1 year)</td>
</tr>
<tr>
<td>Matezky et al. (66)</td>
<td>60</td>
<td>LTA</td>
<td>PCI in STEMI patients</td>
<td>MACE (6 months)</td>
</tr>
<tr>
<td>Migliorini et al. (98)</td>
<td>215</td>
<td>VerifyNow</td>
<td>PCI with stenting</td>
<td>Cardiac mortality (3 years)</td>
</tr>
<tr>
<td>Patti et al. (99)</td>
<td>160</td>
<td>VerifyNow</td>
<td>PCI (all comers)</td>
<td>MACE (30 days)</td>
</tr>
<tr>
<td>Price et al. (70)</td>
<td>380</td>
<td>VerifyNow</td>
<td>PCI with DES implantation</td>
<td>MACE (6 months)</td>
</tr>
<tr>
<td>Sibbing et al. (8)</td>
<td>1,608</td>
<td>Multiplate</td>
<td>PCI with DES implantations (elective and ACS)</td>
<td>Definite ST (30 days)</td>
</tr>
<tr>
<td>Siller-Matula et al. (72)</td>
<td>416</td>
<td>Multiplate</td>
<td>PCI (all comers)</td>
<td>ST (6 months)</td>
</tr>
<tr>
<td>Trenk et al. (52)</td>
<td>797</td>
<td>LTA</td>
<td>Elective PCI</td>
<td>MACE (1 year)</td>
</tr>
</tbody>
</table>

Table 3: Selected studies linking high platelet reactivity to clinical outcome. ACS, acute coronary syndrome; DES, drug-eluting stent; LTA, light transmission aggregometry; MACE, major adverse cardiovascular event; PCI, percutaneous coronary intervention; ST, stent thrombosis.
able to predict event occurrence. Some assays for platelet function testing were not included in the POPULAR study, most notably the Multiplate and VASP assays. These latter two devices were compared for their value in predicting the occurrence of stent thrombosis in a study by Siller-Matula et al. (72). The authors reported that the Multiplate device was able to predict stent thrombosis whereas the VASP assay was not. This observation might be related to the fact that the VASP assay is insensitive for the detection of low levels of P2Y12 receptor inhibition (73), which may result in an appropriately high proportion of patients being classified as HPR patients (74). Such observations raise the questions concerning the advisability of measuring P2Y12 receptor inhibition specifically. Against this aggregation-based methods offer a superior and easier approach to measure the effect of ADP receptor antagonists, providing a global measure of HPR in the setting of clopidogrel treatment. Further studies are urgently needed to clarify this issue.

Consensus definitions on HPR

Widespread adoption of platelet function testing in clinical practice and its incorporation into clinical guidelines has been delayed by the lack of consensus values on how to define HPR with the most commonly used assays. In 2010 the “Working Group on High On-treatment Platelet Reactivity” provided a consensus opinion on the definition of HPR to ADP for the most commonly used methods in literature and clinical routine (7). These methods are LTA, Multiplate, VerifyNow P2Y12 and VASP. The specific cut-off values defining HPR are >46% maximal aggregation in response to 5 μM ADP with LTA, ≥468 aggregation units (AU) x min for the Multiplate analyser, 235 platelet reactivity units (PRU) for the VerifyNow P2Y12 assay, and >50% platelet reactivity index (PRI) for the VASP assay. Definition of these cut-off values is a major step forward for standardising research as well as clinical decision-making in guiding tailored antiplatelet treatment. Methods with limited clinical experience such as the PFA-100 assay (including the newly developed INNOVANCE PFA P2Y* cartridge) or Plateletworks still lack such consensus values, and additional studies are required before adopting these assays for platelet function testing in the setting of P2Y12 receptor inhibition.

Fiction on HPR and clinical outcome

Predictive value of different platelet function assays

Receiver operating characteristic curve (ROC) analysis allows defining a threshold value that provides the greatest sum of sensitivity and specificity. The recently published consensus document employed ROC-based cut-off values for defining HPR, and also summarised the different hazard ratios (HR) for the available devices and their association of HPR with ischaemic events (7). On the basis of these ROC-based cut-points, one should note that the predictive value of different platelet function assays is not identical (7). More specifically, in terms of the occurrence of ischaemic events, a comparatively high predictive value, corresponding to the HR reported for HPR status and event occurrence, was reported for conventional light transmission aggregometry (75) and for the Multiplate analyser (8). Lower HR were reported for the VerifyNow P2Y12 assay (76) or for Plateletworks (77) and the lowest HR was reported for the VASP assay (77). Interestingly, the predictive value seems to be independent of whether the test system uses whole blood or prepared blood components for testing. Indeed taken in conjunction with results from studies like GRAVITAS (Gauging Responsiveness with a VerifyNow Assay: Impact on Thrombosis and Safety) (78) these observations emphasise that reports of antiplatelet treatment response in PCI-treated patients using any given assay for testing cannot be extrapolated to other available assays. For guidance of tailored antiplatelet treatment within or outside of clinical studies available assays have to be tested separately owing to the different predictive values they are associated with.

