Early determination of clopidogrel responsiveness by platelet reactivity index identifies patients at risk for cardiovascular events after myocardial infarction

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Introduction

Thienopyridines such as clopidogrel inhibit multiple pro-aggregationary actions of the platelet agonist adenosine diphosphate (ADP) by selectively and irreversibly blocking the P2Y₁₂ platelet ADP receptor. Following absorption, 85% of the inactive prodrug clopidogrel are already hydrolysed by circulating blood esterases to an inactive metabolite before the remaining 15% undergo hepatic metabolism by cytochrome P450 isoenzymes to generate the active metabolite (1). This active metabolite forms disulfide bridges with the extracellular part of the P2Y₁₂ receptor and inactivates the receptor in this way irreversibly. Large clinical trials demonstrated the superiority of clopidogrel to aspirin for preventing ischaemic events in selected patients with atherosclerotic disease (2, 3). Nevertheless, some patients experience thromboembolic events despite dual anti-platelet therapy, and so-called clopidogrel “non-responsiveness” has been observed (4–8). When impaired responsiveness is defined based on the risk for potential stent thrombosis, a high proportion of patients in a real-world setting are suboptimal responders to clopidogrel in daily practice (9, 10). Following elective percutaneous coronary intervention (PCI), it is recommended that clopidogrel 75 mg daily should be given for at least four weeks after stent implantation with a 300 mg loading dose of clopidogrel at least 6 hours (h) prior to PCI (11, 12). Such pre-treatment periods are, however, not applicable in the setting of acute ST-elevation myocardial infarction (STEMI).

Acute STEMI is the sudden and life-threatening manifestation of coronary artery disease (CAD) leading to occlusion of a major coronary artery. It usually develops due to atherosclerotic plaque disruption and subsequent formation of an occluding platelet thrombus (13). Current guidelines favour primary PCI if performed within 90–120 minutes (min) after first medical contact (13, 14). This aims for achieving more effective revascularisation, reducing reocclusion, improving cardiac function and clinical outcome (15).

Summary

While acute myocardial infarction (MI) is associated with impaired clopidogrel responsiveness, systematic evaluation is lacking due to the inability of functional aggregation-based assays to analyse clopidogrel responsiveness in the presence of glycoprotein IIb/IIa inhibitors. Using the P2Y₁₂-specific, non-aggregation-based platelet-reactivity-index (PRI) we assessed clopidogrel responsiveness in patients with acute MI. Clopidogrel responsiveness was determined 24 hours (h) after loading with 600 mg clopidogrel in 54 patients with acute MI admitted for coronary intervention. A PRI >50% was considered as suboptimal inhibition. Overall response in MI patients was suboptimal with a median PRI of 58%. Diabetes, low high-density lipoprotein and pre-hospital clopidogrel loading were associated with impaired clopidogrel responsiveness. Patients loaded at first medical contact had a significantly weaker platelet inhibition by clopidogrel after 24 h (PRI 63%) compared to those loaded peri-interventionally (PRI 54%, p=0.014). Clinical outcome was assessed as a combination of cardiac death, non-fatal MI, stent thrombosis, ischaemic stroke, and urgent target vessel revascularisation after 12 months. The pre-selected cut-off of PRI ≤50% yielded a sensitivity of 87% at a specificity of 26%, whereas a PRI ≤57% determined by receiver-operating characteristics (ROC)-analysis yielded a sensitivity of 80% at a specificity of 56% (event rate: PRI ≤57%: 12.0%; PRI >57%: 41.4%, p=0.0136). In conclusion, PRI detects clopidogrel responsiveness in acute MI patients requiring glycoprotein IIb/IIa antagonism; and impaired clopidogrel responsiveness predisposes to clinical events. Pre-hospital clopidogrel loading was associated with impaired response and more adverse events challenging the concept of earliest oral clopidogrel loading in MI patients.

