Multiple electrode aggregometry and vasodilator stimulated phosphoprotein-phosphorylation assay in clinical routine for prediction of postprocedural major adverse cardiovascular events

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Summary
Reduced antiplatelet effect of clopidogrel assessed with multiple electrode aggregometry (MEA) and vasodilator stimulated phosphoprotein-phosphorylation (VASP-P) assay has been proven to predict major adverse cardiovascular events (MACE) after coronary stenting. So far no consecutive registry has evaluated the usefulness of different adenosine diphosphate-based platelet function tests to predict outcome in unselected patients. Hence, our objective was to determine the feasibility of MEA and VASP-P for clinical routine and whether low-response to clopidogrel as determined by MEA and/or the VASP-P assays predicts MACE in a “real-life” population undergoing coronary stenting. Three-hundred consecutive patients were included in this prospective registry. Blood was sampled 6–24 hours after stenting to measure MEA and VASP-P. The use of glycoprotein-IIb/IIIa-blockers limited MEA to 196 measurements. Concerning the VASP-P assay, 300 measurements were achieved. Receiver Operating Characteristics (ROC)-curves of sensitivity and specificity estimates for MACE were plotted for VASP-P assay. The area under the ROC-curve was 0.683 (p=0.014) for the platelet reactivity index (PRI) calculated from median fluorescence intensities (FI) with an optimal cut-off at 60.2% PRI. Patients above 60.2% had a significantly increased risk for MACE at six months follow-up (p=0.007). Estimating the cut-offs for the PRI from mean FI (52%) or from geometric mean FI (56.6%) led to clinically relevant differences. VASP-P assay is feasible for clinical routine to measure clopidogrel effects and to predict post-procedural MACE in unselected patients. With regard to differing cut-offs, exact standardisation of the VASP-P assay is mandatory. The use of GP-IIb/IIIa-blockers prevents MEA testing and limits its usability in unselected patients.

Keywords
Platelet function testing, clopidogrel, high on-treatment platelet reactivity, prospective registry

Introduction
Clopidogrel is a thienopyridine inhibiting the P2Y_{12}-receptor on the platelet surface, which results in inhibition of platelet activation and aggregation (1). Over the last two decades substantial evidence has been generated regarding the beneficial effects of acetylsalicylic acid (ASA) and clopidogrel in patients with acute coronary syndromes (ACS) or after percutaneous coronary intervention (PCI) (2–4). Despite dual antiplatelet therapy (ASA and clopidogrel), the subsequent incidence of athero-thrombotic events, including stent thrombosis, remains high. Low-response to clopidogrel, i.e. high on-treatment platelet reactivity (HTPR), has been called to account repeatedly (5, 6). HTPR after short-term therapy apparently is associated with an increased cardiovascular risk ranging from a modest increase up to odds ratios (OR) of 8, depending on the patient population and test systems studied (7–13).

The above mentioned trials usually focused on HTPR in non-emergent patients [i.e. patients with stable coronary artery disease (CAD) or unstable angina (UA)/non-ST-segment elevation myocardial infarction (NSTEMI)], but the issue of HTPR of patients needing emergent intervention [i.e. patients with ST-segment elevation myocardial infarction (STEMI)] or patients receiving glycoprotein-blockers has been less intensively investigated (14–17), although this is a common situation in a “real-life” setting. There is so far no consecutive registry that evaluates the clinical outcome in unselected patients that have been admitted for PCI and coronary stenting with regard to different platelet function tests. We selected two tests using adenosine diphosphate and prostaglandin E_{1} (ADP and PGE_{1}) to evaluate the usefulness of the assays in the daily routine and to study the effect of impaired response to clopidogrel on the occurrence of ischaemic events in a “real-life” setting: a receptor specific vasodilator stimulated
Materials and methods

Patients

The Wilhelminenhospital Monitoring of Antiplatelet Activity (WILMAA)-registry is a prospective single center observational study, based on implication of recent international guidelines (28). It includes 300 consecutive patients of the 3rd Medical Department, Cardiology and Emergency Medicine, Wilhelminenhospital (Vienna, Austria) between May 2009 and February 2010. All consecutive patients, who underwent PCI and coronary stenting with no contraindication for dual antiplatelet therapy for up to one month, aged >18 years were eligible. All participants gave their informed consent and the study was approved by the Ethics Committee of the City of Vienna.

