Cardiovascular Death and Nonfatal Myocardial Infarction in Acute Coronary Syndrome Patients Receiving Coronary Stenting Are Predicted by Residual Platelet Reactivity to ADP Detected by a Point-of-Care Assay

A 12-Month Follow-Up

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Background — The clinical impact of platelet aggregation assessed by point-of-care assays is unknown. We sought to evaluate whether high residual platelet reactivity (RPR) to ADP during clopidogrel therapy, measured by a point-of-care assay, predicts adverse clinical events in acute coronary syndrome patients undergoing percutaneous coronary intervention.

Methods and Results — We used the VerifyNow P2Y12 assay (Accumetrics Inc, San Diego, Calif) to determine RPR to ADP in 683 patients with acute coronary syndrome undergoing dual-antiplatelet therapy who underwent percutaneous coronary intervention with bare-metal or drug-eluting stent implantation. All patients received a single 600-mg clopidogrel loading dose followed by 75 mg of clopidogrel daily and 100 to 325 mg of aspirin daily. The end points of the study at follow-up of 12 months were cardiovascular death, nonfatal myocardial infarction (MI), and target-vessel revascularization. At a 12-month follow-up, we found 51 ischemic events (24 cardiovascular deaths [3.5%), 27 nonfatal MIs [3.9%]) and 40 target-vessel revascularizations (5.8%). By receiver operating characteristic curve (ROC) analysis, the optimal cutoff value in predicting 12-month cardiovascular death and nonfatal MI was P2Y12 reaction unit values ≥240. RPR, defined in the presence of P2Y12 reaction unit values above this cutoff, was found to be a significant and independent predictor of cardiovascular death and nonfatal MI in a model that adjusted for cardiovascular risk factors, renal failure, reduced left ventricular ejection fraction, multivessel disease, total stent length, bifurcation lesions, number of lesions treated, type of stent, and use of glycoprotein IIb/IIIa inhibitors (cardiovascular death: hazard ratio 2.55, 95% CI 1.08 to 6.07, \( P=0.034 \); nonfatal MI: hazard ratio 3.36, 95% CI 1.49 to 7.58, \( P=0.004 \)). No significant association was found between high RPR and the risk of target-vessel revascularization.

Conclusions — RPR to ADP with clopidogrel therapy, measured by the point-of-care assay VerifyNow P2Y12, is able to detect acute coronary syndrome patients at risk of 12-month cardiovascular death and nonfatal MI. The optimal cutoff value was identified as being 240 P2Y12 reaction units.

Key Words: aspirin ■ clopidogrel ■ platelets ■ bedside testing ■ acute coronary syndrome

Dual-antiplatelet treatment with aspirin and clopidogrel in patients undergoing percutaneous coronary intervention (PCI) has dramatically reduced the rate of major adverse cardiac events.\(^1\text{-}^2\) A growing body of evidence demonstrates that an in vitro high residual platelet reactivity (RPR) in patients undergoing dual-antiplatelet treatment is associated with an increased risk of adverse cardiovascular events such as stent thrombosis and cardiovascular death.\(^3\text{-}^8\)

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Clopidogrel nonresponsiveness as assessed by light transmittance aggregometry induced by ADP 10 \( \mu \)mol/L is an independent predictor of drug-eluting stent–implantation thrombosis.\(^7\) Light transmittance aggregometry is considered to be the standard method for assessment of platelet function, but logistic problems make its routine use difficult. In recent
years, point-of-care assays of platelet function have become available, including the VerifyNow system, which provides values of RPR after ADP stimulus correlated with those found by ADP light transmittance aggregometry.9 One study based on 380 patients undergoing PCI and clopidogrel treatment has demonstrated that high ADP RPR, as measured by VerifyNow, is associated with postdischarge (6-month follow-up) adverse events after PCI with drug-eluting stents.8 The aim of the present study was to evaluate whether the VerifyNow assay is able to predict 12-month clinical recurrences in a large sample of patients with acute coronary syndrome (ACS) treated by PCI.

Methods

Study Population

Patients with ACS who underwent PCI and who had an anticipated compliance to dual-antiplatelet treatment for 12 months were considered eligible for the study. Informed written consent was obtained from all patients, and the study was approved by the local ethics review board.

PCI and Antiplatelet Management

All interventions were performed according to current standard guidelines, and the type of stent implanted and the use of glycoprotein IIb/IIIa inhibitors were at the discretion of the operator. All patients received 1 clopidogrel loading dose of 600 mg followed by a daily dose of 75 mg. All patients received unfractionated heparin 70 IU/kg during the procedure and acetylsalicylic acid 500 mg IV followed by a daily dose of 100 to 325 mg PO.