Keywords

ADP receptors, platelet activation, diabetes, P2Y₁₂, myocardial infarction

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Guidelines recommend that "clopidogrel should be given as soon as possible to all patients with STEMI undergoing PCI" (13). This advice for a loading dose of at least 300 mg or 600 mg at the earliest possible time has led to the introduction of oral clopidogrel loading in STEMI patients by ambulance-based emergency physicians in some countries or by smaller hospitals referring patients for primary PCI. However, data are absent regarding absorption during the acute STEMI state including nausea, vomiting and stress as well as pharmacological interventions, which might eventually influence oral drug uptake such as morphine-based analgesia. These considerations might be of particular importance as only oral thienopyridines are currently clinically available.

Systematic analysis of clopidogrel responsiveness in acute STEMI was previously hampered by the available techniques: aggregation as well as point-of-care assays are dependent on fibrinogen-binding which is potently inhibited by glycoprotein IIb/IIIa inhibitors routinely administered for PCI in STEMI. The platelet reactivity index (PRI) is a flow-cytometry based assay assessing the ability of ADP to inhibit adenylyl cyclase independent from other antiplatelet agents such as acetylsalicylic acid or glycoprotein IIb/IIIa antagonists, which are administered during acute STEMI.

In the present study, we investigated the response of STEMI patients to clopidogrel loading using the PRI. We further assessed whether timing of clopidogrel loading in STEMI as well as the strength of its effect 24 h after the initial loading dose would influence clinical outcome.

Methods

Study population

Fifty-four consecutive patients with acute STEMI admitted to the Division of Cardiology at the University Hospital Würzburg, Germany between November 2008 and May 2009 were analysed for this study and served as cases. Patients with CAD (n=50), who underwent elective PCI at the same institution, were used to solely compare the efficacy of clopidogrel loading on a functional basis. Since cardiovascular risk differs too extensively between stable CAD patients and those suffering an acute STEMI, we did not intend to compare any clinical outcome between these populations. The STEMI population, which was prospectively observed and followed-up, was retrospectively stratified for a subanalysis by the timing of clopidogrel loading to pre-hospital (e.g. before immediate transfer or by an ambulance-based emergency physician, n=34, time from clopidogrel loading to first balloon inflation 88 ± 4 min) vs. peri-interventional (post-PCI in the catheterisation laboratory, n=20, time from first balloon inflation to clopidogrel loading 15 ± 3 min) application of the clopidogrel loading dose. The idea for the stratification was based on the CRUSADE registry, which was associated with an increased rate of post-admission myocardial infarction if clopidogrel loading had been given very early (pre-hospital) vs. peri-interventional in a STEMI population (16). Only patients, who could actively swallow clopidogrel were included in the observation in order to exclude a paralysed gastrointestinal tract as a biasing factor as observed in sedated and ventilated patients given clopidogrel by a gastric tube (17). Our study was performed in accordance with the Declaration of Helsinki and following approval by the local Ethics Committee. No sex-based or racial/ethnic-based differences were present in the reported study.

Blood sample collection

Blood sampling was performed 24 h after intake of the clopidogrel loading dose of 600 mg in all subjects (cases and control patients). ADP-mediated inhibition of PGE1-stimulated platelet vasodilator-stimulated phosphoprotein (VASP)-phosphorylation was determined using the P2Y12, platelet reactivity index (PRI).

Platelet reactivity index (PRI)

The P2Y12 PRI was determined through assessment of the phosphorylation status of the VASP using flow cytometry (FACSCalibur, Becton Dickinson, Heidelberg, Germany). VASP phosphorylation (VASP-P) was quantified with labelled monoclonal antibodies using a commercially available kit (PLT VASP/P2Y12 Test kit, BioCytex, Marseille, France), for which platelets were stimulated with PGE1 ± ADP followed by fixation with formaldehyde. Following permeabilisation 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 (18). 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 following 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 the stronger is P2Y12 receptor inhibition.

