ADP-induced platelet aggregation frequently fails to detect impaired clopidogrel-responsiveness in patients with coronary artery disease compared to a P2Y12-specific assay

Andreas Schäfer1, Sarah Weinberger1, Ulrike Flierl1, Martin Eigenthaler2, Stefan Störk1, Ulrich Walter2, Georg Ertl1, Johann Bauersachs1

1Medizinische Klinik und Poliklinik I, Universitätssklinikum Würzburg, Julius-Maximilians-Universität Würzburg, Germany; 2Institut für Klinische Biochemie und Pathobiochemie, Universitätssklinikum Würzburg, Julius-Maximilians-Universität Würzburg, Germany

Summary
Incomplete P2Y12-inhibition during clopidogrel treatment is associated with increased cardiovascular events and mortality after coronary intervention. We investigated the incidence of impaired individual clopidogrel-responsiveness using a P2Y12-specific and pre-treatment-independent assay in a real world situation. One hundred consecutive patients with coronary artery disease (CAD) on combined acetylsalicylic acid and clopidogrel treatment (75 mg/d) and 33 patients on aspirin only were screened for platelet ADP-induced signalling by conventional aggregometry, platelet P-selectin expression and the platelet reactivity index (PRI). Impaired P2Y12-specific inhibition by clopidogrel was defined as a PRI>50%. Functional platelet reactivity was significantly lower in clopidogrel-treated patients compared to controls. Impaired individual response to treatment was diagnosed in 69% of clopidogrel-treated patients. Conventional assessment of maximum ADP-induced platelet aggregation failed to detect impaired P2Y12 inhibition in 36% of patients identified by PRI to have an impaired clopidogrel response. Impaired clopidogrel response was associated with lower HDL levels and a history of hyperlipidaemia. In conclusion, PRI as a P2Y12-specific assay to evaluate the treatment effect of clopidogrel in patients with CAD revealed insufficient P2Y12-inhibition in two thirds of patients in a real-world scenario indicating a markedly higher incidence than previously assumed. PRI detected significantly more patients with impaired response than conventional platelet aggregation.

Keywords
ADP receptors, platelet activation, diabetes, P2Y12, clopidogrel

Introduction
Given the integral role of platelets in the progression of atherosclerosis and in acute clinical events, such as myocardial infarction and ischemic stroke, oral antiplatelet agents - particularly acetylsalicylic acid (ASA) and the thienopyridines clopidogrel and ticlopidine - constitute a cornerstone of therapy for vascular disease. Adenosine-5'-diphosphate (ADP), one of the most important mediators of thrombosis, activates platelets through two G-protein-coupled P2 receptors, P2Y1 and P2Y12. The P2Y1 receptor mediates the initiation of ADP-induced aggregation through calcium mobilization, while the P2Y12 receptor is involved in completion and amplification of the aggregation response (1). P2Y12-receptor activation inhibits cyclic adenosine monophosphate (cAMP) production by adenyl cyclase (2). The thienopyridines impede multiple pro-aggregatory actions of ADP by blocking the platelet P2Y12-receptor and preventing the secondary ADP-triggered amplification of platelet activation. Approximately 85% of the inactive prodrug clopidogrel are hydrolyzed by circulating blood esterases to an inactive metabolite, and the remaining 15% need hepatic metabolism to generate the active metabolite (3). Large clinical trials demonstrated the efficacy of clopidogrel per se or in combination with aspirin to prevent ischemic events in selected patients with atherosclerotic disease (4, 5). Current ACC/AHA/SCAI guidelines recommend (class IB) that clopidogrel 75 mg daily in addition to ASA should be given for at least four weeks after bare metal stent implantation (6). This conventional daily dose was chosen based on initial data demonstrating approximately 50% inhibition of platelet aggregation by this regimen (7). However, many patients...
experience thromboembolic events despite daily antiplatelet therapy, and so-called aspirin and clopidogrel “non-responsive-ness” have been observed and described (8, 9).

Clopidogrel “non-responsiveness” is associated with increased risk of stent thrombosis (10–12) and adverse cardiovascular events following coronary stenting (13). Patients undergoing elective percutaneous coronary intervention (PCI) display marked interindividual variability of platelet inhibition in response to clopidogrel (14) and increased platelet reactivity is associated with higher incidence of myonecrosis following PCI (15).