RPR Assessment

Venous blood samples anticoagulated with sodium citrate 0.109 mol/L (ratio 9:1) were taken from each patient within 24 hours after 600-mg clopidogrel loading. For patients who received both the loading dose of clopidogrel and a glycoprotein IIb/IIIa inhibitor in the catheterization laboratory, blood samples were obtained 6 days afterward, while the patient was taking the 75-mg maintenance dose of clopidogrel.

The VerifyNow system (Accumetrics, San Diego, Calif) is a turbidimetry-based optical detection device that measures platelet-induced aggregation in a system containing fibrinogen-coated beads. The instrument measures changes in light transmission and thus the rate of aggregation in whole blood. In the cartridge of the VerifyNow P2Y12 assay, there is a channel in which inhibition of the ADP P2Y12 receptor is measured. This channel contains ADP as platelet agonist and prostaglandin E1, as a suppressor of intracellular free calcium levels, to reduce the nonspecific contribution of ADP binding to P2Y1 receptors. Results are expressed as P2Y12 reaction units (PRU).

The reference interval in a sample of 98 healthy volunteers obtained in our laboratory is 244 to 382 PRU (5th to 95th percentile of control distribution, n=98). Samples from 5 control subjects and 5 coronary artery disease patients taking dual-antiplatelet therapy and the VerifyNow assay wet quality control (level 1=normal and level 2=abnormal) were assessed 4 times to determine our laboratory coefficient of variation for the assay. The mean coefficients of variation were 3.5% in control subjects, 3.2% in coronary artery disease patients, and 2.5% and 3.4% for level 1 and 2 quality controls, respectively.9

Data Collection and Follow-Up

All data were collected prospectively and entered into a central database. Clinical follow-up information was obtained by contacting all patients at 12 months, and source documents of potential events were obtained.

The end points of the study were as follows: (1) Cardiovascular death, defined as death in the presence of ACS, significant cardiac arrhythmia, or refractory congestive heart failure; 2) nonfatal myocardial infarction (MI; a rise in serum troponin I or an increase in creatine kinase-MB isoenzyme at least twice the upper normal limits with at least 1 of the following: acute onset of prolonged ≥20 minutes) typical ischemic chest pain; ST-segment elevation of at least 1 mm in 2 or more contiguous ECG leads, or ST-segment depression ≤0.5 mm in ≥2 contiguous leads; or T-wave inversion >1 mm in leads with predominant R waves; and (3) target-vessel revascularization by repeat PCI or CABG.

Statistical Analysis

Continuous variables are presented as median (range). Categorical data are reported as frequencies. Differences in continuous variables were compared by the Student t test or Mann–Whitney U test, as appropriate. Dichotomous variables were compared by χ² test or Fisher’s exact test, as appropriate.

A receiver operating characteristic curve analysis was used to determine the ability of the VerifyNow P2Y12 assay to distinguish between patients with and without postdischarge (6-month) adverse events after PCI. The optimal cutoff point was calculated by determining the posttreatment PRU that provided the greatest sum of sensitivity and specificity. Cumulative survival curves for patients with and without high RPR (RPR as defined by PRU ≥240) were constructed by the Kaplan–Meier method, and the log-rank test was used to assess statistical differences between these 2 survival curves. After assessment of the proportional hazard assumption, univariate and multivariate hazard regression models of Cox were used, respectively, to identify risk factors for clinical end points and to adjust for potential confounders that were associated with clinical end points on univariate analysis (cardiovascular risk factors, renal failure, left ventricular ejection fraction <40%, multivessel disease, total stent length, bifurcation lesions, number of lesions treated, type of stent used, and use of glycoprotein IIb/IIIa inhibitors).

A significance level was defined as P<0.05. All analysis was performed with SPSS 14.0 (SPSS Inc, Chicago, Ill). The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

From January 2005 to March 2006, 683 patients were enrolled in the present study. Baseline characteristics including RPR are shown in Table 1. PRU values were normally distributed. The mean platelet reactivity was 193.6±86.9 PRU. Table 2 shows clinical outcomes at 12 months. The 1-year follow-up rate was 100%. There were 51 adverse events: 24 cardiovascular deaths (3.5%), 27 nonfatal MIs (3.9%), and 40 target-vessel revascularizations (5.8%).