Definition of impaired clopidogrel responsiveness

The threshold for impaired clopidogrel responsiveness was defined at P2Y12 PRI >50% according to the current consensus on high on-treatment platelet reactivity (19). This cut-off was based on its association with stent thrombosis as described earlier (20, 21). In fact, recent studies have demonstrated cut-off values for VASP-PRI in the range of 48–53% as a prognostic indicator for stent thrombosis following percutaneous interventions and coronary stenting (21–23). Further PRI-guided repeated clopidogrel loading with a target PRI below 50% has been associated with improved outcome (24).
Clinical follow-up

After initial presentation with STEMI, clinical outcome for a combined cardiovascular endpoint consisting of cardiac death, non-fatal myocardial infarction, stent thrombosis, ischaemic stroke, and urgent target vessel revascularisation was assessed after 12 months by telephone interview (100% complete). Event-rate analysis included time-to-first event, whereby only two out of the three non-fatal MIs had urgent target vessel revascularisation as a second event.

Substances

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

Data analysis

Numbers are given as n (%), mean ± SEM, or median (25th-75th percentile) in the tables, and median with individual measurement points in the figures. Group comparisons were made using the Mann-Whitney U-test. Correlates of PRI were sought using linear regression analysis, and PRI was entered as a log-normalised variable. To assess the clinical utility of the pre-defined cut-off value for PRI (≤50% vs. >50%), receiver operating curves with area under the curve and 95% confidence intervals were computed. Kaplan-Meier plots were constructed and differences in outcome were assessed using the log rank test. Data were analysed using GraphPad Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS (Version 17.1).

Table 1: Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Elective PCI</th>
<th>STEMI total</th>
<th>P-value</th>
<th>STEMI pre-hospital</th>
<th>STEMI peri-intervent.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>50</td>
<td>54</td>
<td></td>
<td>34</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Sex (m/f, m[%])</td>
<td>37/13 (74)</td>
<td>46/8 (85)</td>
<td>0.33</td>
<td>27/7 (79)</td>
<td>19/1 (95)</td>
<td>0.31</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66 ± 2</td>
<td>64 ± 1</td>
<td>0.18</td>
<td>65 ± 2</td>
<td>60 ± 3</td>
<td>0.10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 ± 0.8</td>
<td>27.7 ± 0.5</td>
<td>0.40</td>
<td>27.5 ± 0.7</td>
<td>28.0 ± 0.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>22</td>
<td>17</td>
<td>0.67</td>
<td>21</td>
<td>10</td>
<td>0.47</td>
</tr>
<tr>
<td>Hyperlipoproteinaemia (%)</td>
<td>70</td>
<td>37</td>
<td>0.004</td>
<td>44</td>
<td>25</td>
<td>0.21</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>84</td>
<td>64</td>
<td>0.11</td>
<td>65</td>
<td>65</td>
<td>0.83</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>48</td>
<td>37</td>
<td>0.68</td>
<td>38</td>
<td>35</td>
<td>0.41</td>
</tr>
<tr>
<td>Family history for MI (%)</td>
<td>38</td>
<td>28</td>
<td>0.39</td>
<td>32</td>
<td>20</td>
<td>0.41</td>
</tr>
<tr>
<td>Adiposity (%)</td>
<td>44</td>
<td>41</td>
<td>0.83</td>
<td>35</td>
<td>50</td>
<td>0.41</td>
</tr>
<tr>
<td>Number of CVRFs</td>
<td>3 (IQR 2–4)</td>
<td>2 (IQR 1–3)</td>
<td>0.008</td>
<td>2 (IQR 1–3)</td>
<td>2 (IQR 1–3)</td>
<td>0.15</td>
</tr>
<tr>
<td>cholesterol (mg/dl)</td>
<td>185±5.8</td>
<td>207.7±8.9</td>
<td>0.04</td>
<td>214 ± 12</td>
<td>196 ± 12</td>
<td>0.44</td>
</tr>
<tr>
<td>CK max (U/l)</td>
<td></td>
<td></td>
<td></td>
<td>2092 ± 397</td>
<td>2104 ± 385</td>
<td>0.79</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>100 ± 4</td>
<td>112 ± 5</td>
<td>0.10</td>
<td>108 ± 6</td>
<td>119 ± 11</td>
<td>0.64</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>46 ± 2</td>
<td>42 ± 2</td>
<td>0.55</td>
<td>42 ± 3</td>
<td>43 ± 3</td>
<td>0.65</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.4 ± 0.2</td>
<td>5.9 ± 0.1</td>
<td>0.06</td>
<td>6.1 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73 m²)</td>
<td>88 ± 4</td>
<td>87 ± 3</td>
<td>0.87</td>
<td>86 ± 4</td>
<td>89 ± 5</td>
<td>0.99</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0.34 ± 0.06</td>
<td>0.88 ± 0.34</td>
<td>0.32</td>
<td>0.54 ± 0.17</td>
<td>0.62 ± 0.26</td>
<td>0.47</td>
</tr>
<tr>
<td>Platelets (n*1,000/μl)</td>
<td>228 ± 7</td>
<td>219 ± 8</td>
<td>0.36</td>
<td>218 ± 10</td>
<td>220 ± 11</td>
<td>0.85</td>
</tr>
<tr>
<td>BMS (%)</td>
<td>52</td>
<td>80</td>
<td>0.008</td>
<td>74</td>
<td>83</td>
<td>0.60</td>
</tr>
<tr>
<td>DES (%)</td>
<td>48</td>
<td>13</td>
<td>0.002</td>
<td>16</td>
<td>11</td>
<td>0.77</td>
</tr>
<tr>
<td>GPlib/IIa inhibitor (%)</td>
<td>4</td>
<td>59</td>
<td>&lt;0.0001</td>
<td>59</td>
<td>60</td>
<td>0.88</td>
</tr>
<tr>
<td>Opiate analgetics (%)</td>
<td>53</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td>0.91</td>
</tr>
</tbody>
</table>