The current reference standard and the method most commonly used to assess treatment efficacy of clopidogrel is ADP-induced light transmittance aggregometry (16, 17). However, it needs rapid in-vitro preparation, is labour-intensive and is not specific for P2Y12. The method has a high interindividual variability and pre- and post-treatment samples need to be obtained in order to adequately assess the treatment effect (18). Point-of-care assays, such as the PFA-100, have failed to reliably detect inhibition of ADP-mediated platelet activation and do not correlate with platelet aggregation (19, 20). The P2Y12-reactivity index (PRI) using vasodilator-stimulated phosphoprotein (VASP) phosphorylation is a standardized P2Y12-specific assay. It is based on the P2Y12-specific inhibition of adenylyl cyclase by ADP and assesses the difference in PGE1-induced, adenylyl cyclase-mediated VASP phosphorylation in the absence and presence of ADP using flow-cytometry not requiring a pre-treatment sample (21).

We evaluated the incidence of impaired clopidogrel responsiveness in patients with coronary artery disease (CAD), who had been on clopidogrel for at least five days after a loading dose of 300–600 mg using the PRI in comparison to ADP-induced platelet aggregation and ADP-induced platelet P-selectin surface expression.

Materials and methods

Study population

Blood samples were obtained from one hundred consecutive patients with stable CAD admitted to the Cardiology Division of the University of Würzburg, who gave informed consent and had been on clopidogrel (75 mg/day) for at least five days after a loading dose of 300–600 mg on the first day. Patients with CAD who had not taken clopidogrel were used as controls. The study was performed in accordance with the Declaration of Helsinki.

Blood sample collection

Blood sampling was performed two to four hours after drug intake and samples were processed within one hour. Blood samples were collected from an antecubital vein using a 21-gauge needle. The first 5 ml of blood were discarded to avoid spontaneous platelet activation. Platelet-rich plasma (PRP) was prepared from citrated blood by centrifugation at 180 g for 10 minutes (min). The platelet count in PRP was adjusted to 250,000/μl by dilution with platelet-poor plasma (PPP), which had been obtained by centrifugation of the blood fraction at 2,000 g for 10 min. PRP was kept at 37°C before use. Platelet function assays included ADP-induced platelet aggregation in PRP, ADP-induced platelet P-selectin surface expression in PRP, and ADP-mediated inhibition of PGE-stimulated platelet VASP-phosphorylation to determine the P2Y12 platelet reactivity index (PRI).

Platelet aggregation

Platelet aggregation was performed with light transmittance aggregometry using a commercial 8-channel platelet aggregation profiler (PAP-8, BioData, Horsham, PA, USA). Aggregation was induced by ADP (20 μM) in PRP. Light transmission was adjusted to 0% with PRP and to 100% using PPP for each measurement. Curves were recorded for six minutes. Aggregation was measured at maximal aggregation (Aggmax). Twenty μM was chosen as the ADP concentration given the fact that this is the concentration applied in the PRI test (14).

P2Y12 platelet reactivity index

The P2Y12 PRI was determined through assessment of the phosphorylation status of VASP using flow cytometry (FACScalibur, Becton Dickinson, Heidelberg, Germany). VASP phosphorylation (VASP-P) was quantified with labeled monoclonal antibodies using a commercially available kit (PLT VASP/P2Y12 Test kit, American Diagnostica, Pfungstadt, Germany), for which platelets were stimulated with PGE1 + ADP followed by fixation with formaldehyde. Following permeabilization with triton X-100, VASP-P at serine 239 was determined using a monoclonal antibody (16C2) and a FITC-labelled secondary antibody, while platelets were counterstained with a PE-labelled anti-CD61 antibody, similarly as described initially (22). The PRI was calculated after measurement of VASP-P levels following stimulation with PGE1 (0.5 μM) by mean fluorescence intensity (MFI PGE1) as well as stimulation with PGE1, in the presence of ADP (20 μM, MFI PGE1 + ADP). The P2Y12 platelet reactivity index is defined as: ([MFI PGE1] - [MFI PGE1 + ADP]) / [MFI PGE1] * 100%, whereby background fluorescence is subtracted from each measurement. The lower the P2Y12 platelet reactivity index is the better clopidogrel inhibits P2Y12 activity.

The calculated intra-assay coefficient of variability was 6.3% determined in triplicate measurements in six individual patients with CAD treated with clopidogrel, similar to previously published data (23).

