Rapid assessment of glycoprotein IIb/IIIa blockade with the platelet function analyzer (PFA-100) during percutaneous coronary intervention

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Background The platelet function analyzer PFA-100 (Dade Behring, Miami, Fla) evaluates platelet function by determining the time to occlusion of an aperture in a membrane coated with collagen and adenosine diphosphate or epinephrine as whole blood flows under shear stress conditions. Platelet aggregation causes aperture occlusion, and results are reported as closure time (CT). Interindividual variability is observed in the level of platelet inhibition achieved with use of the current abciximab dosing regimen (0.25-mg/kg bolus + 10-µg/min infusion for 12 hours). The relationships between specific levels of platelet inhibition and clinical efficacy and safety have not been fully established.

Methods and Results We prospectively studied platelet function in 27 patients receiving abciximab during percutaneous coronary intervention. This evaluation included determinations of platelet-rich plasma aggregometry, receptor occupancy studies (D3 assay), and CT measurements at baseline and 10 minutes, 4 hours, 12 hours, and 24 hours after the bolus. All patients received abciximab, aspirin, and heparin; patients undergoing coronary stent implantation received aspirin and ticlopidine after the procedure. CT results were reported within 10 minutes after initiation of testing. For 96% of patients, CT was 300 seconds [maximum CT] immediately after abciximab bolus and remained so throughout the infusion. At 24 hours we observed variable recovery from platelet inhibition and in 72% of patients CT returned to normal (≤130 seconds).

Conclusions Findings with the PFA-100 were similar to results observed with platelet aggregometry and receptor occupancy measurements. Most patients treated with abciximab exhibit normalized platelet function at 24 hours despite moderate levels of receptor occupancy, suggesting dissociation between occupancy and function. (Am Heart J 2001; 141:226-33.)
evaluation including platelet aggregometry and receptor occupancy studies. Patients were eligible for this study if they were men or nonpregnant women more than 18 years old, were undergoing PCI with intended concomitant abciximab use, provided written informed consent, and had received aspirin at least 12 hours before the procedure. Patients were ineligible if they had sustained a cerebrovascular accident in the last 2 years, were actively bleeding, had recent major surgery within 1 month, had a known bleeding diathesis, were intolerant of abciximab, or had leukopenia, thrombocytopenia, or impaired renal function. This study was approved by the research ethics committee at both participating centers.

**Pharmacodynamic studies**

Patients had PFA-100 measurements performed at baseline and 10 minutes, 4 hours, 12 hours, and 24 hours after the abciximab bolus. Concomitant platelet aggregometry and receptor occupancy studies were also performed at these time points. All blood samples were collected in 3.2% buffered sodium citrate by sampling from the vascular access sheath or by direct venipuncture. The physicians performing PCI were blinded to the results of the pharmacodynamic studies.

**Platelet function analyzer**

PFA-100 is an instrument and test cartridge system (Figure 1) that provides a rapid quantitative assessment of primary hemostasis in whole blood flowing under high shear conditions.7,8 The disposable test cartridge consists of a sample reservoir, capillary, and biochemically active membrane with a central 150-µm aperture (Figure 2). The membrane is coated with collagen and either adenosine diphosphate (ADP, 50 µg) or epinephrine (10 µg). The instrument provides a constant vacuum that aspirates citrated whole blood (900 µL in this study) through the capillary in the test cartridge where it contacts the membrane and aperture. Platelets adhere and aggregate until the aperture occludes, causing cessation of blood flow. The time to cessation of flow is reported as the closure time (CT); in an experimental adaptation, the total aspirate volume (in microliters) can also be reported (Figure 3).9 The actual testing process takes about 10 minutes for blood samples with fully inhibited platelets. Previous studies have shown the collagen/epinephrine cartridge to give abnormal CT results with aspirin therapy and congenital platelet dysfunction.10 The collagen/ADP cartridge has a high local concentration of ADP and is less sensitive to the presence of aspirin and short-term ticlopidine.11 Because all patients were taking aspirin, only the collagen/ADP cartridge was used for this study. Whole blood specimens were kept at room temperature and analyzed within 1 hour of sample collection. In a recent study the CT reference range for the normal population was 59 to 120 seconds (3.8% sodium citrate).9 Because the study subjects were taking aspirin often in conjunction with ticlopidine or unfractionated heparin at baseline, we calculated a baseline reference range for our PCI population (3.2% sodium citrate). By use of the 90% central interval of the CT distribution in 225 patients at baseline, a normal CT was defined as 60 to 130 seconds. In this study CT results were reported as either normal (<130 seconds), abnormal (≥130 seconds but <300 seconds), or nonclosure (=300 seconds) (Figure 3). At each time point testing was performed in duplicate and results were reported as a mean CT. If the result of a duplicate test varied by >2 times the value of the first test, a third test was performed. Furthermore, if one result was nonclosure and the other replicate was normal, a third test was also performed. If the third result was also nonclosure, then the mean CT was reported as nonclosure (300 seconds). If the third test result was normal, then the mean CT was the average of this third result and 300 seconds. In a recent study using this device, the duplicate coefficient of variation in 206 healthy adults was 12.8% and 10.2%, respectively, for the collagen/epinephrine and collagen/ADP cartridges.10

