Enhanced Shear-Induced Platelet Aggregation in Patients Who Experience Subacute Stent Thrombosis
A Case-Control Study

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OBJECTIVES
The goal of this study was to identify differences in shear-induced platelet aggregation (SIPA) between patients who did or did not experience subacute stent thrombosis (SAT).

BACKGROUND
Despite dual antiplatelet therapy, SAT after coronary stenting occurs in approximately 1% of patients. There is no accepted platelet function test to identify patients at risk.

METHODS
We analyzed platelet aggregation in 10 patients who had experienced SAT (cases), 22 stented patients without SAT (controls), and 17 healthy volunteers (normals). All patients except normals were treated with both aspirin and clopidogrel.

RESULTS
Shear-induced platelet aggregation was higher in cases than in controls at both shear rates of 200 s⁻¹ (40.9 ± 12.2% vs. 18.2 ± 18%, p = 0.013) and 4,000 s⁻¹ (57.4 ± 16.4% vs. 23.4 ± 21.2%, p = 0.009). Moreover, SIPA in cases was significantly higher than in normals both at 200 s⁻¹ (p = 0.013) and 4,000 s⁻¹ (p = 0.009).

CONCLUSIONS
Shear-induced platelet aggregation is increased in patients experiencing SAT compared with controls receiving dual antiplatelet therapy and to normals receiving no antiplatelet therapy, which suggests increased intrinsic patient-related platelet reactivity in patients with SAT. The predictive value of SIPA for SAT requires prospective investigation. (J Am Coll Cardiol 2005;45:1753–56) © 2005 by the American College of Cardiology Foundation

Percutaneous coronary intervention (PCI) with stenting is now an established treatment for coronary artery disease. Dual antiplatelet therapy with aspirin and a thienopyridine (ticlopidine or, more recently, clopidogrel) has strikingly improved the results of PCI through a marked reduction in myocardial infarction (7) and has been shown to be insensitive to aspirin (9) but sensitive to the combined therapy with aspirin and thienopyridine (7,10). We performed a prospective study of individuals (identified post hoc) with SAT to test the hypothesis that SIPA might identify differences between patients with or without SAT despite dual antiplatelet therapy.

PATIENTS AND METHODS

Patients. Between March 2001 and October 2003, 1,600 consecutive patients underwent urgent or elective PCI and stenting in our institution. All patients were treated with aspirin 75 to 250 mg daily. Clopidogrel was administered either with a 75-mg daily dose beginning at least four days before stenting, or with a loading dose (300 mg) given the day of the procedure. Following the procedure, all patients received clopidogrel 75 mg daily for at least one month. Intravenous glycoprotein IIb/IIIa inhibitors were used at the operator’s discretion. From this group of patients, 19 (1.19%) experienced SAT, of whom 9 either died early after the event, were unavailable for the study (for technical reasons), or had clear evidence of noncompliance to antiplatelet therapy. The 10 remaining patients were available for study and had been compliant with antiplatelet therapy. These 10 cases had experienced an SAT of 9.2 ± 6.2 days (range, 2 to 21 days) after PCI. All presented with acute chest pain; among them, six had ST-segment elevation and four had unstable angina and ST-segment depression. At emergency angiography, all had complete occlusion (i.e., Thrombolysis In Myocardial Infarction flow grade 0 to 1) of

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the previously stented vessel. Nine were treated with emergency PCI to recanalize the infarct-related artery using, in seven cases, intravenous glycoprotein IIb/IIIa inhibitors during the procedure. The last patient underwent emergency coronary artery bypass grafting. One patient received thrombolytic therapy on the way to the catheterization laboratory. A control group was constituted of 22 patients who were enrolled during the same period and who did not experience SAT after stenting and receiving dual antplatelet therapy. Finally, a normal group of 17 healthy volunteers that had taken no medication during the two previous weeks was constituted (identified hereafter as normals). Informed consent was obtained from all patients and volunteers.