Receiver operating characteristic curve analysis demonstrated that PRU was able to distinguish between patients with and without subsequent ischemic events (namely, cardiovascular deaths and nonfatal MI) at 12-month follow-up (area under the curve 0.66, 95% CI 0.57 to 0.78, P<0.001; Figure 1). Table 3 shows that the addition of RPR to a model that included classic and procedural risk factors moderately but significantly improved the area under the curve for the detection of 12-month follow-up cardiovascular deaths and nonfatal MIs. A PRU ≥240 was identified as the optimal cutoff to predict cardiovascular death and nonfatal MI at 12-month follow-up, providing a sensitivity of 61% (95% CI 47.0% to 75.8%), a specificity of 70% (95% CI 66.4% to 73.5%), a negative predictive value of 96% (95% CI 94.6% to 98.0%), and a positive predictive value of 12% (95% CI 0.7% to 1.6%). Patients with RPR PRU above the optimal cutoff value were significantly older, more likely to be female, more
likely to be diabetic, and more likely to have reduced left ventricular ejection fraction than patients without RPR (Table 1).

The event-free survival curves for cardiovascular death and nonfatal MI according to the presence of RPR are shown in Figures 2 and 3. On univariate Cox regression analysis, RPR measured by VerifyNow (cutoff ≥240 PRU) was associated with a significantly higher risk of both cardiovascular death (hazard ratio [HR] 2.38, 95% CI 1.15 to 5.20, \( P = 0.031 \)) and nonfatal MI (HR 2.73, 95% CI 1.54 to 5.01, \( P = 0.006 \)), whereas no significant association was detected with target-lesion revascularization (HR 1.48, 95% CI 0.78 to 2.78, \( P = 0.225 \)). These results were confirmed after adjustment for cardiovascular risk factors, renal failure, reduced ejection fraction, multivessel disease, total stent length, bifurcation lesions, number of lesions treated, type of stent used, and use of glycoprotein IIb/IIIa inhibitors (cardiovascular death: HR 2.55, 95% CI 1.08 to 6.07, \( P = 0.034 \); nonfatal MI: HR 3.36, 95% CI 1.49 to 7.58, \( P = 0.004 \)).

The HR for cardiovascular death and nonfatal MI was also analyzed with respect to PRU quartiles (Figure 4). The highest quartile, which corresponded to PRU values ≥258, was associated with a significantly increased risk for ischemic recurrences (HR 3.6, 95% CI 1.5 to 9.09, \( P_{\text{for trend}} = 0.005 \)).

We also tested the association between RPR and 12-month follow-up cardiovascular events using the cutoff previously calculated in the study by Price et al* (PRU values ≥235). Two hundred thirty-one patients (33.8%) had PRU values ≥235; on univariate Cox regression analysis, PRU ≥235 was associated with an increased risk of both cardiovascular death (HR 2.37, 95% CI 1.06 to 5.30, \( P = 0.035 \)) and nonfatal MI (HR 2.94, 95% CI 1.37 to 6.34, \( P = 0.006 \)). These results were confirmed after adjustment for classic and procedural cardiovascular risk factors (HR 2.41, 95% CI 1.01 to 5.72, \( P = 0.046 \) and HR 3.12, 95% CI 1.38 to 7.02, \( P = 0.006 \), respectively, for cardiovascular death and nonfatal MI).

