Attainment and Maintenance of Platelet Inhibition Through Standard Dosing of Abciximab in Diabetic and Nondiabetic Patients Undergoing Percutaneous Coronary Intervention

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Background—Although the effectiveness of abciximab (c7E3 Fab; ReoPro) in large populations of patients undergoing a percutaneous coronary intervention has been consistently proved in clinical trials, it is unknown whether all patients achieve and maintain target inhibition during treatment. Diabetic patients in particular are a subgroup of patients with known underlying platelet abnormalities whose long-term response to abciximab has been shown to vary from that of nondiabetic patients.

Methods and Results—Forty-nine diabetic and 51 nondiabetic patients who received adjunctive abciximab therapy during percutaneous coronary interventions were evaluated prospectively. The degree of platelet function inhibition was determined immediately after the abciximab bolus, 8 hours after the bolus (during the 12-hour abciximab infusion), and the next morning (13 to 26 hours after the bolus) with the use of a rapid platelet function assay (Accumetrics). After the abciximab bolus, platelet function was inhibited by 95±4% (mean±SD). By 8 hours, the average percent inhibition had decreased to 88±9%, with 13% of patients with <80% inhibition. The next morning (mean 19 hours after the bolus), mean inhibition was 71±14%. A difference was not found between diabetics and nondiabetics, nor was any physiological parameter found to be predictive of the response to abciximab.

Conclusions—Although the majority of patients achieve and maintain ≥80% platelet inhibition during the 12-hour infusion with standard-dose abciximab, there is substantial variability among patients. Diabetic status does not appear to influence this variability. (Circulation. 1999;100:1977-1982.)

Key Words: platelets ■ diabetes mellitus ■ abciximab ■ angioplasty

The platelet glycoprotein (GP) IIb/IIIa (αIIbβ3) is a platelet-specific integrin that mediates the final common pathway of platelet aggregation stimulated by physiological agonists. Blockade of this receptor is capable of preventing platelet aggregation and, therefore, intracoronary thrombus formation. Abciximab (c7E3 Fab; ReoPro), a chimeric human-murine monoclonal antibody Fab fragment, and a number of peptide and nonpeptide (small-molecule) antagonists of this receptor have been developed and extensively tested in clinical trials.

Early studies in animal models found that blockade of >80% of the platelet GP IIb/IIIa receptors, corresponding to ≥80% inhibition of platelet aggregation induced by 20 μmol/L ADP, was necessary to prevent thrombus formation in the setting of a severe thrombogenic stimulus.1 Subsequent pharmacodynamic studies confirmed that this level of receptor blockade in humans also corresponded to a reduction in platelet aggregation to <20% of baseline.2 The clinical importance of achieving and sustaining this level of receptor blockade and suppression of aggregation was demonstrated with the time course of repeat, urgent revascularizations after percutaneous coronary intervention (PCI) in the EPIC (Evaluation of 7E3 for the Prevention of Ischemic Complications) trial.3 Compared with placebo, in which patients began requiring urgent interventions immediately after initial PTCA, patients receiving a bolus of abciximab alone were nearly completely protected for the first 4 to 6 hours, during which maintenance of ≥80% of both receptor blockade and reduction in aggregation would be expected.

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*Dr Coller is an inventor of the RPFA and contributed to the scientific review of the manuscript, but he did not participate in obtaining the patient data or statistical analysis of the data. Dr Coller is a paid consultant to Accumetrics, and in compliance with federal law and the patent policy of Mount Sinai School of Medicine, he also shares in the royalties paid to Mount Sinai School of Medicine by Accumetrics. Dr Coller is also an inventor of abciximab, and in compliance with federal law and patent policy of the Research Foundation of the State University of New York, he shares in the royalties paid to the foundation based on sales of abciximab.

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However, in patients treated with a bolus and a 12-hour infusion of abciximab, protection from urgent revascularization extended throughout almost the entire infusion period, during which receptor blockade was more likely to be maintained at the level achieved with the bolus.

Despite the consistent clinical benefit achieved in populations of patients treated with abciximab, it is possible that specific patients or subgroups of patients may not derive the same degree of benefit. This could be due to underlying differences in platelet structure or function that might influence the achievement or maintenance of adequate platelet inhibition with standard abciximab dosing. Diabetics are a particularly important subgroup of patients whose platelets have been shown to differ physiologically from those of nondiabetics. Interestingly, in both the EPIDOG (Evaluation in PTCA to Improve Long Term Outcome with Abciximab GPIIb/IIIa Blockade) and EPISTENT (Evaluation of Platelet IIb/IIIa Inhibitor for Stenting) trials, the revascularization rate at 6 months among abciximab-treated patients was significantly influenced by the diabetic status of the patients.