Demographic and procedural characteristics in the STEMI populations and patients with stable coronary artery disease. 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; CK max=maximum level of creatinine kinase; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; HbA1c=glycosylated haemoglobin; GFR=glomerular filtration rate estimated using the MDRD equation (Modification of Diet in Renal Disease Study Group); BMI=body mass index; BMS=bare metal stent; DES=drug-eluting stent; IQR=interquartile range; MI=myocardial infarction.
Results

Patient characteristics of the different groups are shown in Table 1. Aspirin (100 mg/day) and clopidogrel (75 mg/day) compliance were assessed as part of the follow-up, and patients included in the analysis had adhered to anti-platelet therapy as recommended.

Clopidogrel responsiveness in STEMI

Twenty-four hours after receiving a 600 mg loading dose of clopidogrel, patients with STEMI displayed less inhibition of ADP-dependent, P2Y12-mediated platelet signalling determined by the PRI compared to stable CAD patients (Fig. 1A). While the response in the elective PCI group was already very heterogeneous with impaired response in a substantial proportion of patients (38%), the majority of STEMI patients (74%) had a P2Y12-specific PRI of >50% (Fig. 1A), i.e. an insufficient platelet inhibition known to increase the risk of post-interventional stent thrombosis (20–22).

Influence of impaired clopidogrel responsiveness on outcome in STEMI patients

The combined clinical endpoint occurred more frequently in STEMI patients within the two higher quartiles of PRI than the lower ones (Fig. 1B).

Since previous studies addressed the sensitivity of the PRI predominantly in non-STEMI patients, we performed a receiver-operator-curve (ROC) analysis to determine whether a PRI >50% would also be appropriate in a STEMI population with regard to risk sensitivity. The pre-selected cut-off of PRI >50% in this STEMI population yielded a sensitivity of 87% at a specificity of 26% over one year, whereas a PRI >57% yielded a sensitivity of 80% at a specificity of 56% over one year (Fig. 2A). The com-
bined cardio- and cerebrovascular end-point over 12 months occurred significantly more often in patients with impaired clopidogrel responsiveness using the ROC-determined cut-off (PRI >57%) (Fig. 2B).

