Usefulness of Platelet Response to Clopidogrel by Point-of-Care Testing to Predict Bleeding Outcomes in Patients Undergoing Percutaneous Coronary Intervention (from the Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty-Bleeding Study)

Giuseppe Patti, MDa, Vincenzo Pasceri, MDb, Vincenzo Vizzi, MDa, Elisabetta Ricottini, MDa, and Germano Di Sciascio, MDa,*

Platelet reactivity predicts ischemic outcomes in patients who undergo percutaneous coronary intervention (PCI), but the correlation of heightened platelet response with bleeding has not been characterized. The aim of this study was to evaluate whether low platelet reactivity by point-of-care measurement after clopidogrel administration correlates with bleeding complications of PCI. A total of 310 patients receiving clopidogrel before PCI were prospectively enrolled. Platelet reactivity was measured with the VerifyNow P2Y12 assay. The primary end point was the 30-day incidence of major bleeding or entry-site complications according to quartile distribution of P2Y12 reaction units (PRU). The primary end point occurred more frequently in patients with preprocedural PRU levels in the lowest quartile compared to those in the highest quartile (10.1% vs 1.3%, p = 0.043), due mainly to entry-site hemorrhages. Absolute PRU levels were lower in patients with major bleeding (171 ± 49 vs 227 ± 68 in patients without, p = 0.002). On multivariate analysis, pre-PCI PRU levels in the first quartile were associated with a 4.5-fold increased risk for major bleeding (odds ratio 4.5, 95% confidence interval 1.9 to 25.9, p = 0.01). By receiver-operating characteristic curve analysis, the optimal cutoff for the primary end point was a pre-PCI PRU value ≤189 (area under the curve 0.76, 95% confidence interval 0.66 to 0.87, p = 0.001). In conclusion, this study suggests that an enhanced response to clopidogrel may be associated with higher risk for early major bleeding or entry-site complications in patients who undergo PCI. Point-of-care monitoring of platelet reactivity after clopidogrel administration may help identify patients in whom individualized strategies are indicated to limit bleeding complications after coronary intervention. © 2011 Elsevier Inc. All rights reserved. (Am J Cardiol 2011;xx:xxx)

Significant interindividual variability in clopidogrel response has been demonstrated,1,2 and various investigations correlated low clopidogrel responsiveness with enhanced risk for early and late adverse cardiac events in the setting of percutaneous coronary intervention (PCI).3–5 Conversely, there is a gap of knowledge on the possible relation between higher clopidogrel responsiveness and increased bleeding risk. Because periprocedural bleeding complications strongly impair prognosis after PCI,6 assays measuring residual platelet reactivity after clopidogrel administration might help rapidly stratify patients according to their bleeding risk. The Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty (ARMYDA) study group3,7–11 has designed the ARMYDA–Bleeding Study (ARMYDA-BLEEDS) to prospectively evaluate levels of platelet reactivity by a point-of-care assay that may correlate with bleeding complications in patients who undergo coronary stent implantation.

Methods

ARMYDA-BLEEDS is a prospective study of 310 consecutive clopidogrel-treated patients who underwent PCI at Campus Bio-Medico University of Rome from April 1 to December 31, 2009. This population represents 69% of patients (310 of 449) who underwent PCI at our institution during the enrollment period. Inclusion criteria were (1) indication for percutaneous coronary revascularization for angina with inducible myocardial ischemia or non–ST-segment elevation acute coronary syndromes (ACS) and (2) clopidogrel therapy initiated before PCI, as a 600-mg load7 given ≥6 hours before intervention (n = 104) or long-term clopidogrel therapy 75 mg/day for ≥5 days (n = 206). Exclusion criteria were indication for long-term therapy with vitamin K antagonists, intervention for ST-segment elevation acute myocardial infarction <24 hours (representing most patients excluded from the study from the whole cohort of PCI patients during the enrollment period), plate-
Platelet reactivity was evaluated in the catheterization laboratory immediately before PCI and at 8 and 24 hours after intervention using the VerifyNow P2Y12 assay (Accumetrics, Inc., San Diego, California), a rapid cartridge-based assay specifically measuring the direct effects of clopidogrel on the platelet P2Y12 receptor. Technical details have been described elsewhere. Results are expressed as P2Y12 reaction units (PRU), which inversely correlate with the degree of P2Y12 receptor inhibition by clopidogrel. Twenty-five randomly selected patients were analyzed to assess intra-assay variability, which was 2.0 ± 1.1% (coefficient of variation 6%). Blood samples were also drawn before and at 8 and 24 hours in all patients to measure hemoglobin levels; further hemoglobin determinations were performed if clinically indicated. Patients were clinically evaluated after PCI for the detection of bleeding events and entry-site complications (hematoma, pseudoaneurysm, or arteriovenous fistula). One-month clinical follow-up was then obtained by office visit in all patients. Each patient gave informed consent to the study.