PCI, antiplatelet therapy and clinical management

The protocol requirements for periprocedural clopidogrel therapy, which were in the design of the GRAVITAS trial (29), were chosen to assure that all patients were at or close to their steady-state level of platelet inhibition at the time of blood sampling. Clopidogrel-naive patients received a 300 or 600 mg loading dose (LD). Patients on chronic clopidogrel therapy with 75 mg clopidogrel of at least seven consecutive days did not receive an additional LD. According to actual evidence, all patients received in parallel ASA (100 mg daily dose). The use of GP-IIb/IIIa-blockers during PCI as well as the choice of the anticoagulant depended on the individual situation and the thrombus load at angiography, and was left to the discretion of the operator.

PCI procedures were carried out according to the international guidelines (28, 30). The selection of the stent type (bare metal stent [BMS], or drug-eluting stent [DES]) was at the discretion of the interventional cardiologist.

Data management and follow-up

Data were collected prospectively and entered into a database. Follow-up information was obtained by outpatient visits at one month (± 2 weeks) and all patients were contacted after six months (± 2 months). Source documents of all possible events were collected. Definition of MACE included: 1) definite and probable ST according to the ARC-definition (31); 2) cardiovascular death, defined as death associated with ACS, significant arrhythmia, or congestive heart failure; and 3) non-fatal STEMI (STEMI: acute onset of prolonged typical ischaemic chest pains, ST-segment elevation of at least 1 mm in 2 or more contiguous electrocardiogram leads and increased biomarkers of myocardial necrosis). All events were classified by independent physicians unaware of the platelet function test results.

MEA and VASP-P

Six to 24 hours (h) after PCI (i.e. the next morning after PCI) venous blood samples along with the routine blood samples were collected via atraumatic venipuncture of the forearm into coagulation tubes (buffered sodium citrate 3.2%, Greiner BioOne, Kremsmünster, Austria), filled to maximum capacity for the VASP-P assay and into lithium-heparin tubes (Greiner BioOne) for MEA. The first tube was only used for routine laboratory measurements.

The VASP-P analysis was performed within 48 h after blood collection by an experienced investigator using PLT VASP/P2Y12 kits (Biocytex, Marseilles, France) according to the manufacturer’s instructions (21). The platelet reactivity index (PRI, %) was calculated from the median fluorescence intensity (MFI) as follows: PRI % = ( MFI_{P2Y12} - MFI_{P2Y12+ADP} / MFI_{P2Y12} ) x 100. For cut-off comparison we also calculated the PRI from the mean and geometric mean fluorescence intensities (FI).

The MEA (Multiple Platelet Function Analyzer, Dynabyte Medical, Munich, Germany) analysis, a point of care assay – was performed within 30 minutes (min) to 3 h after blood sampling. MEA is based on impedance aggregometry (24, 33). A 1:2 mixture

<table>
<thead>
<tr>
<th>Overall</th>
<th>PRI≤60.2%</th>
<th>PRI&gt;60.2%</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Patients, n</td>
<td>300</td>
<td>114</td>
<td>186</td>
</tr>
<tr>
<td>Age, years</td>
<td>62 ± 16</td>
<td>63 ± 19</td>
<td>62 ± 14</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>95 (31.7)</td>
<td>39 (34.2)</td>
<td>56 (30.1)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.9 ± 4.5</td>
<td>26.8 ± 4.4</td>
<td>28.5 ± 4.5</td>
</tr>
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<td>Hypertension, n (%)</td>
<td>224 (74.7)</td>
<td>86 (75.4)</td>
<td>138 (74.2)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>82 (27.3)</td>
<td>27 (23.7)</td>
<td>55 (29.6)</td>
</tr>
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<td>Hypercholesterolemia, n (%)</td>
<td>208 (69.3)</td>
<td>75 (65.8)</td>
<td>133 (71.5)</td>
</tr>
<tr>
<td>Active Smoking, n (%)</td>
<td>82 (27.3)</td>
<td>28 (24.6)</td>
<td>54 (29)</td>
</tr>
<tr>
<td>Family History of CAD, n (%)</td>
<td>130 (43.3)</td>
<td>46 (40.4)</td>
<td>84 (45.2)</td>
</tr>
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<td>Prior PCI, n (%)</td>
<td>73 (24.3)</td>
<td>28 (24.6)</td>
<td>45 (24.2)</td>
</tr>
<tr>
<td>Peripheral arterial occlusive disease, n (%)</td>
<td>30 (10)</td>
<td>9 (7.9)</td>
<td>21 (11.3)</td>
</tr>
<tr>
<td>Prior definite ST</td>
<td>4 (1.3)</td>
<td>1 (0.9)</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>68 (22.7)</td>
<td>27 (23.7)</td>
<td>41 (22)</td>
</tr>
</tbody>
</table>