**Contributors to impaired clopidogrel responsiveness in STEMI**

With regard to classical cardiovascular risk factors, PRI was higher in diabetics (70 ± 17% vs. 56 ± 14%, p=0.014), and was positively associated with glycosylated haemoglobin (HbA1c) (T=2.3, p=0.026). Furthermore, we found an inverse association with high-density lipoprotein (HDL) (T=−2.1, p=0.040), similar to our previous report in patients on a maintenance dose of clopidogrel (10). Of note, other important risk surrogates as body mass index, renal function and C-reactive protein were not associated with PRI. In multivariable analysis, a trend for HbA1c was maintained (T=2.0, p=0.060) where the association with HDL was lost.

When the STEMI population was stratified by the timing of clopidogrel loading, patients with out-of-hospital clopidogrel loading in our cohort showed a significantly weaker platelet inhibition than patients receiving clopidogrel in the catheterisation laboratory (Fig. 3A): mean levels of PRI 62 ± 17% vs. 52 ± 14%, p=0.0141. Since impaired responsiveness was more common in patients loaded with clopidogrel in the pre-hospital phase, outcome analysis was also performed by stratifying patients by the timing of clopidogrel loading. Patients with pre-hospital clopidogrel loading had worse outcome than patients loaded peri-interventionally (Fig. 3B). The individual endpoints are listed in Table 2. All three non-fatal-MIs (2 NSTEMI, 1 STEMI) during the follow-up period were type 1 MIs as defined by the universal definition of MI (e.g. spontaneous myocardial infarction to ischaemia due to a primary coronary event) (25).

**Discussion**

In the present prospective observational study we found that the PRI is useful to reliably detect early clopidogrel responsiveness in STEMI patients. Particularly administering the initial loading dose of clopidogrel (600 mg) pre-hospitally significantly attenuated the response assessed by PRI compared to peri-interventional loading. Patients with impaired response to clopidogrel suffered more frequently from cardiovascular events during a 12-month follow-up. Usage of glycoprotein IIb/IIIa antagonists previously impeded the evaluation of the acute response to clopidogrel in STEMI pa-

### Table 2: First major adverse cardio- or cerebrovascular event following STEMI.

<table>
<thead>
<tr>
<th></th>
<th>STEMI PRI ≤57%</th>
<th>STEMI PRI &gt; 57%</th>
<th>STEMI pre-hospital</th>
<th>STEMI peri-intervent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>14</td>
<td>40</td>
<td>34</td>
<td>20</td>
</tr>
<tr>
<td>CV death</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>non-fatal MI</td>
<td>0</td>
<td>3*</td>
<td>3*</td>
<td>0</td>
</tr>
<tr>
<td>stent thrombosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ischaemic stroke</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>uTVR</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>12</td>
<td>14</td>
<td>1</td>
</tr>
</tbody>
</table>

Distribution of the individual adverse clinical events in STEMI patients dependent on achievement of PRI cut-off and time of loading with clopidogrel. * subtype of non-fatal MI (2 NSTEMI, 1 STEMI).

**Figure 3: Influence of pre-hospital clopidogrel loading in STEMI patients.** Distribution of clopidogrel responsiveness in the STEMI cohort stratified by receiving the clopidogrel 600 mg loading dose either pre-hospitally or peri-interventionally was determined by the platelet-reactivity index (PRI). Horizontal lines indicate the respective median value (A). The primary endpoint – a composite of cardiac death, non-fatal myocardial infarction, stent thrombosis, ischaemic stroke, and urgent target vessel revascularisation assessed after 12 months – occurred significantly more often in STEMI patients receiving the clopidogrel loading dose pre-hospitally than in STEMI patients treated peri-interventionally (5.3% vs. 43.7%; HR 10.8; 95% CI 1.63 to 12.6; p=0.0038) (B).
Clopidogrel efficacy in STEMI