Flow cytometry for P-selectin surface expression

PRP samples were stimulated with ADP (20 μM) for 10 min. The samples were incubated with an FITC-labeled mouse monoclonal anti-human anti-P-selectin (CD62P) antibody (555523, Becton Dickinson, Heidelberg, Germany) for 10 min at room temperature. Following incubation with the antibodies, platelets were fixed with methanol-free formaldehyde (1.5%) for 10 min, and subsequently analyzed in a Becton Dickinson FACScalibur at a low flow rate. The platelet population was identified on its forward and side scatter distribution, and 20000 events were analyzed using CELLQuest Pro software; unspecific binding was arbitrarily adjusted to a mean fluorescence of 10. Additionally, the ratio of CD62P+ platelets was determined following stimulation of PRP.
Definition of impaired clopidogrel responsiveness
The threshold for impaired clopidogrel responsiveness was defined by 20 μM ADP-induced Agg_{max} >50%, and a P2Y_{12} PRI >50%. The Agg_{max} threshold value was chosen based on previous findings demonstrating that patients with such degrees of post-treatment platelet reactivity are at a higher risk of ischemic events as recently discussed in the literature (9). The cut-off for the P2Y_{12} PRI at >50% was based on its association with stent thrombosis as described earlier (24, 25). In fact, more recent studies have demonstrated cut-off values for VASP-PRI in the range of <48% to <53% for differentiating between likelihood and unlikelihood of stent thrombosis following percutaneous interventions and coronary stenting (25–27).

Substances
Unless stated otherwise, all chemicals were obtained from Sigma (Deisenhofen, Germany) in the highest purity available.

Data analysis
Numbers are given as n (%), means ± SEM, or median (25th–75th percentile) in the table, and median with individual measurement points in the figures. Group comparisons were made using the Mann-Whitney U-test. The association between dichotomized dependent variables (e.g. PRI >50%) and influencing factors was assessed using logistic regression. Independent determinants were sought by backward selection from variables listed in Table 1, using the likelihood criterion with a p<0.20 for inclusion into the model. Model fit was assessed by the Hosmer and Lemeshow chi-square goodness of fit test, and Nagelkerke’s R^2 was computed to indicate the degree of explained variance. Odds ratios (OR) with 95% confidence intervals (CI) are reported. A p-value <0.05 was considered statistically significant.

Coefficients of variance for each method were calculated from the 33 CAD patients in the clopidogrel-untreated group and were as follows: Agg_{max} 20.2%, PRI 8.5%, CD62P-MFI 32.7%, CD62P^+ 28.7%.

Results
Patient characteristics of the different groups are shown in Table 1. Determining platelet inhibition by clopidogrel five days after a loading dose might be influenced by non-complete P2Y_{12}-inhibition, however, clopidogrel loading doses are usually given either the day before percutaneous interventions are performed.

Table 1: Patient characteristics.