**PCI**

- Due to STEMI, n (%) | 89 (29.7) | 20 (17.5) | 69 (37.1) | 0.001 |
- Due to UA/NSTEMI, n (%) | 103 (34.3) | 47 (41.2) | 56 (30.1) | |
- Due to stable CAD, n (%) | 108 (36) | 47 (41.2) | 61 (32.8) | |
- DES, n (%) | 196 (65.3) | 80 (70.2) | 116 (62.4) | 0.168 |
- Stents per patient | 1.5 ± 0.8 | 1.5 ± 0.8 | 1.4 ± 0.8 | 0.473 |
- Total stent length, mm | 31.1 ± 18.8 | 31.3 ± 17 | 31 ± 20 | 0.899 |
- Multivessel, n (%) | 175 (58.7) | 67 (58.8) | 108 (58.7) | 0.990 |

**Co-medications**

- Clopidogrel | 300 (100) | 114 (100) | 186 (100) | - |
- Chronic treatment, n (%) | 56 (18.7) | 26 (22.8) | 30 (16.1) | 0.277 |
- 300 mg LD, n (%) | 79 (26.3) | 26 (22.8) | 53 (28.5) | |
- 600 mg LD, n (%) | 165 (55) | 62 (54.4) | 103 (55.4) | |
- LD prior PCI, hours (median (25th-75th percentiles)) | 5.8 (1.3–22.5) | 15.5 (2.3–29.3) | 3.4 (0.9–20.4) | 0.001 |
- ASA, n (%) | 300 (100) | 114 (100) | 186 (100) | - |
- GP-IIb/IIIa blockers | 104 (34.7) | 33 (28.9) | 71 (38.2) | 0.103 |
- UFH, n (%) | 228 (76) | 86 (75.4) | 142 (76.3) | 0.164 |
- Bivalirudin, n (%) | 57 (19) | 19 (16.7) | 38 (20.4) | |
- Enoxaparin, n (%) | 15 (5) | 9 (7.9) | 6 (3.2) | |
- Angiotensin converting enzyme-inhibitor, n (%) | 159 (53) | 63 (55.3) | 96 (51.6) | 0.539 |
- Angiotensin receptor blocker, n (%) | 56 (18.7) | 20 (17.5) | 36 (19.4) | 0.696 |
- Beta-blocker, n (%) | 233 (77.7) | 87 (76.3) | 146 (78.5) | 0.660 |
- Calcium channel blocker, n (%) | 30 (10) | 11 (14.4) | 17 (9.1) | 0.526 |
- Proton pump inhibitor, n (%) | 244 (81.3) | 95 (83.3) | 149 (80.1) | 0.486 |
- Statin, n (%) | 265 (88.3) | 101 (88.6) | 164 (88.2) | 0.911 |