Patients by aggregation-based assays requiring fibrinogen binding. PRI is not influenced by glycoprotein IIb/IIIa antagonism (see Suppl. Fig. 1 available online at www.thrombosis-online.com), since the assay is independent from fibrinogen binding, and therefore allows to extend the assessment of clopidogrel responsiveness to a population requiring glycoprotein IIb/IIIa antagonism such as STEMI patients. High variability in clopidogrel responsiveness in general has been widely described (26, 27). Using the P2Y₁₂-specific PRI to assess clopidogrel responsiveness, suboptimal responses of up to 60% of patients have been noted in real-world patients being on maintenance clopidogrel treatment (10, 28). In particular, a PRI >50% is associated with increased stent thrombosis and major adverse cardiovascular events (20–23). Especially patients with high peri-interventional platelet reactivity after receiving a 600 mg loading dose of clopidogrel had worse outcome after elective PCI (29) and adjusting clopidogrel dosing by PRI to <50% in patients undergoing PCI reduces cardiovascular events (24). However, several studies have determined slightly different cut-off values; Barragan et al. initially determined a mean PRI of 69.7% in patients suffering stent thrombosis compared to a PRI of 39.8% in patients not suffering stent thrombosis and suggested a cut-off around 50% (20). Subsequently others suggested cut-offs for PRI of 48% in PCI with high-risk for stent thrombosis (22), of 50% in elective PCI for major adverse cardiovascular events (21), and of 53% for recurrent ischaemic events after stenting for non-ST elevation acute coronary syndromes (ACS) (23). In the current study, we chose a PRI >57% as cut-off determined by ROC-analysis in order to enhance the specificity of the analysis.

The risk factors associated with impaired clopidogrel responsiveness in STEMI patients in this study, low HDL and diabetes, represent long-term variables which are not acutely modified by STEMI treatment. Impaired clopidogrel responsiveness has been reported in patients with diabetes (30) and was linked to an increased risk of stent thrombosis (31). More efficient platelet inhibition using prasugrel in diabetic patients with ACS reduced cardiovascular events and stent thrombosis (32).

Our previous work in stable patients on chronic clopidogrel treatment suggested that low HDL levels and a history of hyperlipidaemia are related to impaired clopidogrel responsiveness (10). Hyperlipidaemia is associated with an increased cholesterol content of the platelet-membrane, and cholesterol-rich platelets are hypersensitive to ADP (33, 34). Lipid raft-associated P2Y₁₂ oligomers represent the functional form of the receptor and partitioning of P2Y₁₂ out of rafts is observed in response to clopidogrel (35). Plasma HDL levels inversely correlate with platelet membrane cholesterol content, and HDL modulates platelet reactivity by alteration of membrane raft associated receptor clustering (36). Low HDL levels are associated with MI in patients undergoing PCI (37).

One problem with oral dosing of clopidogrel in STEMI patients is the compound’s highly variable responsiveness per se, another one is the unpredictable absorption in the emergency situation. While absorptive issues might have an impact on the acute response and on early events, diabetes and low HDL, which were also associated with impaired clopidogrel responsiveness in our study, might have influenced the different event-rates during chronic follow-up. In the present study, STEMI patients showed a weaker response to clopidogrel loading than stable CAD patients undergoing PCI. Knowing that clopidogrel takes several hours to achieve its maximal effect, guidelines recommend the earliest possible loading if PCI is intended (13), e.g. by using the time needed to transfer a patient to primary PCI. However, in our study STEMI patients with clopidogrel loading in the pre-hospital phase displayed significantly lower inhibition of P2Y₁₂-mediated signalling and had worse clinical outcome. One factor which could contribute to such a phenomenon is compromise of absorption and metabolism of the oral drug clopidogrel. Indeed, Heestermans et al. previously demonstrated that maximal plasma levels of clopidogrel and of its active metabolite are much lower in STEMI patients compared to healthy controls, time to maximal plasma levels is longer, and exposition to active clopidogrel metabolite is significantly worse (38). In contrast to our study, Fefer et al. recently reported that pre-treatment with high-dose clopidogrel in patients undergoing PCI for STEMI would be useful in preventing cardiovascular events (39). However, the most pronounced and statistically significant effect of clopidogrel pre-treatment in their study was demonstrated in patients with door-to-balloon times of more than 90 min, whereas patients with shorter door-to-balloon times had no benefit. Furthermore patients with heart failure, who appear most susceptible to impaired clopidogrel responsiveness (40), were overrepresented in their post-PCI group. Similarly, the influence of ambulance-based clopidogrel loading was assessed in the randomised-controlled CIPAMI trial (41), and the first results indicated no difference between placebo and clopidogrel loading in patients with rapid clopidogrel-to-ballon times of less than 1 h (42). A more recent comparison between patients presenting in a PCI center vs. transfer patients showed a significant benefit for patients with longer transfer and door-to-balloon times, however; these patients with delayed transfer routinely received a fibrinolytic agent, and the PCI center had a median door-to-balloon time above 60 min (43).