**Platelet aggregometry**

A 3-hour time limit was imposed from blood draw to completion of the aggregation studies.12 All samples were maintained at room temperature. Platelet aggregation was determined by the turbidometric method13 with 20 µmol/L ADP as the agonist. Assays were performed in platelet-rich plasma with a Bio/Data aggregometer (Bio/Data, Hatboro, Pa). Platelet aggregation was quantified as the maximum change in light transmittance occurring within 5 minutes after addition of agonist. For each sampling time, platelet aggregation was quantified as a percentage of the subject’s baseline aggregation. Percent inhibition of platelet aggregation (%IPA) was determined by the following formula:
Receptor occupancy studies

Assessment of glycoprotein IIb/IIIa receptor occupancy was determined by use of the D3 monoclonal antibody binding assay described by Jennings and White. The platelet binding of phyocerythrin-conjugated monoclonal antibody D3 (PE-D3) was compared with that of a PE-conjugated mouse IgG (PE-IgG) negative control. Because platelet-bound abciximab does not result in D3 binding, an inverse assay was used to first quantify the total number of unoccupied glycoprotein IIb/IIIa receptors in the baseline sample and at each respective time point. Eptifibatide (4 µmol/L), which causes the induction of the D3 ligand-induced binding sites, was added to each sample in vitro to express ligand-induced binding sites on unoccupied glycoprotein IIb/IIIa receptors in the baseline sample and at each respective time point. Eptifibatide (4 µmol/L), which causes the induction of the D3 ligand-induced binding sites, was added to each sample in vitro to express ligand-induced binding sites on unoccupied glycoprotein IIb/IIIa receptors in the baseline sample and at each respective time point. Eptifibatide (4 µmol/L), which causes the induction of the D3 ligand-induced binding sites, was added to each sample in vitro to express ligand-induced binding sites on unoccupied glycoprotein IIb/IIIa receptors in the baseline sample and at each respective time point. Eptifibatide (4 µmol/L), which causes the induction of the D3 ligand-induced binding sites, was added to each sample in vitro to express ligand-induced binding sites on unoccupied glycoprotein IIb/IIIa receptors in the baseline sample and at each respective time point.

To calculate receptor occupancy (%RO) by abciximab at a given time point, the receptor occupancy resulting from eptifibatide binding (in the presence of bound abciximab) was
first determined and then subtracted from the total receptor occupancy at baseline:

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\%RO_{\text{time point}} = \frac{\text{Total glycoprotein IIb/IIIa receptors}_{\text{baseline}} - \text{Unoccupied glycoprotein IIb/IIIa receptors}_{\text{time point}}}{\text{Total glycoprotein IIb/IIIa receptors}_{\text{baseline}}} \times 100%
\]

where "unoccupied glycoprotein IIb/IIIa receptors" refers to those glycoprotein IIb/IIIa receptors capable of D3 binding after the blood sample had been exposed to eptifibatide.