### Table 1. Clinical Characteristics of Patients Investigated

<table>
<thead>
<tr>
<th></th>
<th>Overall Group (n=683)</th>
<th>RPR* (n=219)</th>
<th>No RPR* (n=464)</th>
<th>( P^\dagger )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>69 (29–94)</td>
<td>73 (46–93)</td>
<td>68 (29–94)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>517 (75.6)</td>
<td>141 (64.3)</td>
<td>376 (81.0)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>178 (26.0)</td>
<td>75 (34.2)</td>
<td>103 (22.2)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>210 (30.8)</td>
<td>68 (31.1)</td>
<td>142 (30.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>460 (67.3)</td>
<td>154 (70.3)</td>
<td>306 (65.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>357 (52.3)</td>
<td>124 (56.6)</td>
<td>233 (50.2)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI &gt;25 kg/m², n (%)</td>
<td>250 (36.6)</td>
<td>87 (39.7)</td>
<td>163 (35.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
<td>132 (19.3)</td>
<td>48 (21.9)</td>
<td>84 (18.1)</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF &lt;40%, n (%)</td>
<td>174 (25.5)</td>
<td>67 (30.6)</td>
<td>107 (23.1)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Renal failure,* n (%)</td>
<td>69 (10.1)</td>
<td>15 (6.8)</td>
<td>54 (11.6)</td>
<td>NS</td>
</tr>
<tr>
<td>STEMI, n (%)</td>
<td>191 (28)</td>
<td>63 (28.7)</td>
<td>128 (27.5)</td>
<td>NS</td>
</tr>
<tr>
<td>ACE inhibitors, n (%)</td>
<td>444 (65)</td>
<td>142 (64.8)</td>
<td>302 (65.1)</td>
<td>NS</td>
</tr>
<tr>
<td>ß-blockers, n (%)</td>
<td>271 (39.6)</td>
<td>88 (40.2)</td>
<td>183 (39.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>410 (60)</td>
<td>131 (59.9)</td>
<td>279 (60.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Pump inhibitors, n (%)</td>
<td>635 (92.9)</td>
<td>201 (91.7)</td>
<td>434 (93.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Glycoprotein IIb/IIIa, n (%)</td>
<td>205 (30)</td>
<td>72 (32.9)</td>
<td>133 (28.6)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of lesions treated</td>
<td>1314</td>
<td>437</td>
<td>877</td>
<td>( \ldots )</td>
</tr>
<tr>
<td>No. of vessels treated</td>
<td>1154</td>
<td>391</td>
<td>763</td>
<td>( \ldots )</td>
</tr>
<tr>
<td>Drug-eluting stent, n (%)</td>
<td>121 (17.7)</td>
<td>52 (23.7)</td>
<td>69 (14.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Bifurcation lesion, n (%)</td>
<td>248 (36.3)</td>
<td>83 (37.9)</td>
<td>165 (35.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Total stent length, mm</td>
<td>37±26</td>
<td>39±31</td>
<td>37±29</td>
<td>NS</td>
</tr>
</tbody>
</table>

*BMI indicates body mass index; CAD, coronary artery disease; LVEF, left ventricular ejection fraction; and STEMI, ST-elevation MI.

*RPR as defined by PRU above the optimal cutoff point by receiver operating characteristic curve analysis (PRU ≥240); renal insufficiency was defined by creatinine levels ≥2.0 mg/dL.

†RPR vs no RPR.

### Table 2. Clinical Outcome at 12-Month Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Overall Group (n=683)</th>
<th>RPR* (n=219)</th>
<th>No RPR* (n=464)</th>
<th>HR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular death and nonfatal MI, n (%)</td>
<td>44 (6.4%)</td>
<td>27 (12.3)</td>
<td>17 (3.6)</td>
<td>2.52 (1.30–5.13)</td>
<td>0.011</td>
</tr>
<tr>
<td>Cardiovascular death, n (%)</td>
<td>24 (3.5)</td>
<td>13 (5.9)</td>
<td>11 (2.4)</td>
<td>2.38 (1.15–5.20)</td>
<td>0.031</td>
</tr>
<tr>
<td>Nonfatal MI, n (%)</td>
<td>27 (3.9)</td>
<td>16 (7.3)</td>
<td>11 (2.4)</td>
<td>2.73 (1.54–5.01)</td>
<td>0.006</td>
</tr>
<tr>
<td>Target-lesion revascularization, n (%)</td>
<td>40 (5.8)</td>
<td>16 (7.3)</td>
<td>24 (5.2)</td>
<td>1.48 (0.78–2.78)</td>
<td>0.225</td>
</tr>
</tbody>
</table>

*RPR as defined by PRU ≥240.
Discussion

In this prospective study of a large number of patients undergoing dual-antiplatelet therapy, we found that RPR to ADP measured by a point-of-care assay was an independent predictor of cardiovascular death and nonfatal MI at 12-month follow-up in patients with ACS who underwent PCI. The cutoff value for the identification of patients at higher risk for ischemic events was 240 PRU. This value is consistent with that revealed by the study of Price et al, based on 380 patients, and by the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study, published during the revision of this report and based on 160 patients. The high negative predictive value (96%) suggests that patients with PRU values ≤ 240 can be labeled as being at low risk of recurrences, whereas because of the low positive predictive value (12%), PRU values ≥ 240 include patients who will not experience an ischemic event.

In the present study, as well as in the study by Price et al, the predictive accuracy of the VerifyNow assay in the identification of high-risk patients was moderate (69%).