**Blood sampling and platelet-rich plasma (PRP) preparation.** Blood samples were obtained from the cases within 4.6 ± 3.4 days (range, 1 to 12 days) after SAT, whereas clopidogrel and aspirin had been initiated at the time of the initial stenting procedure (5). They were obtained from the controls at least three days after the initiation of clopidogrel. Blood was drawn in evacuated container tubes (Vacutainer, Becton-Dickinson, Plymouth, United Kingdom) containing 0.129 mol/l trisodium citrate (1 vol/9 vol blood). Platelet-rich plasma was obtained as previously described (11) and was adjusted to 300 g/l by adding homologous platelet-poor plasma.

**Agonist-induced platelet aggregation.** Platelets in PRP were stimulated with 3.2 μmol/l adenosine diphosphate (ADP) (Biodata, Horsham, Pennsylvania), 2 μg/ml collagen (Horn, Nycomed, München, Germany), or 1.3 mmol/l arachidonic acid (Chronolog, Havertown, Pennsylvania). Aggregation was measured at 37°C in a Lumi-Aggregometer model 490 (Chronolog) and expressed as the maximal percent change in light transmittance from baseline at 5 min after the addition of the agonist, with platelet-poor plasma as a reference.

**SIPA.** Shear-induced platelet aggregation was measured by means of a coaxial cylinder shearing device, as previously described (11). Platelets in PRP (300 g/l) were exposed to a shear rate of 200 or 4,000 s⁻¹ for 2 min at 20°C in the presence of 1 mmol/l CaCl₂. Samples were fixed with 1% paraformaldehyde, and the number of single platelets was measured by flow cytometry (Coulter Epics XL, Beckman Coulter, Roissy, France) before and after exposure to shear. Shear-induced platelet aggregation was expressed as the percentage of disappearance of single platelets, i.e., disappearance of single platelets (DSP) = (n₀ - n)/n₀, where n₀ represents the single platelet population of the nonsheared sample and n the single platelet number in the sheared sample. Each PRP was tested in duplicate.

**von Willebrand factor (vWF) assay.** The plasma level of vWF antigen was measured by enzyme-linked immunosorbent assay (Asserachrom vWF antigen, Diagnostica Stago, Asnières, France).

**Statistics.** Data were analyzed using the StatView software package (version 5, SAS Institute, Cary, North Carolina). Baseline characteristics of the two patient groups were compared using the chi-square test for categorical variables and with the Student unpaired t test for continuous variables. Comparisons between groups were performed using the nonparametric Kruskal-Wallis test. Comparisons between subgroups used the Mann-Whitney U test with Bonferroni correction for multiple comparisons, thus yielding statistical significance if p < 0.0167. All tests were two-tailed. All data were presented as mean values and SD.

## RESULTS

**Clinical data.** The baseline clinical and procedural characteristics as well as the rate of use of medications were similar between groups (Table 1).

**Agonist-induced platelet aggregation.** A significant decrease in platelet response to either collagen or ADP was observed in controls compared with normals (p < 0.001), as expected from patients receiving clopidogrel and aspirin (Figs. 1A and 1B). Cases did not differ from controls. A normal aggregation in response to ADP (defined as >45% of the maximal light transmittance) despite clopidogrel

### Table 1. Patients’ Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 10)</th>
<th>Controls (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>62 ± 12</td>
<td>56 ± 15</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>9 (90%)</td>
<td>19 (83%)</td>
</tr>
<tr>
<td>Risk factors for CAD, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td></td>
<td></td>
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<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of CAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial presentation, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA/NSTEMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable angina/silent ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline procedural characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stents/patient, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total stent length, mm</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Maximum stent diameter, mm</td>
<td>17 ± 6</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Maximum inflation pressure, atm</td>
<td>3.0 ± 0.4</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>Use of IV GP IIb/IIIa blockers, n (%)</td>
<td>8 (80%)</td>
<td>17 (77%)</td>
</tr>
<tr>
<td>Extent of CAD, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>5 (50%)</td>
<td>11 (50%)</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>5 (50%)</td>
<td>11 (50%)</td>
</tr>
<tr>
<td>In-hospital statins statins</td>
<td>8 (80%)</td>
<td>22 (100%)</td>
</tr>
</tbody>
</table>

CAD = coronary artery disease; GP = glycoprotein; IV = intravenous; MI = myocardial infarction; NA = not available; NSTEMI = non–ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina.
therapy was observed in 3 of 10 cases and 4 of 22 controls (p = NS). All cases and controls displayed an absence of response to arachidonic acid (data not shown), providing objective evidence of adherence to aspirin therapy.