<table>
<thead>
<tr>
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<th>Control</th>
<th>Clopidogrel</th>
<th>Clopidogrel impaired response</th>
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<tbody>
<tr>
<td>N</td>
<td>33</td>
<td>100</td>
<td>69</td>
</tr>
<tr>
<td>Sex (m/f, m[%])</td>
<td>24/9 (73)</td>
<td>70/30 (70)</td>
<td>48/21 (70)</td>
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<tr>
<td>Age (years)</td>
<td>70 ± 2</td>
<td>68 ± 1</td>
<td>68 ± 1</td>
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<tr>
<td>BMI (kg/m^2)</td>
<td>27.8 ± 0.7</td>
<td>28.1 ± 0.7</td>
<td>28.5 ± 0.9</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>26</td>
<td>30</td>
<td>34</td>
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<tr>
<td>Hyperlipoproteinaemia (%)</td>
<td>71</td>
<td>61</td>
<td>76</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>100</td>
<td>81</td>
<td>90</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>13</td>
<td>24</td>
<td>28</td>
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<tr>
<td>Family history for MI (%)</td>
<td>35</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>Number of CVRFs</td>
<td>3 (IQR 2–4)</td>
<td>3 (IQR 2–4)</td>
<td>3 (IQR 2–4)</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>182.1 ± 8.0</td>
<td>189.1 ± 4.3</td>
<td>184.8 ± 4.4</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>108.9 ± 7.6</td>
<td>103.3 ± 3.9</td>
<td>101.5 ± 3.7</td>
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<tr>
<td>HDL-C (mg/dl)</td>
<td>46.4 ± 2.5</td>
<td>46.8 ± 1.4</td>
<td>44.6 ± 1.3</td>
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<td>HbA1c (%)</td>
<td>6.3 ± 0.2</td>
<td>6.2 ± 0.1</td>
<td>6.2 ± 0.1</td>
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<tr>
<td>Estimated GFR (ml/min/1.73 m^2)</td>
<td>75.9 ± 5.5</td>
<td>81.4 ± 3.0</td>
<td>83.4 ± 3.7</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>2.69 ± 0.75</td>
<td>1.96 ± 0.37</td>
<td>1.96 ± 0.43</td>
</tr>
<tr>
<td>Platelets (n/1000/μl)</td>
<td>224 ± 10</td>
<td>252 ± 8</td>
<td>236 ± 8</td>
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<tr>
<td>ASA (%)</td>
<td>85</td>
<td>88</td>
<td>85</td>
</tr>
<tr>
<td>ACEi/ARB (%)</td>
<td>89</td>
<td>84</td>
<td>90</td>
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<tr>
<td>Beta-blocker (%)</td>
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<tr>
<td>Statin (%)</td>
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</tr>
<tr>
<td>PPI (%)</td>
<td>39</td>
<td>70</td>
<td>71</td>
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</table>

BMI, body-mass index; Hyperlipoproteinaemia, total cholesterol >240 mg/dl or statin use for raised cholesterol; Hypertension, blood pressure >140/90 mmHg or on antihypertensive therapy for raised blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; GFR, glomerular filtration rate estimated using the MDRD equation (Modification of Diet in Renal Disease Study Group); ASA, acetylsalicylic acid; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; CVRF, cardiovascular risk factor; MI, myocardial infarction; PPI, proton pump inhibitor.
or even shortly after the procedure. The treatment is supposed to protect the patient from acute and sub-acute stent thrombosis. Therefore, we chose day five for the assessment of clopidogrel efficacy to represent a realistic scenario.

**Assessment of clopidogrel-induced inhibition of ADP-mediated platelet signalling**

Platelet signaling determined by maximum ADP-induced aggregation was significantly lower in clopidogrel-treated patients (Fig. 1A). Similarly, ADP-mediated inhibition of PGE-induced VASP phosphorylation assessed by the PRI (Fig. 1B) as well as surface-expression of P-selectin (CD62P) determined by mean fluorescence, as well as D) ratio of CD62P⁺ platelets. Horizontal lines indicate the respective median value. P-values are given for Mann-Whitney U test.

**Figure 1: Platelet reactivity in clopidogrel-treated CAD patients.** A) ADP-induced platelet signalling in clopidogrel treated vs. untreated patients was determined by maximum ADP-induced aggregation, B) ADP-mediated inhibition of PGE-induced VASP phosphorylation assessed by the PRI, and C) surface-expression of P-selectin (CD62P) determined by mean fluorescence, as well as D) ratio of CD62P⁺ platelets. Horizontal lines indicate the respective median value. P-values are given for Mann-Whitney U test.

**Figure 2: Correlation of platelet function tests.** In the patients treated with clopidogrel, correlations were performed for A) PRI with Aggₘₐₓ and B) CD62P⁺ platelets. Percentage values given in (A) indicate the ratio of patients with PRI>50% for Aggₘₐₓ<50% (25%), Aggₘₐₓ≥50% (44%), and of all patients (69%) (A).

\[
r^2=0.44, \ p<0.0001
\]

\[
r^2=0.31, \ p<0.0001
\]
ADP-stimulated platelet P-selectin expression (Fig. 1C, D) were also significantly lower in patients treated with clopidogrel. While a highly significant effect of clopidogrel could be observed overall, the response in the clopidogrel group was very heterogeneous resulting in substantial proportions of patients fulfilling the criteria of impaired response: 69% of clopidogrel-treated patients had a P2Y$_{12}$-specific PRI $>50\%$, an impaired state of platelet inhibition associated with post-interventional stent thrombosis as previously shown (24–26). When using a combination of PRI and Agg$_{\text{max}}$, although these tests were significantly correlated as previously shown in healthy volunteers (28), 44% of clopidogrel-treated patients still had impaired responses in both tests. More importantly, conventional aggregation failed to detect insufficient P2Y$_{12}$-inhibition in 25% of all patients representing 36% of the impaired responders defined by PRI (Fig. 2A). There were no patients in the upper quartile of Agg$_{\text{max}}$ that fell into the lowest quartile of PRI and there was only one patient in the upper quartile of PRI that fell into the lowest quartile of Agg$_{\text{max}}$. Thus, the analysis of interquartile ranges suggests that major, excessive differences between both tests occurred rarely. The correlation of PRI with P-selectin expression appeared weak (Fig. 2B).

