Post-Treatment Platelet Reactivity Predicts Long-Term Adverse Events Better Than the Response to Clopidogrel in Patients With Non-ST-Segment Elevation Acute Coronary Syndrome

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Introduction and objectives: Poor response to antiplatelet therapy has been associated with adverse long-term outcomes. The objective of this study is to assess the relationship between response to clopidogrel and post-treatment platelet reactivity (PPR) and 1-year major adverse cardiovascular events (MACE) in patients with non-ST-segment elevation acute coronary syndrome (NSTEMI).

Methods: Patients with NSTEMI undergoing early coronary angiography were enrolled in this prospective, observational study. The VerifyNow® analyzer was used to measure clopidogrel response and PPR immediately before coronary angiography.

Results: Of the 179 patients included (97 percutaneous coronary intervention, 21 coronary artery bypass graft), 161 (90%) completed 1-year follow-up and 18 (11%) incurred MACE: 10 deaths, 6 myocardial infarctions, 2 strokes, 5 revascularizations. Lower response to clopidogrel (31±21% vs. 43±21%; P=0.049) and higher PPR (204±60 vs. 155±67 platelet reaction units [PRU]; p=0.006) were significantly associated with MACE occurrence. Multivariate analysis confirmed PPR (OR per 10-unit increase, 1.12, 95% CI, 1.01-1.24; p=0.020) as an independent predictor of MACE. A PPR cut-off value of 175 PRU was associated with an adjusted OR for 1-year MACE occurrence of 3.9 (95% CI, 1.2-15.4; p=0.024).

Conclusions: PPR predicts adverse long-term outcomes better than response to clopidogrel in patients with NSTEMI. Patients with PPR values above 175 PRU were identified as being at higher risk for adverse long-term events.

Key words: Platelet aggregation inhibitors. Acute coronary syndrome. Major adverse cardiovascular events.
METHODS

Patient Screening and Procedures

A prospective observational study was conducted, including patients with NSTEACS who underwent early diagnostic coronary angiography at our hospital. NSTEACS was defined as typical prolonged chest pain at rest (>20 min) with ST-segment changes or T-wave abnormalities in the electrocardiogram, or troponin-T levels ≥0.03 g/L. At admission, the patients received a loading dose of clopidogrel 300 mg followed by 75 mg/d and a loading dose of aspirin 250 mg followed by 100 mg/d. The main exclusion criteria were long-term oral anticoagulant therapy, contraindication or allergy to aspirin, clopidogrel, or heparin, active treatment with glycoprotein IIb-IIIa inhibitors before diagnostic coronary angiography, active bleeding, and thrombocytopenia (<100 000/mL). The study was approved by our hospital’s ethics committee and all patients gave written informed consent to participate.

Antithrombotic therapy and abciximab, in the case of PCI, were prescribed according to our hospital’s protocol in accordance with clinical practice guidelines. At discharge, patients received aspirin 100 mg/d indefinitely and clopidogrel 75 mg/d for 9 months (clopidogrel therapy at 75 mg/d was extended to 12 months in patients who received a drug-eluting stent). The patients who underwent coronary artery bypass grafting (CABG) discontinued clopidogrel at least 5 days prior to surgery.

Platelet Function Assessment

A blood sample was obtained through the arterial introducer (discarding the first 10 mL) for platelet aggregation analysis before the diagnostic coronary angiography began. The samples (2 mL) were placed in tubes with 3.2% sodium citrate (Vacuette®, Greiner Bio-One, Monroe, North Carolina, USA) and platelet aggregation was measured with the VerifyNow® analyzer (Accumetrics Inc., San Diego, California, USA) within 1 hour of sample collection. Most of these studies have been conducted with optical aggregometry, a method too labor-intensive for routine clinical practice. Rapid platelet-function analyzers, such as the VerifyNow® system (Accumetrics Inc., San Diego, California, USA),19-22 make this analysis more useful in clinical practice.