**Blood testing at admission**

- Platelet count, G/l | 234.9 ± 71.4 | 241.3 ± 76.5 | 231.1 ± 68.1 | 0.237 |
- White blood cell count, G/l | 9.5 ± 3.8 | 8.7 ± 2.9 | 10 ± 4.3 | 0.005 |
- Haemoglobin, g/dl | 14 ± 1.6 | 13.8 ± 1.6 | 14.1 ± 1.6 | 0.084 |
- Glucose, mg/dl | 138 ± 62.5 | 127.8 ± 56.8 | 144.4 ± 65.2 | 0.029 |
- C-reactive protein, mg/l | 13.9 ± 34.2 | 12.6 ± 27.9 | 14.7 ± 37.7 | 0.623 |
- Creatinine, mg/dl | 1.0 ± 0.5 | 1 ± 0.4 | 1 ± 0.5 | 0.828 |
- Creatinine clearance (MDRD) | 64.6 ± 11.1 | 64.6 ± 11.2 | 64.6 ± 11.1 | 0.985 |
- Cholesterol, mg/dl | 187.2 ± 48.9 | 185.8 ± 50.7 | 188.2 ± 47.7 | 0.728 |
- Low density lipoproteins, mg/dl | 111.9 ± 42.1 | 109.2 ± 41.5 | 113.8 ± 42.6 | 0.449 |
- High density lipoproteins, mg/dl | 46.1 ± 13.9 | 47.5 ± 14 | 45.1 ± 13.9 | 0.216 |
of 0.9% sodium-chloride and whole blood (0.3 ml) anticoagulated with lithium-heparin was incubated and stirred at 37°C for 3 min in the test cuvettes. ADP (6.5 μM) and PgE1 (9.4 μM) (Dynabyte Medical) were added and the increase in electrical impedance was recorded for 6 min. The increase in impedance was then changed into arbitrary aggregation units (AU) and plotted against time. The mean values of the two independent determinations are expressed as the area under the curve (AUC) of arbitrary units (AU x min). To simplify the numbers, 10 AU x min correspond to 1 unit (U). Variability of MEA has been reported to be <6% (34).

Statistical analysis

Previous studies investigating coronary stent-implantation with ASA alone versus dual antiplatelet therapy (ASA + thienopyridine) have shown 85% relative risk reduction in patients treated with dual antiplatelet therapy (35, 36). Based on this assumption we estimated a sample size of 280 patients would provide 80% power to detect 85% relative risk reduction in the group of responders versus non-responders at a one-sided significance level of <0.05. To compensate for potential drop-outs we included 20 patients more. Therefore we included 300 patients in the present registry. Data are presented as mean ± SD for normally distributed continuous variables (normal distribution confirmed via Kolmogorov-Smirnov test) unless depicted otherwise. Median values (25th, 75th percentiles) are shown for not normally distributed continuous variables. Continuous variables were compared by t-test or the Wilcoxon rank sum test, as appropriate. Differences in PRI between groups were calculated by use of Kruskal-Wallis test and complemented by the Dunn’s test for multiple comparisons. Differences between PRI-calculations were compared by the Friedman test and adjusted by the Dunn’s test for multiple comparisons. Categorical variables were compared by the Pearson's Chi-square (Chi²) test or the Fisher’s exact test, as required. The correlation of PRI and MEA measurements was analysed via Spearmann's correlation coefficient, denoted as R. Previously detected variables influencing the response to clopidogrel, as well as factors influencing the response to clopidogrel in our study [clinical entity at presentation, time from LD to PCI, body mass index (BMI), admission C-reactive protein, admission white blood cell count, admission platelet count, admission glucose, as well as treatment with calcium channel blockers, protone pump inhibitors and statins] were included in a multivariable logistic regression model. A receiver-operating characteristic (ROC) curve analysis was calculated to determine the ability of the platelet function assay results to distinguish between patients with or without MACE. The exploratory cut-off value was defined at a sensitivity >90%. The predictive significance of MEA and PRI for time to first MACE was tested in Cox regression analysis as continuous variables, as well as in a Kaplan-Meier analysis with a log-rank test as a dichotomised variable according to the calculated cut-off for PRI.

A two-sided p<0.05 was defined as significant. Data were processed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA), and Graph Pad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Patients

Three-hundred consecutive patients who fulfilled the inclusion criteria were enrolled in the present study. Mean age was 62 ± 16 years and 32% were women. The majority of patients underwent non-emergent PCI due to stable angina (36%) or UA/NSTEMI.

All authors had significant input into the study, had full access to and take full responsibility for the accuracy of the data and have read and agreed to the present manuscript.