The ability to characterize the response of an individual patient or a subgroup of patients to GP IIb/IIIa inhibition has been limited by currently available methods of platelet function determination. Recently, a simple and rapid platelet function assay (RPFA) that is sensitive to GP IIb/IIIa receptor blockade was developed and refined into an automated version (Accumetrics) that correlated well with traditional hematological parameters and confirmed through chart review.

Infarction (within 1 week), was defined by the attending physician for unpaired data was used to compare mean values among various subgroups. Linear regression models were used to assess the relationships of the hematological parameters with the baseline RPFA range and the change in percent inhibition at selected time points. The coefficient of determination ($r^2$) and the $P$ value were reported for each of the linear models. $P<0.05$ was considered statistically significant.

### Results

A total of 100 patients were entered into the study (49 were diabetic and 51 were nondiabetic). Three patients (2 diabetics and 1 nondiabetic) were evaluated but excluded from the primary analysis due to deviations from the standard abciximab bolus-and-infusion regimen. The nondiabetic patient received one-half dose of the standard bolus (0.125 mg/kg) followed by the standard infusion, and the other 2 patients had their abciximab infusions stopped in the catheterization laboratory due to a procedural complication (n=1) and the inability to cross the planned lesion with a guidewire (n=1).

All patients were being treated with long-term aspirin therapy before the procedure. Three patients received ticlopidine as a 500-mg loading dose within 6 hours before the procedure. Along with radiolabeled binding assays ($r=0.95$ and GP IIb/IIIa receptor blockade as judged with radiolabeled binding assays ($r=0.96$).

We sought to determine the level of platelet inhibition achieved with a standard bolus and an infusion of abciximab in patients undergoing PCI at 3 time points and to evaluate whether any patient characteristics, in particular, diabetic status, affected this response.

### Methods

The study protocol was approved by the institutional review board of the Cleveland Clinic Foundation. Diabetic status was determined before the procedure. Three patients received ticlopidine as a 500-mg loading dose within 6 hours before the procedure. Along
with abciximab, all patients also received a weight-adjusted heparin bolus before the start of the intervention. The mean peak ACT achieved for all patients was 312 seconds. There was no significant difference between the mean peak ACTs of the diabetic and nondiabetic patients (317 versus 306 seconds, respectively; \( P = 0.37 \)). No patients received a heparin infusion or began to receive warfarin after the procedure. All patients continued on a regimen of 325 mg/d noncoated aspirin while in the hospital. In addition to the 3 patients who started to receive ticlopidine before the procedure, an additional 62 patients began to receive ticlopidine as a 500-mg loading dose after receiving a heparin bolus before the start of the intervention. The mean peak ACT achieved for all patients was 312 seconds. There was no significant difference between the mean peak ACTs of the diabetic and nondiabetic patients (317 versus 306 seconds, \( P = 0.37 \)).

Demographic data for all patients are shown in Table 1. Nondiabetic patients were on average heavier, more often male, and more likely to be current smokers. Baseline hematological parameters are shown in Table 2. As has been reported by other investigators, the MPVs of diabetic patients were significantly greater than those of nondiabetic patients. The mean PC, mean platelet mass (PC×MPV), mean platelet density [PC/(1–hematocrit (HCT)), and mean platelet mass density (PC×MPV)/(1–HCT)] were not significantly different in diabetic and nondiabetic patients. The mean hemoglobin A1C of the diabetic cohort was 7.4% (normal 4.0% to 6.0%).

A significant correlation (\( r^2 = 0.58, \) \( P = 0.0001 \)) was found between HCT and the baseline RPFA slopes for the entire group (regression line \( y = 1670 – 31x \)), presumably reflecting the optical effect of the erythrocytes. Baseline platelet function as determined with the pretreatment slope of the RPFA did not differ significantly between diabetic and nondiabetic patients.