Table 3. Area Under the Receiver Operating Characteristic Curve of Different Regression Models for the Detection of Cardiovascular Death and Nonfatal MI at 12-Month Follow-Up

<table>
<thead>
<tr>
<th>Model</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: Classic</td>
<td>0.67 (0.58–0.77)</td>
</tr>
<tr>
<td>Model 2: Model 1 +</td>
<td>0.71 (0.62–0.80)</td>
</tr>
<tr>
<td>procedural risk factors†</td>
<td></td>
</tr>
<tr>
<td>Model 3: Model 2 +</td>
<td>0.79 (0.72–0.86)</td>
</tr>
<tr>
<td>residual platelet</td>
<td></td>
</tr>
<tr>
<td>reactivity</td>
<td></td>
</tr>
</tbody>
</table>

AUC indicates area under the curve.

P = 0.004, model 3 vs model 1; P = 0.021, model 3 vs model 2.

Type of stent, bifurcation lesion, total length of stent, No. of vessels treated, No. of stents implanted, use of glycoprotein IIb/IIIa inhibitors.

Figure 1. Receiver-operating characteristic curve for the VerifyNow P2Y12 assay.

Figure 2. Survival free from cardiovascular (CV) death in patients with and without PRU ≥ 240.

Figure 3. Survival free from nonfatal MI in patients with and without PRU ≥ 240.

The addition of RPR according to VerifyNow P2Y12 to the classic and procedural cardiovascular risk factors moderately but significantly enhanced the predictive ability to define the risk of recurrences. In a recent study, a higher (95%) predictive accuracy of a platelet aggregation test for ischemic events was obtained when platelet function was assessed by arachidonic acid and collagen in addition to ADP stimulation, which emphasizes that a single pathway assessment does not encompass the complexity of the platelet role in thrombotic events. Currently, a number of assays for platelet reactivity by different methods and agonists are under laboratory and clinical evaluation. Among these, a flow-cytometric vasodilator-stimulated phosphoprotein phosphorylation assay was able to detect a reduced response to clopidogrel and to successfully drive the antiplatelet therapy in 162 patients undergoing PCI.

We are aware that platelet reactivity at the time of ACS may be influenced by a number of clinical and laboratory factors.
parameters, including the high level of the inflammatory state17–19 and the increased platelet turnover.20–23 Consequently, a percentage of patients with RPR in the acute phase of disease might subsequently return to an adequate platelet inhibition level after a standard dose of clopidogrel. Therefore, the present results strengthen the evidence that an impaired and reduced inhibition of platelet function by clopidogrel in the acute phase of the disease is associated with subsequent worse clinical follow-up, which underscores the importance of optimal platelet inhibition in the acute phase of the disease. This paradigm mirrors the clinical relevance of the optimization of anticoagulation therapy in the acute phase of deep venous thrombosis to the risk of clinical recurrence, ie, the better the anticoagulation therapy in the acute phase, the lower the risk of clinical recurrence. However, the availability of a simple test for the assessment of this biological entity (persistent in vitro platelet hyperreactivity with therapy) in the panel of clinical, laboratory, and procedural risk factors may allow better risk stratification and identification of patients in whom an “aggressive” blood is likely to play a key role in making the patient a “vulnerable” patient.

In conclusion, the results of the present study indicate that platelet hyperreactivity, measured by the point-of-care assay VerifyNow P2Y12, is able to identify ACS patients at higher risk of 12-month adverse clinical events and that the cutoff value of 240 PRU can be used for clinical and intervention trials.

Sources of Funding
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Disclosures
None.

References

Figure 4. HRs for cardiovascular (CV) death and nonfatal MI by quartiles of PRU values. Q indicates quartile.
CLINICAL PERSPECTIVE

A growing body of evidence shows that an in vitro residual platelet reactivity (RPR) in patients undergoing dual-antiplatelet (aspirin plus clopidogrel) treatment is associated with an increased risk of adverse cardiovascular events in high-risk vascular patients. Light transmittance aggregometry is considered the standard method for assessment of platelet function, but logistic problems make its routine use difficult. In recent years, point-of-care assays of platelet function have become available, including the VerifyNow P2Y12 system, which provides values of RPR after ADP stimulus correlated with those found by light transmittance aggregometry induced by ADP. In 683 patients with acute coronary syndrome treated by percutaneous coronary intervention, we sought to verify whether the VerifyNow P2Y12 assay is able to predict clinical recurrences, and we found that RPR to ADP measured by a point-of-care assay is an independent predictor of cardiovascular death and nonfatal myocardial infarction at 12-month follow-up. The availability of a simple test for assessment of this biological entity (persistent in vitro platelet hyperreactivity with therapy) in the panel of clinical, laboratory, and procedural risk factors may allow for better risk stratification and identification of patients in whom an aggressive blood is likely to play a key role in making the patient a vulnerable patient.
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