**Coronary intervention**

All PCI procedures were performed via the femoral approach with standard techniques. Coronary stent deployment was followed by high-pressure balloon dilations (12-20 atmospheres). All patients received aspirin 325 mg by mouth before the procedure and all patients received low-dose weight-adjusted heparin (an initial bolus of 70 U/kg at the beginning of PCI, with additional heparin as needed to attain an activated clotting time of 200-250 seconds). Abciximab was given as an intravenous bolus of 0.25 mg/kg, followed by a 12-hour infusion at 0.125 \(\mu\)g/kg/min to a maximum of 10 \(\mu\)g/min. Oral aspirin was administered daily after PCI. Patients undergoing stent implantation also received oral ticlopidine 250 mg twice daily for 4 weeks after PCI. In anticipation of stent implantation, most patients received oral ticlopidine 500 mg on the evening before PCI or on the morning of the PCI procedure.

**Definitions and end points**

The efficacy end points were angiographic success and clinical success. Assessment of clinical efficacy end points was limited to those events occurring up to hospital discharge. Angiographic success was defined as achievement of a final <50% residual stenosis after PCI (by visual assessment) with Thrombolysis In Myocardial Infarction (TIMI) grade 3 antegrade flow. Major adverse cardiovascular events included death, myocardial infarction, or urgent target vessel revascularization either by repeat PCI or bypass surgery. Death included all-cause mortality. The definition of myocardial infarction depended on the timing of the event. Within 24 hours after PCI, myocardial infarction was defined as the development of new pathologic Q waves (≥40 ms) in two or more contiguous leads or elevation of serum creatine kinase MB isoenzyme to more than twice the upper limit of normal. After 24 hours after PCI, myocardial infarction was defined by the development of Q waves (as defined above) or elevation of the MB isoenzyme to more than twice the upper limit of normal. Cardiac enzymes were obtained at baseline and 8, 16, and 24 hours after PCI. Urgent coronary bypass surgery and urgent PCI were defined as unanticipated procedures performed before hospital discharge for the management of abrupt closure or recurrent ischemia. The primary safety end points were intracranial hemorrhage or stroke, major bleeding requiring red blood cell transfusion, vascular access site complications including pseudoaneurysm, arteriovenous fistula, retroperitoneal bleeding, and the development of thrombocytopenia <100 \(\times\) 10⁹/L. Platelet counts were monitored at 2 to 4 hours and 24 hours after the PCI procedure.

**Statistical analysis**

Baseline characteristics and clinical events are reported as frequencies, and percentages, or medians with 25th and 75th percentiles when appropriate. The pharmacodynamic profile of abciximab is presented as a graphic display of median levels of platelet inhibition and median levels of receptor occu-
Results

Baseline clinical characteristics, procedural data, and clinical outcomes are outlined in Tables I and II. The effect of abciximab therapy on platelet function as assessed by CT measurements, aggregometry, and receptor occupancy is shown in Figure 4.

PFA-100 measurements

The median time from sample collection to result was 28 minutes and most test results were reported within 10 minutes of initiation of testing. At each time point the maximum number of correctly obtained sam-
samples are presented. Two patients were discharged from the hospital before 24-hour samples could be obtained, leaving 25 remaining patients for pharmacodynamic analysis. Regarding the 12-hour time point, one patient had premature termination of abciximab infusion at 9 hours and incorrect sampling or recording of sampling times occurred in another 3 patients. In 24 of 25 patients (96%), PFA-100 detected maximal platelet inhibition (nonclosure) immediately after administration of the abciximab bolus. Nonclosure was maintained in the majority of patients throughout the abciximab infusion, as reflected by CT measurements at 4 hours (25/25 patients) and 12 hours (18/21 patients), respectively. At 24 hours we observed variable degrees of recovery from platelet inhibition: in 18 of 25 patients (72%) platelet function had already normalized (mean CT <130 seconds) and in 4 of 25 patients (16%) we observed persistent nonclosure (Figure 4, A and B).

**Platelet aggregometry and receptor occupancy studies**

Maximum inhibition of platelet aggregation and glycoprotein IIb/IIIa receptor occupancy was observed at the earliest time point (10 minutes after abciximab bolus) (Figure 4, C). At this time point, the median inhibition of platelet aggregation for all patients was 92% (range: 41%-99%) and the median receptor occupancy for all patients was 76% (range: 30%-89%), respectively. During the abciximab infusion a steady-state level of platelet inhibition was observed corresponding to roughly 80% median inhibition of platelet aggregation and 70% receptor occupancy. The median inhibition of platelet aggregation and median receptor occupancy both declined to 42% and 58% at 24 hours, respectively.