The primary end point of ARMYDA-BLEEDS was the 30-day incidence of major bleeding or significant entry-site complications in relation to periprocedural quartile distribution of platelet reactivity measured by PRU assay. Major
bleeding was defined according to the Thrombolysis In Myocardial Infarction criteria. Significant entry-site complications were defined as hematoma >10 cm in diameter, pseudoaneurysm, or arteriovenous fistula. Secondary end points were (1) evaluation of absolute PRU values in patients with or without major bleeding and (2) correlation of PRU values with minor bleeding or post-PCI hematoma ≤10 cm in diameter.

Continuous variables were compared using Student’s t tests for normally distributed values; otherwise, Mann-Whitney U tests were used. Proportions were compared using Fisher’s exact test when the expected frequency was <5; otherwise, chi-square tests were applied. Odds ratios and 95% confidence intervals investigating the independent predictive role of PRU quartiles on the occurrence of the primary end point were assessed by logistic regression. The following parameters were first evaluated in a univariate model: PRU quartile, age, gender, body mass index, diabetes mellitus, clinical presentation (stable angina vs ACS), chronic renal failure, hemoglobin levels, previous transient ischemic attack or stroke, previous major bleeding, use of bivalirudin versus unfractionated heparin, and use of glycoprotein IIb/IIIa inhibitors. Variables with p values ≤0.15 were then entered into the final model of multivariate logistic regression analysis. The ability of the assay to discriminate between patients with and without major bleeding at 30 days was evaluated using receiver-operating characteristic curve analysis. The optimal cut-off value was calculated by determining the PRU value providing the greatest sum of sensitivity and specificity. Results are expressed as mean ± SD. Two-tailed p values <0.05 were considered significant. Analysis was performed using SPSS version 12.0 (SPSS, Inc., Chicago, Illinois).

Results

Clinical and procedural characteristics according to pre-intervention PRU quartiles are listed in Table 1. Procedural success was achieved in 98% of patients, without need for repeat PCI or coronary artery bypass grafting at 30 days.

The overall incidence of major bleeding or entry-site complications was 4.8% (15 of 310 patients); 3 patients had gastrointestinal bleeding, 2 had bladder or urethral bleeding, and the remaining had entry-site hematomas >10 cm. Patients in the lowest PRU quartile before PCI had a higher incidence of major bleeding at 1 month (10.1%) compared to those in the highest quartile (1.3%, p = 0.043) and the third quartile (1.4%, p = 0.05) (Figure 1). Absolute PRU values before PCI were lower in patients with compared to those without major bleeding at 30 days (171 ± 49 vs 227 ± 68, p = 0.002). Periprocedural PRU values in patients with or without major bleeding are reported in Figure 2; PRU increased at 8 hours after PCI and decreased over 24 hours.

Multivariate analysis identified pre-PCI PRU level in the lowest quartile as an independent predictor of increased major bleeding risk at 30 days (odds ratio 4.5, 95% confidence interval 1.9 to 25.9, p = 0.01; Figure 3). Age >70 years and periprocedural use of glycoprotein IIb/IIIa inhibitors were also associated with a significantly higher risk for major bleeding. On multivariate analysis, there was a trend toward increased major bleeding in patients with body mass indexes <22 kg/m². Moreover, PRU values in patients in the lowest body mass index quartile tended to be lower than those of patients in the highest quartile (220 ± 68 vs 237 ± 69, p = 0.09).

Receiver-operating characteristic curve analysis showed that PRU levels significantly discriminated between patients with and without 30-day major bleeding, with an area under the curve of 0.76 (95% confidence interval 0.66 to 0.87, p = 0.001). A PRU value ≥189 was identified as the optimal cut-off point to predict 30-day bleeding outcome, with sensitivity of 87% and specificity of 70%. The incidence of major bleeding at 1 month was 11.6% in patients with pre-PCI PRU values ≥189 and 1.9% in those with PRU values >189 (p <0.001).

The occurrence of 30-day minor bleeding was significantly higher in patients with pre-PCI PRU values ≤189 (13.7%) compared to those with PRU values >189 (5.1%) (p <0.001). No relation was found between bleeding com-
applications and PRU levels measured at 8 and 24 hours after intervention (p ≥0.39), probably because entry-site bleeding complications occurred earlier, while in the hours after PCI, there was an increase of platelet reactivity due to the procedure in the groups of patients with and without major bleeding.

Discussion

This prospective study indicates that low residual platelet reactivity after clopidogrel, as measured by a point-of-care assay at the time of intervention, is associated with a significantly higher incidence of 30-day major bleeding or entry-site complications after PCI.