### Table 1. Patient Demographic Data

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>11 (21)</td>
<td>16 (33)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>13 (25)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Average weight, kg</td>
<td>98.1</td>
<td>87.7</td>
</tr>
<tr>
<td>Procedure indication, n (%)</td>
<td>11 (21)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Stable angina</td>
<td>30 (59)</td>
<td>27 (56)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>10 (20)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Aspirin use, n (%)</td>
<td>51 (100)</td>
<td>49 (100)</td>
</tr>
<tr>
<td>Ticlopidine preprocedure, n (%)</td>
<td>0 (0)</td>
<td>3 (6)</td>
</tr>
</tbody>
</table>

### Table 2. Baseline Hematological Data

<table>
<thead>
<tr>
<th></th>
<th>Range for All Patients</th>
<th>Nondiabetic (Mean±SD)</th>
<th>Diabetic (Mean±SD)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT</td>
<td>22.7–47.8</td>
<td>38.1±4.0</td>
<td>36.7±4.9</td>
<td>0.11</td>
</tr>
<tr>
<td>MPV</td>
<td>8.1–12.4</td>
<td>9.84±0.90</td>
<td>10.22±0.85</td>
<td>0.029</td>
</tr>
<tr>
<td>PC×1000</td>
<td>99–374</td>
<td>217±57.2</td>
<td>212±67.0</td>
<td>0.683</td>
</tr>
<tr>
<td>Platelet mass (PC×MPV)</td>
<td>1079–3346</td>
<td>2108±495</td>
<td>2142±614</td>
<td>0.756</td>
</tr>
<tr>
<td>Platelet density (PC/(1–HCT))</td>
<td>160–592</td>
<td>352.0±99.5</td>
<td>336.5±108.0</td>
<td>0.448</td>
</tr>
<tr>
<td>Platelet mass density (PC×MPV)/(1–HCT))</td>
<td>1736–6107</td>
<td>3427±872.1</td>
<td>3403±1005</td>
<td>0.897</td>
</tr>
</tbody>
</table>

Figure 1. Scattergram of percent platelet function inhibition at various time points in 97 patients (291 data points). Dashed horizontal line demarcates 80% inhibition. Dotted vertical line indicates end of 12-hour infusion.

(506±168 versus 460±153 mV/10 sec, \( P = 0.144 \)) and between smokers and nonsmokers (442±149 versus 501±189 mV/10 sec, \( P = 0.498 \)). Female gender was associated with a significantly greater baseline slope (female 634±186 mV/10 sec, male 439±153 mV/10 sec, \( P = 0.002 \)), but this difference could be explained on the basis of differences in HCT between men (39±4) and women (35±5).

For the entire 97-patient cohort, the percent inhibition immediately after abciximab bolus was 95±4%, with all except 1 patient achieving >80% inhibition. At 8 hours after the bolus, during the 12-hour abciximab infusion, mean inhibition of platelet function was 88±9% compared with baseline, but 13% (13 of 97) of patients demonstrated <80% inhibition (Figure 1). By the next morning (13 to 26 hours, mean 19 hours after bolus), the mean percent inhibition was 71±14%, with 29% (28 of 97) of patients still having >80% inhibition of platelet function. As shown in Figure 2, individual levels of inhibition were quite variable and not related to the time elapsed after completion of the abciximab infusion. Diabetic status did not influence the degree of platelet inhibition at the 3 time points (Figure 3), and diabetic and nondiabetic patients had a similar distribution in the change in platelet function per hour over the course of the study (Figure 4). Subgroup analysis that evaluated the influence of gender, cigarette smoking, and clinical indication also did not demonstrate a correlation with platelet inhibition (Table 3). The patient’s weight was not associated with the level of...
inhibition at any of the time points. In addition, the baseline HCT did not correlate with the percentage inhibition of RPFA slope after the bolus ($r^2 = 0.01$), at 8 hours ($r^2 = 0.11$), or the next morning ($r^2 = 0.04$).

Concomitant ticlopidine therapy did not influence the level of measured platelet inhibition. Ticlopidine-treated patients (n=65) were compared with those not receiving ticlopidine (n=33). The mean±SD percent inhibition after the bolus, at 8 hours, and at 24 hours was 95±5% versus 95±3%, 87±9% versus 86±13%, and 69±14% versus 70±17%, respectively ($P=NS$ for all). Similarly, no difference was found when the rate of change in the percent inhibition from the 8-hour to the 24-hour sampling points was compared between these patient groups (1.8±1.1%/h versus 1.6±0.6%/h, $P=0.214$).

Because the correlation between the baseline RPFA slope and HCT was not recognized before this study, a postprocedural HCT was not routinely obtained as part of the study protocol. However, the HCT was determined in 59% of the study patient population on the morning after the procedure at the discretion of the attending physician. These data were used to determine the influence of a decrease in HCT on the change in the percent inhibition during the infusion (after bolus to 8 hours) and the change from the 8-hour sample until the morning-after sample. Importantly, a change in HCT could not account for the change in platelet inhibition during the infusion ($r^2=0.02$) or from 8 hours into the infusion until the next morning ($r^2=0.063$).