High platelet reactivity in diabetic patients with coronary artery disease is associated with a higher risk of long-term adverse cardiovascular events despite standard chronic dual antiplatelet therapy (29). Using similar cut-offs for impaired response as in our study, a high incidence of non-response was recently reported in diabetic patients on clopidogrel (30). We therefore compared clopidogrel-treated diabetic (Fig. 3B) and non-diabetic patients (Fig. 3A), who exhibited very similar aggregometry data (Fig. 3C, D). Of note, by using the PRI to define an impaired response to clopidogrel more specifically than aggregation, we found that the impaired clopidogrel response in the general patient population was as high as recently reported for diabetics (30).

**Influence of comorbidities and concomitant medications on clopidogrel responsiveness**

The heterogeneous response to clopidogrel led us to investigate whether established cardiovascular risk factors or comorbidities in our patients or concomitant medications as listed in Table 1 might be associated with increased incidence of clopidogrel non-responsiveness. In univariable logistic regression analysis selected from variables listed in Table 1, only reduced levels of HDL cholesterol (OR=0.95 per 10 mg/dl, 95%CI 0.92–0.98, p=0.006), a history of hyperlipidaemia (OR=2.68, 95%CI 1.08–6.67, p=0.034) and a lower platelet count (OR=0.99 per 50000, 95%CI 0.97–0.99, p=0.017) were associated with an increased risk of impaired clopidogrel responsiveness as detected by PRI, while neither statin treatment nor BMI were associated with impaired clopidogrel responsiveness. The variables identified by univariable logistic regression analysis remained independently associated if explored further in multivariable analysis as detailed in Table 2.

![Figure 3: Clopidogrel efficacy in diabetes.](image-url)
Discussion

Even after a loading dose of clopidogrel and more than five days of continuous treatment, ADP-induced P2Y\textsubscript{12}-mediated platelet signaling was not sufficiently (>50%) inhibited in 69% of clopidogrel-treated patients with CAD assessed by the P2Y\textsubscript{12}-specific PRI. Conventional platelet aggregation response to ADP failed to detect significant P2Y\textsubscript{12}-mediated platelet signaling in 36% of patients with an impaired PRI response. Reduced HDL and hyperlipidemia were determined as independent risk factors for impaired clopidogrel responsiveness.

Platelet aggregation, P-selectin expression and P2Y\textsubscript{12}-mediated signalling determined by PRI was significantly reduced in clopidogrel-treated CAD patients, which is well in accordance with prospective trials demonstrating an advantage of clopidogrel + ASA over clopidogrel alone in CAD patients (4, 5, 31). However, our data demonstrate a very heterogeneous distribution of the inhibitory effects of clopidogrel on platelet aggregation and PRI in an unselected cohort of patients with CAD. When evaluating “non-” or “impaired-”responsiveness to clopidogrel, the deviation of the observed from the intended effect is of particular concern: the currently recommended daily dose of 75 mg clopidogrel was originally intended to achieve >50% inhibition of platelet activation (7), which—as demonstrated in Figure 1—is actually achieved on average in a real world patient cohort treated for at least five days.

The term clopidogrel “non-response” was used to describe a state of impaired clopidogrel-efficacy because higher than predicted rates of stent thrombosis and adverse effects were observed. Later, several studies demonstrated that Agg\textsubscript{max} of >50% as well as a PRI >50% were associated with increased stent thrombosis and major adverse cardiovascular events (11, 24, 26, 32, 33). A PRI cut-off value of >50% can predict postprocedural adverse events, while the negative predictive value is 100% (25, 26). Currently recommended doses deliberately aim for a partial effect on platelet reactivity, which puts a significant number of patients below these thresholds at increased risk for stent thrombosis after interventions, because the response to platelet inhibition is highly variable. A significant interindividual variability in pharmacological response to clopidogrel has been demonstrated which suggests a potential benefit by increasing the maintenance dose of clopidogrel in poorly responding patients. Trials using higher loading or maintenance doses suggest that a suboptimal bioavailability of the active metabolite might contribute to clopidogrel non-responsiveness (30, 34–36).