HPR group size and positive predictive value of testing

Depending on the method used for platelet function testing and also depending on the cut-off value chosen to define HPR, the proportion of patients that can be defined as patients with HPR differs largely across commonly used assays. Whereas the proportion of HPR patients was found to be relatively small (<20%) when using the Multiplate analyser (8), a significantly higher proportion of patients (>50%) was classified as demonstrating HPR when using the VASP assay in a similar setting (74). Keeping in mind, however, the relatively low rate of clinical treatment failure – <2% of PCI-treated patients manifest early stent thrombosis (79) – rates for HPR close to or exceeding 50% seem to clearly overestimate the true proportion of patients that are at risk for suffering ischaemic events. Apart from that, the positive predictive value of measurements is low for all assays used in clinical settings. This is primarily related to the low prevalence of the ischaemic endpoint under investigation (e.g. <2% for early stent thrombosis) but also serves as a reminder that the overall proportion of patients with HPR suffering ischaemic events following stenting procedures is low – an observation that should be borne in mind when considering intensification of antiplatelet treatment in patients with a high bleeding risk.

Stability of the HPR phenotype

As mentioned already, platelet function measurements rely on a number of complex cellular functions that act in concert and finally result in a certain level of platelet. It follows from this that we should not expect measurements of platelet reactivity in patients to remain stable over time, as they are influenced not only by in-
trinsic (e.g. genetic) but also by a number of extrinsic (e.g. co-
medication, control of diabetes, presence of ACS) factors that are
subject to continuous change over time.

The importance of genotyping of specific genetic variants with-
in the cytochrome P450 (CYP) system that impact on clopidogrel
bioactivation was highlighted in a recent commentary, where it
was argued that platelet function testing may provide inconsistent
and dynamic results measured at different time points in one and
the same patient (80). Against this, initial studies by Gurbel et al.
using LTA have shown relatively stable measurements over time in
clopidogrel treated patients with coronary artery disease (81, 82).
Recently, we assessed the stability of the HPR phenotype over time
in patients receiving chronic clopidogrel treatment by testing pla-
telet function with both LTA and multiple electrode aggregometry
(MEA) simultaneously (83). We found that the HPR phenotype is
stable over time in the majority of treated patients using both
methods for testing, although some patients could be misclassified
based on single testing and showed an unstable phenotype over
serial measurements. It must me acknowledged that only patients
with stable coronary disease were investigated in our study and
that especially in ACS patients the situation is even more difficult.
Pathophysiological processes accompanying ACS are known to ac-
tivate platelets and this is why ACS patients show higher aggre-
gation values in the acute setting (18, 19), which are likely to settle
later on. Looking to the future, it may well be that serial measure-
ments of platelet reactivity – similar to sampling methods em-
ployed in monitoring the efficacy of antihypertensive drugs by
measuring blood pressure on a regular basis – may prove prefer-
able in guiding tailored antiplatelet treatment.

Predictive value of HPR for early and late events
A further question to be addressed is the predictive value of platelet
function measurements for early versus late events. Indeed, at our
center we observed a stronger association of HPR with the occur-
rence of early events (≤30 days) after the procedure, with little or
no association with late events (>30 days) (84). This finding seems
self-evident since there is no reason to believe that insufficiency to
generate the active metabolite of clopidogrel for whatever reason
would lead to events some months after the stenting procedure
rather than in the immediate aftermath. In support of this, Geisler
et al. observed a similar relationship of platelet function measure-
ments on early versus late event occurrence using LTA (85). Sur-
prisingly, in the POPULAR study cohort (71) the strongest abso-
lute difference in the event rate between patients with and without
HPR was found several months after the procedure when test re-
results for LTA and VerifyNow P2Y12 were compared, the reason for
which remains unclear. In contrast, other studies using LTA or the
VerifyNow assay showed an early separation of Kaplan-Meier
curves for ischaemic events in patients with versus without HPR
(68, 70). As it stands, further studies are needed to test for the value
of platelet function testing for predicting early and/or late events.

Therapeutic window of platelet reactivity
Similar to managing hypertension therapy by monitoring blood
pressure or to adjusting diabetes mellitus control by monitoring
blood glucose levels it can be assumed that the monitoring of anti-
platelet treatment regimens will become an integral component of
future routine cardiovascular care. In the field of antithrombotic
treatment management, the international normalised ratio (INR),
commonly used for guidance of tailored coumarin-derivative
Figure 3: Levels of P2Y12 receptor inhibition and adverse events. 30-day incidence of definite or probable stent thrombosis (black line) and incidence of in-hospital major bleeding events (red line) in a study cohort (9) of 2533 patients undergoing coronary stenting with clopidogrel mediated P2Y12 receptor inhibition. Patients are stratified into groups of patients with low platelet reactivity (area under the aggregation curve [AUC] ≤ 188), patients with normal reactivity (AUC 189 to 467), and patients with high platelet reactivity (AUC ≥ 468). Patients with normal platelet reactivity showed the lowest risk for adverse events [odds ratio (OR) 0.40; p=0.003] as compared to the remaining patients.