The purpose of this study is to determine the relationship between recurrence of MACE at 1 year and the response to clopidogrel and PPR results in patients with NSTEACS, using the VerifyNow® analyzer.
to the effect of thienopyridines (baseline reactivity) is measured with the first channel; the PPR remaining after inhibition of the clopidogrel-mediated P2Y12 receptor is then measured with the second channel (values expressed in PRU). Clopidogrel response is calculated as follows:

\[
[1 – (\text{PPR in PRU} / \text{baseline reactivity in PRU})]|<100
\]

**Events**

The primary endpoint of the study was 1-year MACE: death from any cause, nonfatal acute myocardial infarction, new revascularization (CABG or PCI) after readmission for NSTEACS, and ischemic stroke. Nonfatal acute myocardial infarction was defined as the appearance of ischemic symptoms lasting more than 20 minutes plus pathological Q-waves in at least 2 contiguous electrocardiographic leads or elevated creatine kinase-MB fraction (creatinine kinase-MB) and troponin-T in at least 2 measurements. Stroke was defined as a new focal neurologic deficit assessed by diagnostic computed tomography and confirmed by a neurologist. Additional endpoints were the individual components of the primary endpoint. Bleeding was classified according to the Thrombolysis In Myocardial Infarction (TIMI) classification.24 Minor bleeding was defined as clinically manifest bleeding accompanied by a decrease of 3 to 5 g/dL in hemoglobin or 9% to <15% in hematocrit. Major bleeding was defined as a >5 g/dL decrease in hemoglobin or ≥15% in hematocrit. Patients were contacted by telephone 1, 6, and 12 months after the procedure to identify those who had experienced a MACE, in which case the medical history or a report from the attending physician was reviewed.

**Statistical Methods**

The study was designed to test the hypothesis that the incidence of the primary endpoint is related to a higher PPR and/or a lower IPA. The PPR and IPA values are expressed as continuous variables and in quartiles; the discrete variables, in absolute numbers and percentages; and the continuous variables, as the mean (SD) or median (interquartile range). We assessed the differences between groups using the \( \chi^2 \) test or Fisher’s exact test for discrete variables. A linear regression analysis was performed to assess differences in the continuous variables. Event-free survival was analyzed using the Kaplan-Meier method and the differences were assessed by the log-rank test. Receiver operating characteristic (ROC) curves were used to analyze the sensitivity and specificity of the platelet function variables for detecting the incidence of MACE. The sample size was calculated from the results of the CURE and CREDO studies.1,2 Thus, if we assume a 10.5% incidence of the primary endpoint, a poor clopidogrel response of 30%,25 and a 10% loss to follow-up to detect a difference of 0.25 with a power of 80% and \( P<.05 \), then the calculated sample size should be ≥175 patients. To identify factors correlated with PPR and the percentage of IPA, univariate and multivariate analyses were performed using a general linear model with PPR and IPA as continuous variables. Multivariate logistic regression models were constructed with the primary endpoint as a dependent variable. The multivariate stepwise forward logistic regression models included all variables (demographic, clinical, and angiographic) that had shown an association with PPR, percentage of IPA, or MACE with a probability value of \( P≤.20 \) in the univariate models. A \( P \) value (2-tail) ≤.05 was considered statistically significant. The statistical analyses were performed using JMP 6 (SAS Institute, Cary, North Carolina, USA).

**RESULTS**

**Patient Characteristics, Clinical Events, and Platelet Function**

A total of 179 patients with NSTEACS were included between January 2005 and February 2006: 97 (54%) patients underwent coronary angioplasty and 21 (12%), CABG. Telephone contact was made with 161 (90%) at 1, 6, and 12 months, a period during which 18 (11%) patients experienced MACE: 10 deaths (9 of cardiovascular cause), 6 nonfatal acute myocardial infarctions, 2 strokes, and 5 new revascularizations after NSTEACS. Of the 18 patients who experienced MACE, 5 presented more than 1. Six minor and 2 major bleeding episodes were reported. The median time-to-event was 53 (range, 27-184) days. The demographic, clinical, and angiographic characteristics of the study population according to the occurrence of MACE are summarized in Table 1.