For the assessment of functional response following clopidogrel treatment it might be worthwhile to selectively investigate P2Y\textsubscript{12}-mediated platelet activation by PRI, which can not be achieved by measuring platelet aggregation that comprises both P2Y\textsubscript{12} and P2Y\textsubscript{13}-mediated effects. One caveat for the assessment of PRI in patients with CAD might be its dependence on PGE\textsubscript{1} stimulation. Patients with stable and unstable angina have decreased platelet responsiveness to the antiaggregatory effects of the endogenous platelet inhibitors nitric oxide (37) and PGE\textsubscript{1} (38). Thus, the sensitivity for PGE\textsubscript{1} is altered in CAD, but it is unknown how this affects PRI measurements.

A limitation of this study is the absence of information on pre-treatment platelet reactivity. Although it is interesting and helpful to have pre- and post-treatment measurements available, in clinical routine, pre-treatment values will often not be available. However, patients with hyperaggregability after receiving a loading dose of clopidogrel have an increased risk for development of major adverse cardiac events after elective PCI (39).

A very high incidence of clopidogrel “non-responsiveness” (62.5%) was recently reported in type II-diabetic patients with CAD (30). We therefore analyzed platelet aggregation and PRI in our patients with regard to established cardiovascular risk factors. While diabetes per se was not associated with higher rates of impaired clopidogrel responsiveness, multivariable analysis of comorbidities and cardiovascular risk factors suggested that low HDL levels and a history of hyperlipidaemia might be related to impaired clopidogrel responsiveness. Hyperlipidaemia is associated with an increased cholesterol content of the platelet-membrane, and clopidogrel-resistant platelets are hypersensitive to ADP (40, 41). Plasma HDL levels inversely correlate with platelet membrane cholesterol content, and HDL modulates platelet reactivity by alteration of membrane raft associated receptor clustering (42). Thus, low HDL levels and hyperlipidaemia observed in metabolic syndrome and diabetes may explain the lower efficacy of clopidogrel in these patients.

However, other factors that are more difficult to address in a clinical study must be taken into consideration: poor compliance with drug treatment, interference with other drugs, and interindividual variations in metabolism of the pro-drug to the active metabolite (8). To ensure compliance, we only included hospitalised patients receiving daily individual drug dispense. Interference with other drugs, which share metabolism or clearance via cytochrome P450 3A4 has widely been discussed as a potential confounder in clopidogrel responsiveness. Whilst we did not detect any influence of statins on clopidogrel responsiveness (Table 1), poor metabolisation of clopidogrel might still be relevant for impaired responsiveness since co-treatment of patients with minimal responses to clopidogrel with the cytochrome P450 3A4 inducer rifampin improved platelet inhibition (43). Incomplete responses to clopidogrel could be overcome in vitro with the direct active P2Y\textsubscript{12} antagonist AR-C69931MX (44), and stronger platelet inhibition was achieved with more potent P2Y\textsubscript{12} inhibitors in vitro.
vivo, which are either better metabolised (45) or do not need metabolisation at all (46). Very recent data suggest a potential impact of proton pump inhibitors, which are frequently administered together with dual anti-platelet therapy to prevent gastrointestinal bleeding, on metabolism of clopidogrel contributing to impaired effectiveness of clopidogrel in the presence of omeprazole (47), however, there was no such association in this study.

Conclusion

The extraordinary heterogeneity in the response to clopidogrel leading to a very high prevalence of individual impaired clopidogrel responsiveness (up to 69%) in patients with CAD in a real-world scenario observed in this study using a P2Y<sub>12</sub>-specific assay underlines the need to determine a useful threshold of platelet inhibition in the future, thus tailoring anti-platelet treatment to the individual with optimal platelet inhibition and low bleeding risk. Recently, a clinical score was proposed to identify patients, who are likely to develop increased platelet reactivity and thus might benefit from individualised treatment (48). Further prospective studies are needed to determine the potential of individual testing under certain circumstances and the risk reduction by adjusted anti-platelet therapy.

References