Future perspectives

Presently, a number of studies (see Table 3) have clearly established high on-treatment platelet reactivity as a risk marker for worse outcome in patients undergoing coronary stenting. Moreover, the availability of consensus definitions (7) will surely help the treating physician for guidance of tailored antiplatelet treatment. Ongoing studies such as TRIGGER-PCI have set their focus on intensifying treatment in a randomised fashion in selected patients, and results of these investigations are awaited eagerly. Whereas initial smaller studies using the VASP assay provided promising results for an individualised treatment approach (88, 89), the large-scale GRAVITAS trial, which implemented the VerifyNow assay and a doubling of the clopidogrel dose in HPR patients, showed no benefit for a tailored antiplatelet treatment approach based on platelet function measurements. Surely, the trial questions the value of platelet function monitoring in clinical practice (78). However, negative study results may be attributed to a general lack of benefit of platelet function monitoring in PCI-treated patients on the one hand, but on the other hand also to the assay used and the HPR cut-off value, or even more likely to the fact that intensifying clopidogrel treatment alone may not be sufficient to achieve an adequate level of platelet inhibition in HPR patients. More potent agents such as prasugrel or ticagrelor may be a better choice for a selected group of patients suffering from HPR.

Notably, although more potent drugs such as prasugrel and ticagrelor have become available, the relative market share of these agents as compared to clopidogrel is small at present and presumably also in the near future. It remains to be confirmed in everyday clinical practice, whether the reported benefit of these drugs within randomised clinical studies, really transfers to a better outcome of patients in a clinical setting beyond well-controlled trials. Practical experience with newer agents will also promote or inhibit the widespread use of platelet function monitoring.

treatment (86), may serve as a role model for successful drug dose titration. An established therapeutic window based on INR measurements helps the physician to reduce the risk of thrombotic complications without increasing the risk for bleeding in an unacceptable fashion. Results of large-scale trials such as TRITON-TIMI 38 (2) suggest that a therapeutic window or “sweet-spot”, similar to that outlined in Figure 2, is likely to exist for antiplatelet treatment in general and for P2Y12 receptor inhibition in particular. Although not investigating bleeding events and ischaemic events simultaneously, the existence of such a therapeutic window for P2Y12 receptor inhibition was suggested by Gurbel et al. some years ago (75). Indeed, in a recently published observational study including 2,533 clopidogrel-treated patients undergoing PCI, we were able to provide initial evidence for the existence of a therapeutic window for P2Y12 receptor inhibition (9). As outlined in Figure 3, the group of patients with “normal platelet reactivity”, defined according to ROC-based cut-off values (8, 87), showed the lowest risk for any adverse events (stent thrombosis or bleeding). These findings await confirmation in further studies.

Nowadays, with the advent of more and more high-potency antiplatelet agents, the armamentarium of the treating physician is considerably expanded, and offers the prospect of individualised treatment strategies to provide optimal treatment for the individual patient. Although guidelines are lacking at present, and a generally accepted algorithm on how to respond to HPR is not currently available, regulatory agencies such as the FDA have already posted statements suggesting that intensification of P2Y12 receptor inhibition is desirable in specific circumstances (e.g. CYP2C19*2 carriage). Ongoing research in this field will help to fix a therapeutic window for platelet inhibition and to establish guidelines for tailored antiplatelet treatment.
The negative results of the GRAVITAS trial has prompted discussion as to what extent HPR should be considered as a risk marker or modifiable risk factor for adverse events. However, data from studies monitoring clopidogrel bioactivation in patients with a prior history of stent thrombosis (62, 90) clearly suggest that HPR should not only be considered as an intrinsic unmodifiable marker of a “multimorbid patient” but also as modifiable risk factor able to distinguish patients that may benefit from an intensified antiplatelet treatment or not. At the same time newer antiplatelet drugs (e.g. prasugrel, ticagrelor) have recently become available and offer intensified inhibition treatment. However, on the other hand, clopidogrel is now widely available in generic form and the question of which P2Y12 inhibitors to use now has economic as well as pharmacological implications. Finally recent findings concerning a therapeutic window of P2Y12 receptor inhibition (2, 9) demonstrate that not all patients are likely to benefit from intensified antiplatelet treatment. Monitoring-directed approaches to pharmacological modulation of platelet function will likely become increasingly widely used in the coming years.

Conflict of interest

Dr. Sibbing has received speaker fees from Dynabrade and has been on the advisory board for AstraZeneca and Eli Lilly.

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