The PPR and the percentage of IPA showed normal distributions, with mean values of 157 (68) PRU and 43% (21%), respectively (Figure 1). There was no significant association between IPA and the other variables analyzed. However, higher PPR was observed in patients previously treated with clopidogrel (191 [67] vs 155 [66] PRU; \( P=.023 \)), TIMI risk score >3 (171 [69] vs 146 [64] PRU; \( P=.028 \)) and LVEF <50% (189 [73] vs 152 [63]; \( P=.01 \)).
A multivariate logistic regression analysis was performed, including all variables associated with the appearance of MACE with a $P$ value $<.20$ (Table 1). The independent predictors of MACE at 1 year were PPR (10-unit increase in PPR is associated with adjusted OR [AOR], 1.12; 95% CI, 1.01-1.24; $P=.02$) and previous platelet therapy (AOR, 4.56; 95% CI, 1.13-23; $P=.033$). IPA was not an independent predictor in this model. To determine the effect of adjusting for this variable, 2 new multivariate analysis models were constructed using the above variables: one in which PPR was excluded, in which IPA was found not to be an independent predictor, and one in which IPA was excluded, in which PPR maintained statistical significance (AOR, 1.2; 95% CI, 1-1.4; $P=.002$). None of the cases were excluded.
PPR values (Figure 2). The ROC curve (Figure 3) had a low area under the curve (0.71); when the PPR threshold was assumed to be 175 PRU, it had a sensitivity of 75% and a specificity of 64% for predicting the appearance of MACE. The positive and negative predictive values were 20% and 96%, respectively. Figure 4 shows that patients with PPR values above the 175-PRU cut-off had a lower event-free survival rate. Table 3 shows the demographic, clinical, and angiographic characteristics, separated according to values below and above the PPR cut-off of 175 PRU. Following adjustment for co-

from these models because of a lack of information on the variables studied.

The incidence of MACE was significantly higher ($P=.009$) among patients in the quartiles with higher

**Figure 1.** Normal distribution of A: post-treatment reactivity platelet (PPR) and B: inhibition of platelet aggregation (IPA). PRU indicates platelet reaction units.

**Figure 2.** Major adverse cardiovascular events (MACE) at 1 year, separated into quartiles of post-treatment platelet reactivity (PPR) measured in platelet reaction units (PRU).

**Figure 3.** Receiver operating characteristic curve (ROC): post-treatment platelet reactivity (PPR) values by discriminating against the appearance of major adverse cardiovascular events (MACE). PRU indicates platelet reaction units.
Our results show that high PPR and low IPA before diagnostic coronary angiography are significantly related to the appearance of MACE in patients with NSTEACS. In addition, the multivariate regression analysis adjusted for other variables showed that PPR predicts the appearance of MACE better than IPA.

Previous studies have related the degree of IPA and PPR to the recurrence of adverse events. Our study corroborates this relationship over a 1-year follow-up period in a high-risk population: patients with NSTEACS. In addition, the multivariate regression analysis adjusted for other variables showed that PPR predicts the appearance of MACE better than IPA.

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Previous studies have related the degree of IPA and PPR to the recurrence of adverse events. Our study corroborates this relationship over a 1-year follow-up period in a high-risk population: patients with NSTEACS and a significant rate of long-term antiplatelet therapy (39%). Various studies have shown a relationship between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term. Cuisset et al have described an association between PPR and MACE recurrence at short-term.

variables, only PPR >175 PRU (OR, 3.9; 95% CI, 1.2-15.4; \( P = 0.024 \)) and prior clopidogrel therapy (OR, 5.4; 95% CI, 1.6-18.9; \( P = 0.007 \)) were associated with the appearance of MACE at 1 year.
therefore, to greater platelet reactivity. However, the multivariate regression analysis, once adjusted for these variables, confirmed PPR as an independent predictor of MACE, as previously described. In our study, a 10-unit increase in PPR was associated with an AOR for 1-year MACE of 1.12 (95% CI, 1.01-1.24; \( P = .02 \)). The EXCELSIOR study showed that a 10% increase in platelet aggregation was associated with an AOR for 30-day MACE of 1.32 (95% CI, 1.04-1.61; \( P = .026 \)). In addition, the 1-year

### TABLE 3. Demographic, Clinical, and Angiographic Characteristics and Major Cardiovascular Adverse Events According to Post-Treatment Platelet Reactivity Above and Below the Cutoff of 175 Platelet Reaction Units