SIPA. Shear-induced platelet aggregation was measured at two different shear rates: 200 s⁻¹ and 4,000 s⁻¹. The normal values of aggregation determined in a normal group of healthy subjects were 26.2 ± 13.8% at 200 s⁻¹ and 40.2 ± 9.9% at 4,000 s⁻¹ (Figs. 2A and 2B). Among controls, the mean values of DSP at 200 s⁻¹ (18.2 ± 18%) and 4,000 s⁻¹ (23.4 ± 21.2%) were lower than those obtained in normals, as was expected from patients treated with clopidogrel, although the decrease reached significance only at 4,000 s⁻¹ (p = 0.0008) (Figs. 2A and 2B). In contrast, DSP values of the cases were significantly higher than the normal values both at 200 s⁻¹ (40.9 ± 12.2%, p = 0.013) and 4,000 s⁻¹ (57.4 ± 16.4%, p = 0.009). Moreover, SIPA was significantly higher in cases than in controls both at 200 s⁻¹ (p = 0.0008) and 4,000 s⁻¹ (p = 0.0002).

Although plasma levels of vWF were increased in both groups, vWF was lower among cases (154 ± 54%) than among controls (203 ± 76%), indicating that the higher values of SIPA observed among cases cannot be explained by variations in vWF levels.

DISCUSSION

The aim of the present study was to assess differences of platelet activation/aggregation in patients with versus without SAT after PCI, despite dual antiplatelet therapy with aspirin and a standard dose of clopidogrel. We used a case-control design, focusing on prospectively identified consecutive cases of SAT.

The analysis of the inhibitory effect of aspirin/clopidogrel on platelet reactivity to physiological agonists and shear stress strongly suggests that interindividual variations in response to aspirin and/or clopidogrel are directly associated with stent thrombosis.

Shear-induced platelet aggregation did not decrease under normal values in patients with SAT, suggesting that these patients might differ from controls by a reduced response to clopidogrel. It has been previously suggested that clopidogrel resistance could be associated with recurrent cardiovascular events (6) and that hypothetical mechanisms for such resistance include cytochrome P450 metabolic activity (12) as well as P₂Y₁₂ polymorphisms (13).

The most striking observation was that SIPA was not only able to discriminate cases from controls but that it was significantly higher among cases than among normals de-
spite the fact that cases and controls but not normals received dual antiplatelet therapy. Increased values in cases were observed both at low and high shear rates, corresponding to shear rates applied in veins and atherosclerotic arteries (14), respectively, indicating that the degree of platelet stress or pre-activation is important in patients with SAT. Comparison of the various tests performed in cases, controls, and normals indirectly suggest that although conventional methods of inhibition of platelet aggregation by turbidimetry really assess the response to aspirin/clopidogrel, SIPA is able to differentiate cases from both controls and normals. This suggests that increased SIPA is really a marker of intrinsic patient-related platelet activation and, therefore, that among patients with SAT, some may present with increased platelet reactivity compared with controls and normals that cannot be overcome by antiplatelet therapy. An alternative explanation is that SIPA may be a better, more physiologically relevant test than other measures of platelet reactivity and response to antiplatelet therapy.

**Study limitations.** The main limitations of this study are its small size and the fact that patients were studied several days after SAT. However, as previously noted (3), SAT is a rare and unplanned event, making prospective studies difficult. It cannot be excluded that the differences in platelet function observed are the consequence rather than the cause of SAT. Additional study at later time points should provide more compelling evidence of the causality of increased SIPA in SAT. The relevance of our results to patients receiving a higher (600 mg) loading dose of clopidogrel, which is now frequently used, as opposed to a “standard” 300-mg dose, is uncertain (15).

In conclusion, this study suggests that SIPA is abnormal in patients experiencing SAT and suggests possible intrinsic differences in platelet reactivity in these patients. Prospective studies are needed to assess the response to dual antiplatelet therapy using SIPA in patients undergoing stenting.

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**REFERENCES**

9. Moake JL, Turner NA, Stathopoulos NA, Nolasco L, Hellums JD. Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin. Blood 1988;71:1366–74.