Figure 1: Platelet function measurements and clinical endpoints. A) MEA measurements, with regard to the recommended cut-off of 47 U. Due to peri-interventional use of GP-IIb/IIIa-blockers it was not possible to obtain valid measurements within clinical routine in 35% of patients. Only seven out of 16 patients with MACE had valid MEA measurements available. There were no valid measurements for patients suffering from post-procedural stent thrombosis. B) PRI, with regard to our cut-off of 60.2%. Within clinical routine it was possible to obtain valid measurements in all patients. Abbreviations: ST: stent thrombosis, CV death: cardiovascular death.

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(34%) and 30% of the patients underwent emergent PCI due to STEMI. The baseline characteristics are summarised in Table 1.

Clinical endpoints

A total of 96% completed the six months follow-up: Three patients (1%) were lost to follow-up and nine patients (3%) died before the six months follow-up visit. All patients were thoroughly interviewed after one month and after six months whether they were compliant with their prescribed therapy in order to guarantee that all events occurred during ongoing clopidogrel treatment. Patients that completed therapy with clopidogrel prior to the six months follow-up visit were censored from analysis at that specific point in time. However, the mean follow-up duration was 189 ± 68 days. Overall, 22 events, concluding three definite stent thromboses, two probable stent thromboses, five STEMI’s and 12 cardiovascular deaths occurred in 16 patients (5.3%) during follow-up.

MEA and VASP-P

The use of GP-IIb/IIIa-blockers in this unselected cohort limited MEA to only 196 valid measurements (65%). All valid measurements with regard to the recommended cut-off (37) and the clinical endpoints are depicted in Figure 1A. MEA measurements evidenced a skewed distribution with a median value of 30 (21–48U). According to the recommended cut-off of 47 U, 29% of patients were classified as HTPR phenotype. We found a moderate correlation of MEA with the obtained VASP-P values (Rs=0.587, p<0.001). Due to missing values predominantly in ACS patients, only limited analyses were calculated regarding MEA.

Concerning the VASP-P assay in this unselected cohort, we received valid measurement in all patients (100%). The results with regard to our cut-off and the clinical endpoints are outlined in Figure 1B. The median PRI was 66.5 (50.7–77.7%). Patients on chronic clopidogrel therapy of at least seven days (18.7%) had slightly better PRI values compared to those who received a LD [61.5 (47.4–73.3) vs. 68.6 (50.7–78.4)%, p=0.041]. There was no difference in PRI whether the patients received a LD of 300 or 600 mg clopidogrel [69.6 (55.7–77.9) vs. 67.1 (50.4–78.8%), p=0.759]. However, patients with STEMI had significantly higher PRI values compared to patients with stable CAD or UA/NSTEMI (Fig. 2A). Also the time from LD to PCI varied significantly within groups (p<0.001) (Fig. 2B). Chronic pre-treatment with clopidogrel was found in 32.4% of stable CAD patients, in 3.4% of STEMI patients and in 17.5% of UA/NSTEMI patients (p<0.001), respectively.

To identify factors that independently influenced HTPR phenotype according to the VASP-P assay we calculated a multivariable logistic regression model. Only time from LD to PCI (OR 0.981, p=0.003) and admission glucose (OR 1.011, p=0.015) could be confirmed as independent variables responsible for a reduced pharmacodynamic effect of clopidogrel.
The ROC-curve analysis exhibited that MEA (limited to 196 measurements) did not distinguish between patients with and without MACE at six months follow-up. The area under the ROC-curve was 0.520 [95% confidence interval (CI) 0.299–0.740; p=0.859]. Based on the rare events no cut-off could be calculated. The positive predictive value (PPV) evidenced 5.7% and the negative predictive value (NPV) was 97.1%.

In the ROC-curve analysis the VASP-P assay PRI distinguished significantly between patients with and without MACE at six months follow-up (Fig. 3). The area under the ROC-curve was 0.683 (95% CI 0.564–0.801; p=0.014). The optimal cut-off value to provide sensitivity >90% for exclusion of MACE was at 60.2% PRI, when the PRI was calculated from the median FI. This cut-off provides 93.8% sensitivity and 39.8% specificity. Using this threshold, 62% of patients were classified as HTPR phenotype. The PPV was 8.1% whereas the NPV was 99.1%.