In comparing PFA-100 with the traditional methods, PFA-100 qualitatively documented information similar to that of aggregometry and receptor occupancy studies. Profound inhibition of platelet function after abciximab administration was followed by gradual recovery from platelet inhibition. Because this study used a fixed dose of abciximab, correlation of PFA-100 measurements with receptor occupancy and aggregometry over increasing doses of abciximab was not possible. We attempted to correlate %IPA with percent maximum CT at the time points 10 minutes, 4 hours, 12 hours, and 24 hours, where the percent maximum CT = ([Mean CT_{time point} – Mean CT_{baseline} × 100)/CT_{baseline}]. Aggregometry measurements and CT were not correlated at 4 hours and 12 hours (r = 0). At these time points, nonclosure was detected in most patients (CT ≥300 seconds). The correlation coefficient was 0.82 and 0.60 at 10 minutes and 24 hours, respectively.

**Clinical outcomes**

Angiographic success was achieved in all 34 lesions attempted. Overall clinical event rates were low. Post-procedural myocardial infarction occurred in one patient. Despite having received the standard abciximab bolus and infusion, this subject failed to achieve significant levels of platelet inhibition immediately after the abciximab bolus (mean CT 137 seconds). This subject did achieve nonclosure by the 4-hour and 12-hour time points, with recovery of platelet function detected at 24 hours (mean CT 78 seconds). Platelet aggregometry and receptor occupancy studies confirmed the apparent lack of adequate initial platelet inhibition in this individual (at 10 minutes after abciximab bolus percent inhibition of platelet aggregation and percent receptor occupancy were 41% and 30%, respectively).

**Discussion**

PFA-100 provides a rapid assessment of platelet function and therefore its results may be useful in the catheterization laboratory or other acute care settings to quickly evaluate the effect of glycoprotein IIb/IIIa inhibitors or other antiplatelet therapies. The instrument identified significant platelet inhibition after an abciximab bolus in the majority of patients studied. This inhibition of platelet function was followed by nonuniform variable recovery from platelet inhibition during and after termination of the abciximab infusion. These findings are consistent with observations from platelet aggregometry and receptor blockade in this study and earlier pharmacodynamic evaluations of abciximab therapy.6,15-17 This variability among individuals in inhibition of platelet aggregation and recovery from platelet inhibition may be related to differences in platelet number, glycoprotein IIb/IIIa receptor density, or affinity of receptors for abciximab. Despite the presence of bound abciximab molecules on the platelets (median receptor occupancy levels of 58%) at the 24-hour time point, the majority of patients (72%) had normal platelet function as assessed by PFA-100. This apparent dissociation between receptor occupancy and platelet function is consistent with previous observations with bleeding time or aggregometry.6,15-17

Qualitatively, PFA-100 provides a similar assessment of platelet inhibition as aggregometry, although from a quantitative perspective the degree of inhibition as measured by each assay was somewhat different, especially at the 24-hour time point. This finding may be due to the related but dissimilar aspects of platelet function that are measured by the respective assays. PFA-100 primarily assesses the initial binding of von Willebrand factor by glycoprotein Ib (platelet adhesion) followed by glycoprotein IIb/IIIa-dependent binding of von Willebrand factor, and possibly collagen, under high shear stress conditions. Aggregometry chiefly measures fibrinogen-mediated platelet aggregation, which is a low shear process that is mostly independent of von Willebrand factor.18

The recommended dosing regimen for abciximab
observations from animal models. The selection of >80% median level of receptor occupancy with suppression of median platelet aggregation to <20% of baseline as a therapeutic target for platelet blockade with suppression of median platelet aggregation with abciximab bolus and infusion, interindividual variability has been observed, with some patients clearly not achieving this level of inhibition.

Clinical studies in PCI have not directly linked the actual level of platelet inhibition to clinical outcomes. In our series the observation of myocardial infarction in one subject who did not achieve adequate platelet inhibition during PCI raises an interesting question of whether the level of platelet inhibition is in fact predictive of future clinical events. Steinshubl et al. reported similar observations in 97 PCI patients evaluated with another whole blood assay. In their study, 13 of 97 patients (13%) did not achieve >80% inhibition of platelet aggregation at 8 hours after abciximab bolus and infusion. Roughly half of these patients (6/13) had a myocardial infarction (defined as a creatine kinase >2 times normal with positive MB fraction). Although these early observations are insufficient to draw firm conclusions, subsequent analyses of our larger cohort and other ongoing investigations may better assess the relationship between platelet inhibition and adverse clinical events.