In fact, the CRUSADE registry showed that clopidogrel loading more than 30 min before PCI in STEMI patients was associated with a higher rate of recurrent MI than administering clopidogrel peri-interventionally (16). Recently, ACS have been identified as a predictor for impaired clopidogrel responsiveness (44).
The rationale for early clopidogrel loading in STEMI patients was to achieve at least some P2Y₁₂-dependent platelet inhibition at the time of primary PCI. However, assessment of P2Y₁₂-specific inhibition in ACS patients in the TRITON-TIMI38 trial demonstrated that there was no detectable P2Y₁₂-inhibition after 1–2 h in the clopidogrel-treated group (45).

While STEMI patients did respond less to clopidogrel than stable CAD patients per se, administering the loading dose during an unstable and unsettled pre-clinical situation resulted in an even higher proportion of suboptimal response. Because of the association of pre-clinical loading and impaired responsiveness to clinical outcome, the general recommendation for the earliest-possible loading of clopidogrel should be scrutinised. Recently, the TRITON-TIMI38 trial showed that prasugrel, which exerts efficient platelet inhibition more rapidly (45) and reduces cardiovascular events in ACS patients (46), prevented more ischaemic events in STEMI patients than clopidogrel (47). Therefore, it might be a useful approach to perform PCI first under glycoprotein IIb/IIIa antagonism, and to apply a more rapid acting and more efficient P2Y₁₂ blockade immediately after PCI.

A limitation of our study is the small sample size: first, it was too small to allow for a meaningful multivariable adjustment of possible confounders. Second, our study is not and was never supposed to be powered for outcome analysis. However, the study contributes to the discussion that impaired clopidogrel responsiveness or high on-treatment platelet reactivity despite clopidogrel treatment is associated with increased numbers of cardiovascular events. One of the first studies to associate impaired clopidogrel responsiveness with increased event rate included a similar number of patients (n=60) with seven patients having events over six months of follow-up (7). Clearly, larger follow-up studies would be needed in order to confirm our findings. However, differences in baseline variables between pre-hospital and peri-interventionally loaded patients were minor and unlikely to account for the observed differences in outcome. The study was started after the guidelines recommended the earliest possible loading, e.g. pre-hospital. Further enrollment was discontinued when long-term clinical follow-up on clopidogrel was no longer possible, as STEMI patients were regularly loaded with and continued on prasugrel based on the data of the TRITON-TIMI38 STEMI cohort. Furthermore, the hope for improved infarct artery patency at the time of primary PCI has been reduced by the data of the CIPAMI trial demonstrating no effect of pre-clinical clopidogrel loading on neither infarct artery patency nor ST segment resolution after PCI. Taken together, it would be methodically useful to increase the sample size of this study, but it seems ethically not feasible given the changes in current medical practice. Nevertheless, the study adds to the discussion that the un- critical early oral treatment might put patients more at risk than it improves care as patients requiring urgent surgical treatment are exposed to higher bleeding risk and patients needing platelet inhibition might not have the benefit of proper clopidogrel loading.

In conclusion, we demonstrated the feasibility of using the PRI to detect early clopidogrel responsiveness in STEMI patients. Clopidogrel loading in STEMI patients is not as efficient as in stable CAD patients, particularly in patients loaded before PCI. Patients loaded pre-clinically and those with suboptimal clopidogrel responsiveness have worse outcome than patients loaded following acute PCI and those achieving sufficient P2Y₁₂ inhibition.

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