Potential interactions between platelet number and volume with both the baseline RPFA slope and percentage inhibition in RPFA slope were also investigated. Nonsignificant correlation trends of baseline slope were observed with platelet mass ($r^2=0.028$, $P=0.10$) and PC ($r^2=0.02$, $P=0.15$), but there was no suggestion of correlation with MPV ($r^2<0.001$, $P=0.97$), platelet density ($r^2=0.001$, $P=0.74$), or platelet mass density ($r^2=0.001$, $P=0.72$). No correlation was found between these platelet parameters and the degree of platelet inhibition found after bolus, at 8 hours, and the next morning.

Although the study was not designed to evaluate clinical outcomes, in-hospital adverse cardiac events, with systematic monitoring of postprocedural myocardial enzymes, are routinely obtained for all patients undergoing PCIs at the Cleveland Clinic. Complete data were available for 88 of the 97 patients receiving a complete 12-hour infusion. In the study cohort, there were no deaths or Q wave myocardial infarctions. Twelve patients had a non-Q wave myocardial infarction (creatinine kinase >2× normal with positive MB fraction); 1 patient also required urgent CABG. Interestingly, of the 13 patients whose RPFA values at 8 hours were inhibited by <80%, 6 (46%) had an adverse cardiac event, whereas only 5 of 75 (7%) patients with ≥80% inhibition at 8 hours had similar events ($P<0.001$).

The patient treated with one half of the normal abciximab bolus dose due to a borderline-low PC (103,000/μL) demonstrated 76% inhibition of the RPFA immediately after the bolus, 49% inhibition at 8 hours during a standard-dose infusion, and 40% inhibition at 20 hours. Both patients who received standard bolus doses but had their infusions terminated within 1 hour had 60% inhibition at 8 hours and <30% inhibition 22 hours later.

**Discussion**

In the present study, we used a new assay of platelet function, the RPFA, to serially test platelet GP IIb/IIIa receptor blockade after treatment with abciximab. The RPFA has
several advantages over turbidimetric aggregometry, including (1) the use of whole blood, thus avoiding the need for sample preparation and eliminating variables in sample preparation; (2) semiautomated format, which avoids operator errors and subjective end point assessments; (3) rapid test completion; (4) digital readout; and (5) duplicate analysis to minimize random errors. Experience with this assay, however, is not as extensive as is that with conventional turbidimetric aggregometry. Studies involving the simultaneous separation; (2) semiautomated format, which avoids operator errors and subjective end point assessments; (3) rapid test completion; (4) digital readout; and (5) duplicate analysis to minimize random errors. Experience with this assay, however, is not as extensive as is that with conventional turbidimetric aggregometry. Studies involving the simultaneous measurement of platelet function measured with RPFA and turbidimetric platelet aggregometry induced with 20 μmol/L ADP of samples treated with increasing doses of abciximab demonstrated a close correlation between the results ($r^2=0.95$), as well as between the RPFA results and the percentage of unblocked GP IIb/IIIa receptors assessed directly with radiolabeled monoclonal antibody binding ($r^2=0.96$). Similarly, the mean difference in measurements between RPFA and aggregometry was only $-4\%$ ($\pm 4\%$ SD), and the mean difference in measurements between RPFA and free GP IIb/IIIa receptors was $-2\%$ ($\pm 6\%$ SD). The RPFA uses a novel platelet agonist, iso-TRAP, in which a thrombin receptor–activating peptide in which an amino-terminal iso-sine is substituted for serine. The dose of iso-TRAP used in the RPFA was chosen based on the similarity of results obtained with the RPFA compared with turbidimetric aggregation induced with 20 μmol/L ADP, a concentration that has previously been used to assess platelet function in patients treated with abciximab. Thus, although the RPFA differs from turbidimetric platelet aggregometry, we believe that it fundamentally reflects the same function. It is important to emphasize, however, that previous animal and human studies of GP IIb/IIIa antagonists used GP IIb/IIIa receptor blockade as measured with a radioimmunometric assay as the primary correlate with efficacy, with turbidimetric aggregometry a secondary correlate. As noted, the RPFA correlates well with both of these assays. The similarity in results in this study with the use of RPFA and in other studies with the use of receptor blockade and turbidimetric aggregometry lends further support for the high correlation between these assays.