### Table 2: Comparison of PRI.

#### A) Calculated PRI values from the median, mean and the geometric mean FI.

<table>
<thead>
<tr>
<th>Comparison of PRI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friedman test</td>
<td>p&lt;0.001</td>
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<tr>
<td>PRI (median FI) vs. PRI (mean FI)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>PRI (median FI) vs. PRI (geometric mean FI)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>PRI (mean FI) vs. PRI (geometric mean FI)</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

#### B) ROC-curve based estimations

<table>
<thead>
<tr>
<th>PRI from median FI</th>
<th>AUC</th>
<th>P-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cut-off value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.683</td>
<td>0.014</td>
<td>93.8%</td>
<td>39.8%</td>
<td>60.2%</td>
</tr>
<tr>
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<td>0.691</td>
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<td>33.1%</td>
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<tr>
<td></td>
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<td>0.011</td>
<td>93.8%</td>
<td>37%</td>
<td>56.6%</td>
</tr>
</tbody>
</table>

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**Cut-off estimation**

The ROC-curve analysis exhibited that MEA (limited to 196 measurements) did not distinguish between patients with and without MACE at six months follow-up. The area under the ROC-curve was 0.520 [95% confidence interval (CI) 0.299–0.740; p=0.859]. Based on the rare events no cut-off could be calculated. The positive predictive value (PPV) evidenced 5.7% and the negative predictive value (NPV) was 97.1%.

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To outline potential differences with regard to PRI calculation, we also calculated the PRI values based on the mean FI and on the geometric mean FI and compared them to the PRI values obtained from the median FI. The PRI from the median FI was significantly higher compared to the PRI obtained from the geometric mean FI or the mean FI [66.5 (50.7–77.7) vs. 64.6 (49.9–77%) and vs. 62.7 (47.9–75.8%), respectively; p<0.05 for both comparisons]. Furthermore, we calculated exploratory ROC-curves for all methods of calculation and compared the obtained cut-off-values at the given sensitivity of 93.8% (the cut-offs are: for PRI from median FI: 60.2%, for PRI from mean FI: 52% and for PRI from geometric mean FI: 56.6%) (Table 2A).

**Prediction of MACE**

To evaluate the predictive value of MEA with regard to MACE within the follow-up period, patients with MACE (n=7) were compared to patients without MACE (n=189). Median MEA measurements in patients with MACE were comparable to patients without [34 (21–53) vs. 30 (21–48U); p=0.859]. The risk for MACE in patients with HTPR as defined by MEA was also comparable to patients without HTPR (5.4% vs. 2.9%, p=0.670). The predictive significance of MEA as a continuous variable for MACE was tested by Cox regression analysis [HR 0.998 (95% CI 0.959–1.039), p=0.919]. Hence, MEA in this limited sample was not associated with an increased risk for MACE. The post-hoc power for MEA to detect 85% relative risk reduction in the group without HTPR was only 67%.

To evaluate the predictive value of the VASP-P assay PRI with regard to predicting MACE within the follow-up period, patients with MACE (n=16) were compared to patients without MACE (n=284). Median PRI in patients with MACE was significantly higher compared to patients without [77.5 (65.8–80.5) vs. 65.5 (49.9–77.1%); p=0.014]. The MACE rate of 8.1% in the HTPR group was significantly higher compared to 0.9% in the group without HTPR (p=0.007). The relative risk reduction was 89% in the group of patients without HTPR compared to the group of patients with HTPR. The predictive significance of PRI as a continuous variable for MACE was tested by Cox regression analysis [HR 1.044 (95% CI 1.004–1.085), p=0.030]. Accordingly, an increase of 10% in PRI was associated with a 1.5-fold increased risk for MACE. Furthermore, in a Kaplan-Meier analysis we could demonstrate that patients with a PRI above the cut-off of 60.2% had a significantly increased risk for MACE at six months follow-up (p=0.007) (Fig. 4).