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PPR ( \leq 175 \text{ PRU (n=97)} )</th>
<th>PPR &gt;175 PRU (n=64)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>66.9 (1.5)</td>
<td>69 (1.8)</td>
<td>.216</td>
</tr>
<tr>
<td>Women</td>
<td>22 (23)</td>
<td>19 (30)</td>
<td>.361</td>
</tr>
<tr>
<td>Hypertension</td>
<td>57 (59)</td>
<td>38 (60)</td>
<td>.786</td>
</tr>
<tr>
<td>Diabetes under treatment</td>
<td>20 (21)</td>
<td>16 (25)</td>
<td>.430</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>53 (55)</td>
<td>33 (52)</td>
<td>.893</td>
</tr>
<tr>
<td>Current smoker</td>
<td>22 (23)</td>
<td>8 (13)</td>
<td>.128</td>
</tr>
<tr>
<td>Platelets, 10^9/L</td>
<td>215.2 (71.2)</td>
<td>214.4 (69.8)</td>
<td>.946</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>76 (30)</td>
<td>75 (30)</td>
<td>.804</td>
</tr>
<tr>
<td>Prior antiplatelet therapy</td>
<td>34 (35)</td>
<td>29 (45)</td>
<td>.176</td>
</tr>
<tr>
<td>Prior clopidogrel therapy</td>
<td>9 (9)</td>
<td>13 (20)</td>
<td>.028</td>
</tr>
<tr>
<td>Duration of clopidogrel treatment, h</td>
<td>67 (30)</td>
<td>75 (31)</td>
<td>.088</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>17 (19)</td>
<td>16 (27)</td>
<td>.259</td>
</tr>
<tr>
<td>History of PCI</td>
<td>11 (11)</td>
<td>9 (14)</td>
<td>.623</td>
</tr>
<tr>
<td>History of CABG</td>
<td>7 (7)</td>
<td>5 (8)</td>
<td>.902</td>
</tr>
<tr>
<td>LVEF &lt;50%</td>
<td>14 (14)</td>
<td>16 (25)</td>
<td>.099</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>71 (73)</td>
<td>45 (70)</td>
<td>.285</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>70 (72)</td>
<td>46 (72)</td>
<td>.737</td>
</tr>
<tr>
<td>Nitrates</td>
<td>60 (62)</td>
<td>41 (64)</td>
<td>.675</td>
</tr>
<tr>
<td>Statins</td>
<td>83 (86)</td>
<td>52 (81)</td>
<td>.204</td>
</tr>
<tr>
<td>Preangiography troponin-T &gt;0.1 µg/L</td>
<td>63 (65)</td>
<td>45 (70)</td>
<td>.431</td>
</tr>
<tr>
<td>TIMI risk score &gt;3</td>
<td>44 (45)</td>
<td>44 (69)</td>
<td>.012</td>
</tr>
<tr>
<td>Extension of coronary disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No significant lesions</td>
<td>19 (20)</td>
<td>7 (11)</td>
<td>.139</td>
</tr>
<tr>
<td>Left main coronary disease</td>
<td>1 (1)</td>
<td>2 (3)</td>
<td>.347</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>36 (60)</td>
<td>46 (51)</td>
<td>.284</td>
</tr>
<tr>
<td>Number of diseased vessels</td>
<td>1.5 (1)</td>
<td>1.9 (1.1)</td>
<td>.056</td>
</tr>
<tr>
<td>Postangiography treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical treatment</td>
<td>31 (32)</td>
<td>21 (33)</td>
<td>.885</td>
</tr>
<tr>
<td>CABG</td>
<td>9 (9)</td>
<td>8 (12)</td>
<td>.531</td>
</tr>
<tr>
<td>Incomplete revascularization( \dagger )</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>1</td>
</tr>
<tr>
<td>Mammary artery graft( \dagger )</td>
<td>9 (53)</td>
<td>8 (47)</td>
<td>1</td>
</tr>
<tr>
<td>PCI</td>
<td>57 (59)</td>
<td>35 (55)</td>
<td>.787</td>
</tr>
<tr>
<td>Incomplete revascularization( \dagger )</td>
<td>16 (28)</td>
<td>12 (34)</td>
<td>.640</td>
</tr>
<tr>
<td>Drug-eluting stent( \dagger )</td>
<td>31 (54)</td>
<td>16 (46)</td>
<td>.193</td>
</tr>
<tr>
<td>MACE at 1 year of follow-up</td>
<td>5 (5)</td>
<td>13 (20)</td>
<td>.003</td>
</tr>
</tbody>
</table>

Abbreviations: ACE inhibitors, angiotensin-converting enzyme inhibitors; CABG, coronary artery bypass graft; LVEF, left ventricular ejection fraction; MACE, major adverse cardiovascular events; PPR, post-treatment platelet reactivity; PRU, platelet reaction units.

*Percentage calculated as the number of events/number of patients treated by PCI or CABG.

Data are expressed as mean (SD) or n (%).

et al\( \dagger \) in a 2-year follow-up of stable diabetic patients.