### Table 3. Subgroup Analysis of Degree of Inhibition Achieved at 3 Time Points After Start of Abciximab

<table>
<thead>
<tr>
<th>Sex</th>
<th>Immediately After Bolus</th>
<th>8 h After Bolus</th>
<th>Morning After Procedure (Mean$\pm$19 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>95±5</td>
<td>87±10</td>
<td>71±14</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>95±4</td>
<td>86±13</td>
<td>68±17</td>
</tr>
<tr>
<td><strong>Clinical indication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable angina</td>
<td>95±4</td>
<td>88±11</td>
<td>75±16</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>94±5</td>
<td>87±11</td>
<td>69±15</td>
</tr>
<tr>
<td>Recent myocardial infarction</td>
<td>97±2</td>
<td>85±11</td>
<td>67±14</td>
</tr>
<tr>
<td><strong>Tobacco use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>95±5</td>
<td>85±8</td>
<td>69±11</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>95±4</td>
<td>87±11</td>
<td>70±16</td>
</tr>
</tbody>
</table>

Values are mean$\pm$SD percent inhibition. All comparisons $P=NS.$

### Diabetic Patients and PCI

Patients with diabetes mellitus are at an increased risk of in-hospital complications after PCI and, in the long term, are at a higher risk of developing restenosis than are nondiabetic patients.$^{11-13}$ Although in the EPILOG trial abciximab treatment decreased acute adverse events in diabetic patients as well as, if not more potently than, in nondiabetic patients,$^{14}$ in the long term, diabetic patients experienced a substantially higher incidence of target vessel revascularization.$^6$ On the other hand, in the 6-month data from the EPISTENT trial, a highly significant 51% decrease (8.1% versus 16.6%, $P=0.021$) in target vessel revascularization at 6 months was noted in stented diabetic patients treated with abciximab compared with stented diabetic patients receiving placebo.$^3$ These clinical findings were corroborated by those of the EPISTENT angiographic substudy, which reported a significant increase in net gain among diabetic patients treated with abciximab compared with diabetic patients who did not receive abciximab.$^5$ Several mechanisms for the influence of diabetic status on outcomes have been proposed, including differences in platelet function. The platelets of diabetics have been shown to be larger$^{20}$ and to have enhanced fibrinogen binding$^{15}$ and impaired ability to mediate vasodilatation.$^{16}$ The larger platelet size correlates with enhanced platelet aggregation,$^{17}$ as well as increased numbers of GP IIb/IIIa receptors (by up to 26%).$^{18}$ There also is increased glycation of the GP IIb/IIIa receptors in some diabetics, which has been hypothesized to contribute to increased aggregation.$^{19,20}$ The results of our study show that these underlying platelet abnormalities do not appear to translate into a difference in the degree of platelet inhibition achieved with abciximab during and early after PCI. Whether the degree or duration of platelet inhibition maintained beyond the initial 24 hours evaluated in this study influences long-term outcomes, or varies based on diabetic status, requires further study.

### Previous Studies of Monitored Platelet Inhibition With Abciximab

Five studies involving a total of 63 patients who received abciximab before PCI and 50 patients who received it unassociated with PCI evaluated the degree of inhibition of platelet...
aggregation achieved in humans. These studies differed in their patient populations and techniques for evaluation of platelet function and dosing regimens, and although none were powered to precisely define interindividual variability in response to abciximab, the majority did demonstrate variability similar to that seen in the present study.

Clinical Implications
The safety of GP IIb/IIIa therapy may be influenced by interindividual variations in response to treatment. An increased bleeding risk with prolonged oral anti–GP IIb/IIIa therapy has been demonstrated in early trials with these agents. Studies of platelet function in patients receiving prolonged oral GP IIb/IIIa inhibitor therapy may help to identify the optimal level of platelet inhibition required to maximize the antithrombotic benefits while minimizing bleeding complications.

Although the strong relation noted in our patients between adverse events and the level of platelet inhibition at 8 hours is of considerable interest for the purpose of hypothesis development, these results require careful interpretation because clinical correlation was not a predefined objective of the study. Thus, although it is tempting to suggest that decreased inhibition of platelet function was permissive in the development of ischemic events, it is possible that decreased systemic platelet inhibition is a marker for an ongoing thrombotic process. Larger prospective trials with platelet function monitoring will be necessary to further characterize the interindividual response to GP IIb/IIIa inhibitors, understand determinants of this variability, and, most important, assess whether this variability in response affects clinical efficacy and safety.

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References
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