**Discussion**

The most important finding of our study is that the VASP-P assay might be useful in clinical routine to detect HTPR in clopidogrel-treated patients and to predict post-procedural MACE after PCI and coronary stenting in an unselected patient cohort. The optimal cut-off for our cohort for predicting MACE was 60.2% PRI when calculated from the median FI. However, using the mean or geometric mean FIs for calculation of PRI leads to clinically relevant differences in the estimated cut-offs.

The convenience and usefulness of MEA for routine clinical use in unselected patients is questionable, because the test cannot be applied in patients treated with GP-IIb/IIIa-blockers (35% of invalid measurements in our patient cohort). Moreover, also for the remaining patients we were unable to show a significant association with MACE and no cut-off for MEA could be estimated. Due to the reduced number of patients, and therefore reduced power to detect the hypothesised effects we cannot exclude that MEA might be predictive for MACE in unselected patients undergoing coronary stenting. The group of patients with valid MEA measurements also seems to be biased towards low risk, as patients that receive GP-IIb/IIIa-blockers with a high thrombus load are per se at increased risk. Very recently another small study investigating 222 patients also reported negative results for MEA in order to predict MACE at 30 days follow-up (38). However, previous studies have shown that MEA is predictive for stent thrombosis and death in patients with planned DES implantation (9, 39). Others showed that MEA predicts stent thrombosis better than the VASP-P assay (8). Interestingly, the conclusion of the latter study was mainly based on ROC curves from three definite stent thromboses. They also ex-
cluded patients that received GP-IIIb/IIIa-blockers from their analysis as the presence of GP-IIIb/IIIa-blockers results in pronounced platelet inhibition in aggregation based platelet function tests.

Maccucci et al. used another functional point of care test (VerifyNow) and have proven the prognostic value of this test system to predict MACE at one year follow-up (15). They included patients with GP-IIIb/IIIa-blockers into their study, but measured platelet function after six days in these patients. This study and test system used is limited by the fact that early cardiovascular event rates could not be predicted.

We strictly included all consecutive patients undergoing coronary stenting to reflect a “real-life” situation, and this differs from other studies, e.g. Price et al screened 901 patients to finally recruit 380 patients (36). For our registry we have defined specific standard operating procedures: Regarding clopidogrel effects, measuring platelet function should be possible and comparable in all patients and clinical presentations. Therefore, blood sampling 6–24 h after PCI seems to be the optimal timeframe in our clinical routine. This allows us to measure the effects of clopidogrel in all patients at or close to their steady-state level of platelet inhibition. With regard to the use of GP-IIIb/IIIa-blockers one solution could be to measure MEA a few days later. However, we decided against this, as: 1) the values obtained several days after PCI are not necessarily comparable to early measurements in the same patients, which might influence the prediction of outcome; 2) a late performance of MEA might be impractical as a majority of patients already has been discharged; and 3) early MACE within the first days could not be predicted, respectively. Another solution would be to perform the test before PCI and stenting. This again is impractical because GP-IIIb/IIIa-blockers frequently are initiated before the cath-lab and/or because the LD of clopidogrel used in emergent patients immediately before the intervention might not be active. Taking these considerations and our results into account, we conclude that MEA is at least impractical to evaluate HTPR in clinical routine in unselected patients.

Measurement of PRI based on the VASP-P assay predicted post-procedural MACE after PCI and coronary stenting in our unselected patient cohort, which included also patients under GP-IIIb/IIIa-blocker therapy. The relative risk for MACE in patients without HTPR was 89% lower compared to patients with HTPR. It has previously been proven that the PRI is not influenced by GP-IIIb/IIIa-inhibitors (17) and, moreover, the VASP-P assay has previously been shown to predict MACE including stent thrombosis (11, 21, 40–42). However, knowledge of the predictive value of the VASP-P assay in high-risk STEMI patients is missing except for one recent publication which outlined that the VASP-P assay might be helpful in predicting cardiovascular death above a cut-off of PRI=61% in unselected patients (43). The high sensitivity (93.8%) as well as the low specificity (39.8%) of the VASP-P assay in our study is comparable with earlier findings (8, 11). It has recently been shown that levels of P2Y_{12}-blockade above 60% are required to block the effects of ADP on PGE{sub 1}-induced phosphorylation of VASP (44). Furthermore, a study in rats has shown that 90% P2Y_{12}-receptor occupancy led to a PRI of 50% (45). This indicates that the VASP-P assay is insensitive to low levels of P2Y_{12}-blockade (46) and might in part explain the poor specificity of the assay, which limits its value for guiding individual therapy. Small randomized controlled trials have recently shown that tailoring clopidogrel LD in non-emergent patients based on a PRI cut-off of 50% significantly improves the patients’ outcome (22, 23). Translating these results to our data, 62% of patients would require reloading or a change in thienopyridine therapy. However, the neutral results of GRAVITAS question anyhow whether routine platelet function testing remains justified (47). Further results of ongoing trials are therefore urgently needed (37).