We did not observe >80% receptor occupancy in all patients with the standard dosing regimen of abciximab as was reported by the radiometric method of Coller. This may reflect differences between the assays used to assess glycoprotein IIb/IIIa blockade (direct or indirect occupancy assessment). Other potential variables such as the anticoagulant source, variation in individual platelet counts over the course of the infusion, or the use of weight-adjusted abciximab in our study may explain these discrepancies. Furthermore, we studied an acute coronary syndrome population undergoing PCI; such patients may exhibit heightened platelet activation with a greater glycoprotein IIb/IIIa receptor pool requiring blockade.

Platelet function assays including aggregometry and receptor occupancy studies have been used as research tools thus far and have not been easily integrated into patient care algorithms. These techniques are not widely available, can be time consuming, require extensive standardization or specialized equipment, and often require a carefully trained technician to perform these procedures. PFA-100 testing has several advantages over traditional methods. The testing technique is easy to learn, uses whole blood, and does not require a highly skilled technician to operate the instrument. Blood can be collected in routinely used Vacutainer blood-collecting tubes (Becton Dickinson, Rutherford, NJ) and kept at room temperature for up to 4 hours before testing, and disposable test cartridges are used. The results are available to the operator in real-time (10 minutes to obtain a test result versus 2-3 hours for platelet aggregometry) and testing may be less costly than traditional methods (estimated costs $8-$12/sample [1 test cartridge, Dade Behring price list] versus $100-$200/sample for platelet aggregometry).

Currently several other functional assays are in various stages of development for point-of-care monitoring of glycoprotein IIb/IIIa inhibitor therapy. These include devices using impedance aggregometry, thromboelastography, shear-induced platelet deposition, or turbidometric fibrinogen-coated bead agglutination. The PFA-100 is a high-shear system that attempts to mimic the environment of a microcapillary in the human body. Currently PFA-100 is used to screen for von Willebrand disease and other congenital platelet disorders, and the 2 different test cartridges can be used to study aspirin effect (prolonged CT with collagen/epinephrine cartridge but normal CT with collagen/ADP cartridge). In the catheterization laboratory, it is anticipated that platelet function assays would provide complementary information to activated clotting time measurements. Such measurements are currently used in many laboratories to titrate concomitant heparin therapy when a glycoprotein IIb/IIIa inhibitor is administered. Specifically, PFA-100 may be helpful to qualitatively monitor selected cases of glycoprotein IIb/IIIa inhibition. For example, in patients with extensive thrombus formation during PCI or bleeding complications, platelet inhibition could be quickly assessed and possibly adjusted; in patients requiring very early readministration of glycoprotein IIb/IIIa inhibitors, subsequent dosing could be tailored to an individual patient’s requirements; in the management of acute myocardial infarction, platelet inhibition during rescue or direct-infarct angioplasty could be monitored.

Study limitations

Because this instrument confines detection of closure to a 300-second window, and because the majority of
patients exhibit nonclosure shortly after abciximab bolus and throughout the infusion, the device may not be sensitive enough to distinguish between those individuals at a higher risk for thrombosis or bleeding. Nonetheless, these early findings suggest that the PFA-100 device may be valuable for qualitatively evaluating platelet function as normal, abnormal, or nonclosure. It appears that an abnormal reading and nonclosure may be desirable states in which to perform PCI with glycoprotein IIb/IIIa inhibition.

In conclusion, the qualitative assessment of glycoprotein IIb/IIIa inhibition by PFA-100 during PCI provided results similar to those observed with platelet aggregometry and receptor occupancy. However, most patients treated with abciximab exhibit normalized platelet function at 24 hours despite moderate levels of receptor occupancy, suggesting possible dissociation between occupancy and function. Larger studies will be needed to determine the relationship between platelet function and clinical outcomes and to delineate the role of PFA-100 in the individualized dosing of glycoprotein IIb/IIIa inhibitors in the interventional catheterization laboratory.

References

Appendix

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