Residual platelet reactivity after clopidogrel administration in an index population follows a Gaussian distribution, reflecting the interindividual variability of drug response; of note, CYP2C19*17 carrier status and some drugs may promote the cytochrome activity and accelerate the rate of clopidogrel activation in the liver, thus causing an enhanced degree of platelet inhibition in response to the drug and a possible increase in bleeding risk. Various studies have focused on PCI patients with low response to clopidogrel, demonstrating a significantly higher incidence of periprocedural myocardial infarction and adverse cardiac events during follow-up. Accordingly, more aggressive antiplatelet strategies in patients with ACS, particularly those who undergo PCI, increase the degree of platelet inhibition and significantly reduce ischemic events, but at the price of higher bleeding complications (aspirin plus clopidogrel vs aspirin alone, prasugrel vs clopidogrel, ticagrelor vs clopidogrel). The prognostic role of bleeding is now largely recognized in interventional cardiology; in particular, a fourfold increase in the risk for death at 30 days was observed in patients with ACS with major bleeding and threefold higher mortality at 1 year in patients with early bleeding complications after PCI. Although ARMYDA–Platelet Reactivity Predicts Outcome (ARMYDA-PRO) and other studies have established an efficacy threshold of platelet inhibition (i.e., 240 PRU by the VerifyNow assay), the optimal safety threshold for bleeding complications has not been identified. In our study, the relation between low PRU values and bleeding complications in the multiple logistic regression model was independent of various predictors of bleeding (i.e., older age, presentation with ACS, low body mass index, renal failure, previous bleeding events, and concomitant antithrombotic therapies). In particular, a pre-PCI PRU value in the lowest quartile was associated with a 4.5-fold increased risk for postintervention major bleeding or entry-site complications compared to the highest quartile. More pronounced platelet inhibition was also associated with higher incidence of minor bleeding at 30 days. In a prospective study, the in-hospital incidence of major bleeding was 3.5-fold higher in PCI patients with enhanced clopidogrel responsiveness (defined as ≤188 aggregation units) measured before the procedure by the multiple electrode aggregometry. In contrast, in a recent investigation on patients who underwent elective PCI, several platelet function tests failed to predict postdischarge bleeding events up to 1 year.

Receiver-operating characteristic analysis in our study indicated that the VerifyNow assay can predict periprocedural bleeding outcomes, with an optimal cut-off point to discriminate patients at higher risk for 30-day major bleeding of ≤189 PRU and sensitivity of 87%. Thus, ARMYDA–BLEEDS confirms the usefulness of a rapid point-of-care assay for monitoring residual platelet reactivity after clopidogrel administration, to identify a clinically driven threshold of platelet reactivity defining patients at increased risk for bleeding complications, in whom individualized therapeutic strategies (i.e., limited use of glycoprotein IIb/IIIa inhibitors, more extensive utilization of bivalirudin, restricted use of drug-eluting stents, and more liberal use of gastroprotective agents) may be indicated. In our study, most bleeding events were large entry-site hematomas; thus, a radial approach, which has been associated with a lower incidence of vascular complications, might be also indicated in patients with higher degrees of platelet inhibition in

Figure 3. Results of multivariate analysis showing that patients with preintervention PRU levels in the lowest quartile had a significantly higher risk for 30-day major bleeding or entry-site complications (odds ratio 4.5, 95% confidence interval 1.9 to 25.9, p = 0.01). BMI = body mass index.
response to clopidogrel. ARMYDA-BLEEDS, by defining the lower threshold for bleeding in clopidogrel-treated patients (189 PRU) represents the “pendant” of the ARMYDA-PRO study,3 in which a cut-off point of PRU ≥240 was identified as a threshold associated with increased risk for major ischemic cardiac events at 30 days (odds ratio 6.1); therefore, the therapeutic range of 190 to 239 PRU could be the most favorable in the clinical setting. On the basis of the results of ARMYDA-BLEEDS and the previous ARMYDA-PRO study, the incidence of ischemic and bleeding events according to PRU values follows a curvilinear distribution (Figure 4) in which, below a certain safety threshold of PRU, ischemic events are not further reduced, to the expense of increased bleeding, and above an efficacy threshold, bleeding is not reduced, but ischemic events may be significantly increased. This curvilinear distribution was also described in a recent research correspondence by Sibbing et al30 in a population of 2,533 patients in whom multiple electrode aggregometry was used to evaluate platelet aggregation: the incidence of bleeding was highest in low responders; a threshold phe-


---

**Figure 4.** Incidence of major adverse cardiac events in the ARMYDA-PRO study and of major bleeding or entry-site complications in ARMYDA-BLEEDS according to preintervention PRU values. The flat portion of the curves may represent the “therapeutic window” for clopidogrel therapy.
The American Journal of Cardiology (www.ajconline.org)