Some of the earlier studies included both stable and unstable patients; however, Geisler et al\( \dagger \) have shown that patients with acute coronary syndrome present higher PPR than patients with stable angina.

In our study population, patients who experienced MACE presented poorer baseline and clinical characteristics. These factors could contribute to a proinflammatory and prothrombotic state and, therefore, to greater platelet reactivity. However, the multivariate regression analysis, once adjusted for these variables, confirmed PPR as an independent predictor of MACE, as previously described. In our study, a 10-unit increase in PPR was associated with an AOR for 1-year MACE of 1.12 (95% CI, 1.01-1.24; \( P = .02 \)). The EXCELSIOR study\( \dagger \) showed that a 10% increase in platelet aggregation was associated with an AOR for 30-day MACE of 1.32 (95% CI, 1.04-1.61; \( P = .026 \)). In addition, the 1-year
incidence of MACE was significantly higher in the quartiles with higher PPR values.

No consensus currently exists regarding the cut-off point to identify patients with poor response to clopidogrel; moreover, clopidogrel response (percentage decrease in IPA) does not take absolute pre-treatment and post-treatment platelet reactivity into account.\(^{27}\) In our study, an analysis of the ROC curve identified a PPR value of 175 PRU as the best discriminatory value for predicting MACE recurrence. In addition, a PPR >175 PRU was associated with an AOR for MACE of 3.9 (95% CI, 1.2-15.4; \(P=.024\)). Except for development of a MACE, previous clopidogrel therapy, and TIMI risk score >3, no differences were found in the demographic, clinical, or angiographic characteristics when comparing patients with PPR below and above the cut-off described. In patients who underwent coronary angioplasty with a drug-eluting stent, Price et al\(^{18}\) established a PPR cut-off of ≥235 PRU (using the VerifyNow® analyzer) to identify patients at a higher risk of experiencing adverse events at 6 months; the differences between the cut-off obtained in our study and the cut-off found by Price et al\(^{18}\) may be due to a longer antiplatelet/antithrombotic treatment period before platelet function was assessed in our group and to population differences.

Of note, the multivariate regression analysis showed that previous antiplatelet therapy is an independent predictor of MACE recurrence. Likewise, a higher number of patients with PPR above the 175-PRU cut-off were previously under clopidogrel therapy. Bliden et al\(^{16}\) reported that patients on long-term clopidogrel therapy scheduled for coronary intervention who presented higher PPR values had an increased risk of recurrence of ischemic events.

**Study Limitations**

The use of clopidogrel loading doses of 300 mg (following the recommendations approved at the start of the study) could be considered a limitation because loading doses ≥600 mg are related to higher IPA.\(^{10-12,28-30}\) However, this difference becomes less relevant because of the extended time between the clopidogrel loading dose and coronary angiography (69.4 [30.2] h), which was never less than 6 h; thus, the drug had reached stable levels in most patients. Compliance with the clopidogrel therapy prescribed may have had some influence on the outcome, particularly in medically managed patients.

Some methodological limitations should be mentioned. Because of the exploratory nature and the limited power of our study, the results should be validated in larger cohorts. Second, the inclusion of too many variables in the multivariate analyses may have led to models that hinder interpretation, and lastly, 10% of all patients included were lost to follow-up, which could affect the results obtained.

**Clinical Implications**

Platelet reactivity measurement in patients with NSTEACS is clinically relevant with regard to the appearance of MACE over the long term. Patients under previous clopidogrel treatment who experience an acute coronary syndrome are at higher thrombotic risk, and more intensive therapeutic strategies should be investigated in this group. In patients with a high PPR, different therapeutic options could reduce MACE recurrence. These options could include using higher maintenance doses of clopidogrel (150 mg/d),\(^{31,32}\) repeating the loading dose,\(^{33,34}\) or using direct thrombin inhibitors or glycoprotein IIb-IIIa inhibitors. Furthermore, new, more potent P2Y12 receptor antagonists are currently under investigation in various clinical trials. Bonello et al\(^{35}\) have recently demonstrated the safety of a clopidogrel reloading strategy until adequate IPA is reached, as well as its efficacy in reducing adverse events.

**CONCLUSIONS**

In patients with NSTEACS, the PPR predicts the recurrence of cardiovascular adverse events over the long term better than clopidogrel response. Our study population represents all “real-world” patients and the results reinforce the predictive value of PPR. Larger clinical studies are needed to determine if a decrease in PPR would result in a significant improvement of the recurrence of long-term events.

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