Previous investigations have found that PRI values between 48 and 53% seemed to be optimal for the prediction of MACE including stent thrombosis (11, 21, 41). While Siller-Matula et al. showed a cut-off at PRI of only 42% (8), another study defined an optimal cut-off at 61% to distinguish between patients with or without cardiovascular death (43). Similarly, we found a cut-off of 60.2% PRI as most sensitive for predicting MACE. Of note, this is higher than a previously recommended consensus cut-off of 50% (37). In this publication the authors did not discuss the importance of strict standardisation of the VASP-P assay, which might explain these differing cut-offs: a PRI calculated from the mean FI is significantly higher than the PRI calculated from the mean FI (48) as confirmed by our data. The consensus cut-off is based on four publications, two of which used the mean FI to calculate the PRI (11, 40), one used the geometric mean FI (21) and another one used the median FI for PRI calculation (41). We are able to show for the first time (Table 2B) that such significant differences in the calculations of PRI also translate into clinically relevant differences in the respective cut-offs. When the mean FI was used to calculate PRI the optimal cut-off seemed to be around 50% (11, 23). We and others (41, 43) used the median FI for calculating PRI and found optimal cut-offs at 60.2%, 53% and 61%, respectively. Therefore, the consensus cut-off of 50% should not be applied to data that are derived from the median FI. To define a clinically relevant and comparable cut-off between trials it would be important to calculate all PRIs with the same method or at least to exactly report the methods used for calculation of PRI.

Most importantly, our aim was not to draw firm conclusions which test better predicts outcome in unselected patients. Nevertheless, in our hands the VASP-P assay – although more time consuming and not a point-of-care assay – seemed more feasible than MEA for clinical routine.

**Strengths and limitations**

One important limitation of our registry is that we did not test for response to ASA and some events might have also been linked to a poor response to ASA. Fortunately, a resistance to ASA is rare in clinical practice and might not have had important impact on our data (49). Moreover, the relative small patient number and event rate might have influenced our results.

In contrast, strengths of the present registry include an exact monitoring of intake of clopidogrel; censoring of all patients when
their antiplatelet therapy was stopped; and drawing of blood for platelet function measurements not earlier than 6 h after PCI, leaving the vast majority of patients in or close to their steady state regarding the pharmacodynamic effect of clopidogrel. Furthermore, as the enrollment was strictly consecutive, a selection bias is unlikely and the investigated patients might be considered representative for an unselected, “real-life” CAD patient cohort.

Conclusion

The results from this prospective registry indicate that the VASP-P assay is feasible for clinical routine to measure clopidogrel effects and to predict MACE in unselected “real-life” patients after PCI and coronary stenting; on the other hand the clinical usefulness is limited by its low specificity. Given the high negative predictive value, the VASP-P assay allows selection of a group of patients at low risk for MACE. So far all reported cut-offs have been derived from relatively small investigations by use of different clinical endpoints and of different methods of calculation of the PRI. Therefore, further validation of the assay in large patient cohorts and with standardised methods is recommended. The use of GP-IIIb/IIa-blockers prevents MEA testing and limits therefore its usability especially in high-risk patients. Thus, the reduced sample size for valid MEA measurements prevents reliable conclusions whether MEA is predictive for MACE in unselected patients.

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Conflict of interest

The authors have no conflict of interest with regard to the present manuscript.

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